Grand Challenges in Biology and Biotechnology

Ihana Aguiar Severo
Juan Carlos Ordóñez
André Bellin Mariano
José Viriato Coelho Vargas *Editors*

Microalgae Horizons

Fundamentals, Innovations, and Industrial Applications

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Grand Challenges in Biology and Biotechnology

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Editors

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Editors
Ihana Aguiar Severo
Center for Advanced Power Systems (CAPS)
Florida State University (FSU)
Tallahassee, FL, USA

Sustainable Energy Research & Development Center (NPDEAS)
Federal University of Paraná
Curitiba, PR, Brazil

André Bellin Mariano
Sustainable Energy Research & Development Center (NPDEAS)
Federal University of Paraná
Curitiba, PR, Brazil

Juan Carlos Ordóñez
Center for Advanced Power Systems (CAPS)
Florida State University (FSU)
Tallahassee, FL, USA

José Viriato Coelho Vargas
Sustainable Energy Research & Development Center (NPDEAS)
Federal University of Paraná
Curitiba, PR, Brazil

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To my family, whose constant support, love, and encouragement have been the foundation of my journey. Their belief in me has given me the strength to pursue my dreams and overcome every challenge.
To my co-editors and mentors, Juan, José, and André, researchers and professors, whose guidance and collaboration have been invaluable at every step of this process. Their experience, dedication, and support have shaped my career.
This book is the result of the collective effort, shared knowledge, and dedication of those who inspire and elevate others in the relentless pursuit of achieving their full potential and goals.

Preface

Microalgae have emerged as one of the most versatile and promising biological resources of our time. With their remarkable ability to convert sunlight and carbon dioxide into valuable biomass, these microorganisms are poised to address some of the most pressing challenges in energy, food security, and environmental sustainability. From their early recognition as ecological powerhouses to their modern applications in biotechnology, nutraceutical production, pharmaceuticals, agricultural uses, and biofuels, microalgae represent a transformative force across multiple industries.

This book reflects the collaborative efforts of leading experts, researchers, and visionaries who have dedicated their work to exploring and advancing microalgae technologies. It provides a comprehensive journey through the evolution of microalgae research, highlighting the milestones that have been achieved and the challenges that remain. With a balanced focus on fundamental knowledge, cutting-edge innovations, and future prospects, this volume serves as both an informative guide for researchers and an inspiration for those new to the field.

The intent of this book is not only to compile the achievements in microalgae biotechnology but also to stimulate new ideas and collaborations. By addressing the interactions between science, technology, and industry, we hope to narrow the gap between laboratory research and large-scale industrial applications, paving the way for a sustainable bioeconomy.

We are deeply grateful to our contributors, whose insights and expertise have enriched this volume. We also extend our appreciation to the institutions and organizations that have supported this endeavor. Most importantly, we hope this book inspires you, our readers, to continue exploring the unlimited potential of microalgae to create a greener, healthier, and more sustainable world.

Tallahassee, FL, USA — Ihana Aguiar Severo
Tallahassee, FL, USA — Juan Carlos Ordóñez
Curitiba, PR, Brazil — André Bellin Mariano
Curitiba, PR, Brazil — José Viriato Coelho Vargas

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Part I
Innovations in Microalgae Processing—From Fundamentals to Scalable Solutions

Introductory Chapter—Microalgae: Past, Present, and Future Directions



Ihana Aguiar Severo, Juan Carlos Ordóñez, José Viriato Coelho Vargas, and André Bellin Mariano

Abstract This chapter provides an overview of the past, present, and future of microalgae, tracing their evolution from essential contributors to the Earth's ecosystem to their modern role as key players in biotechnology and sustainability. Here, we describe the early scientific interest in microalgae as potential sources of food and biofuels, highlighting significant advances in cultivation and industrial applications over the years. The chapter also discusses current advances in microalgae-based systems for the production of biofuels, food, pharmaceuticals, and other bioproducts, and environmental bioremediation. Future challenges and the promising potential of large-scale production of microalgae for the creation of a circular bioeconomy are also discussed.

Keywords Microalgae · Biotechnology · Photobioreactors · Large-scale cultivation · Bio-based economy · Environmental sustainability

I. A. Severo (✉)
Department of Mechanical Engineering, FAMU-FSU College of Engineering, Energy and Sustainability Center, Center for Advanced Power Systems (CAPS), Florida A&M University, Florida State University, Tallahassee, FL, USA

Sustainable Energy Research & Development Center (NPDEAS), Federal University of Paraná (UFPR), Curitiba, PR, Brazil
e-mail: is23@fsu.edu

J. C. Ordóñez
Department of Mechanical Engineering, FAMU-FSU College of Engineering, Energy and Sustainability Center, Center for Advanced Power Systems (CAPS), Florida A&M University, Florida State University, Tallahassee, FL, USA

J. V. C. Vargas · A. B. Mariano
Sustainable Energy Research & Development Center (NPDEAS), Federal University of Paraná (UFPR), Curitiba, PR, Brazil

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1 Microalgae: A Brief History

Microalgae have been quietly influencing our planet for over 4 billion years, with an immeasurable number of species having evolved since life first appeared here. These microorganisms are fundamental to life on Earth because they were among the first to perform photosynthesis, a process that transformed the atmosphere, filling it with the oxygen we depend on today. Although invisible to the naked eye, microalgae have always been colossal in their ecological impact, producing a significant portion of the world's oxygen and forming the basis of aquatic food chains (Chisti, 2023).

These distinct, single-celled microscopic organisms are found in both freshwater and marine environments. They are a diverse group of microorganisms capable of converting sunlight, carbon dioxide, and nutrients into energy and biomass through photosynthetic mechanisms. Microalgae are incredibly diverse, with thousands of species varying in size, shape, and function (Fay, 1983), and they play a critical role in the global carbon cycle, contributing to carbon sequestration and oxygen production (Kruse et al., 2005). Besides, microalgae have the unique advantages of rapid growth, adaptability, versatility, and an impressive range of valuable by-products, making them a key bioresource in the search for renewable feedstocks and sustainable solutions across multiple industries.

It was recognized very early on that we have more species than functions, and therefore, there was a need to group them taxonomically. With the development of classification and nomenclature systems, the number of described species grew exponentially. Around 12 years ago, Guiry (2012) reported in the online database AlgaeBase (https://www.algaebase.org) that more than 30,000 species had been described. This number has continued to rise, and in his recent article "How many species of algae are there? A reprise. Four kingdoms, 14 phyla, 63 classes and still growing" (Guiry, 2024), he updated the database to include more than 50,500 living species and about 10,556 fossil species. Still, millions of species remain undescribed. Historically, they have been largely ignored by science and industry, hiding in plain sight until a convergence of environmental and technological changes highlighted their immense potential.

In the last century, the scientific community began to recognize the enormous promise of microalgae (mostly Spirulina and Chlorella). Early studies emphasized their role in ecosystems, but it wasn't long before researchers discovered the versatile properties of microalgae biomass, from high levels of proteins and essential fatty acids to pigments, vitamins, and other valuable compounds (Barbosa et al., 2023). During the mid-twentieth century, research on microalgae gained momentum

with early explorations of their potential as a nutritional source, especially during periods of food shortages in countries concerned about food security after World War II. Scientists observed that certain species of microalgae could produce considerable amounts of protein and nutrients in a fraction of the time required by traditional land crops, sparking interest in their role in human nutrition. This marked the beginning of the "modern era" of microalgae biotechnology, with important developments such as the first Symposium on Mass Culture of Algae, held at Stanford University in 1952, as well documented in the paper by Coaldrake (2021), who reviewed historical events and more recent sources on algae research.

Later, as the world struggled with the environmental consequences of fossil fuel dependence and unsustainable agricultural practices, microalgae came back into the spotlight. In the second half of the twentieth century, biofuel research increased, and microalgae became a focal point. Their ability to produce lipids that could be converted into biodiesel, coupled with their high growth rate and ability to grow on non-arable land, offered a tempting alternative to conventional terrestrial crops for biofuel production. Thus, the last few decades have witnessed a paradigm shift in how we view microalgae—from ecological curiosities to potential biofuel producers, sustainable food sources, and environmental remediators (Borowitzka & Moheimani, 2013).

Today, microalgae research is at the nexus of biotechnology, environmental science, and industrial innovation. Advances in cultivation technology, photobioreactor design, and genetic engineering have taken microalgae research to new heights. Scientists are now able to precisely manipulate environmental factors, cultivating microalgae in controlled systems that maximize yield and optimize the quality of the end product. This shift has enabled the large-scale production of not only bulk commodities such as food, feed, chemicals, and biofuels but also high-value products such as omega-3 fatty acids, natural colorants, and bioactive compounds used in pharmaceuticals, cosmetics, and biomedical products (Wijffels et al., 2013; Borowitzka, 2013; Lafarga et al., 2020; Severo et al., 2022, 2024; Sirohi et al., 2022; Costa et al., 2024) (Fig. 1). Box 1 highlights additional information on microalgae cultivation systems over time.

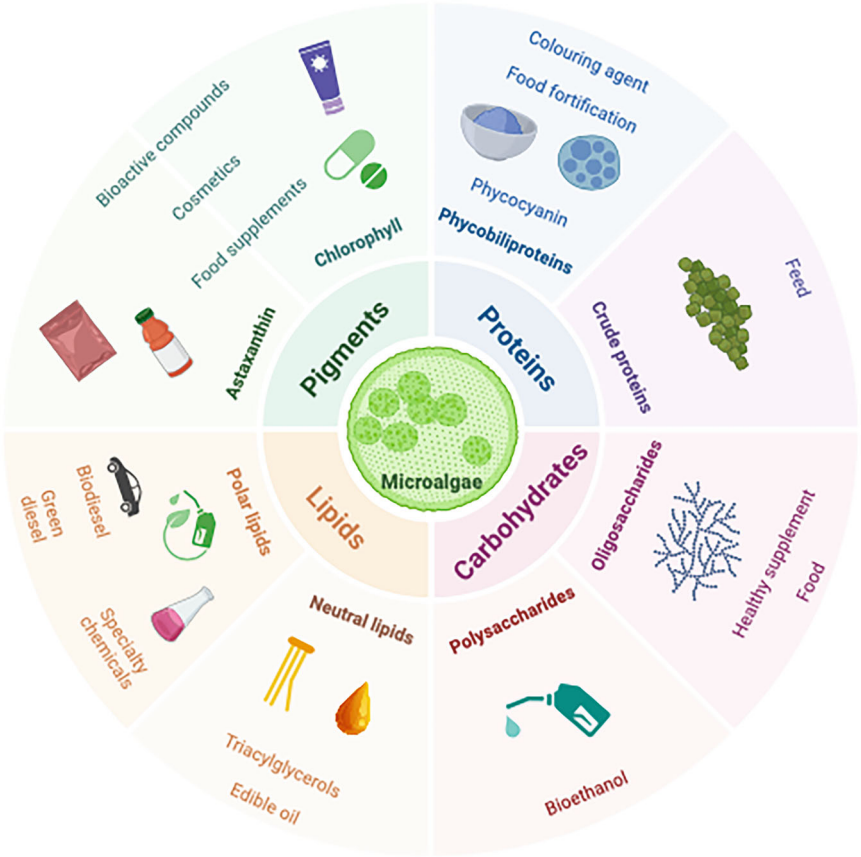


Fig. 1 Schematic representation of various microalgae applications

Box 1: Evolution of Microalgae Cultivation Systems

Over time, the evolution of cultivation systems has mirrored the progress of microalgae research itself, progressing from rudimentary open systems to highly sophisticated closed designs. These systems play a critical role in optimizing microalgae cultivation by providing controlled environments that maximize growth and productivity. Broadly categorized into open and closed systems, each type presents distinct advantages and challenges (Garrido-Cardenas et al., 2018; Acién-Fernández et al., 2021).

(continued)

Box 1 (continued)

1. **Open systems: Past**

Open systems such as raceways and circular ponds have been the primary methods for growing microalgae due to their simplicity and low operating costs. These systems were widely used in early large-scale trials, particularly for biofuel production. However, as researchers sought greater control over contamination, evaporation, environmental fluctuations, and productivity, closed photobioreactors emerged (Borowitzka, 1999).

2. **Closed systems: Present**

Today, closed systems such as tubular, airlift, and flat plate panels offer the precision needed to grow high-value compounds and pharmaceuticals by providing controlled environmental conditions, protecting cultures from external contamination, and allowing for precise regulation of light, temperature, and nutrient levels, thereby increasing the yield and purity of target compounds. However, photobioreactors have higher operating costs. The choice between open and closed systems depends on the specific goals of the production process, whether for biofuels, pharmaceuticals, or high-value compounds, and the scale at which production is intended (Acién-Fernández et al., 2021).

3. **Hybrid and next-generation systems: Future**

Research is focused on developing hybrid systems that combine the affordability of open systems with the control of closed systems. Advances in automation and energy efficiency are expected to reduce costs and further enhance productivity, enabling large-scale production for diverse applications from biofuels to pharmaceuticals (Shahhoseyni et al., 2024).

Along with technological advances, microalgae have found applications in environmental regeneration. Microalgae-based wastewater treatment systems have been and are being developed to remove contaminants while producing biomass, capture carbon dioxide, and even combat urban heat islands through building-integrated photobioreactors. Cities of the future may well incorporate green facades and architecture-integrated algae bioreactors, contributing to urban sustainability efforts (Talaei et al., 2020).

The economic, environmental, social, and regulatory implications of large-scale microalgae production cannot be ignored. As industries increasingly adopt microalgae-based systems, the need for economic models and market structures that support such innovation has become apparent (Stephens et al., 2010). Furthermore, patent applications have increased significantly in recent years, highlighting the competitive race to unlock and protect new technologies in microalgae

cultivation and bioproduct extraction (Severo et al., 2021). These factors have spurred international collaboration, policy formation, and funding to promote sustainable microalgae research and commercialization.

Looking ahead, the future of microalgae is full of promise. Emerging genetic tools and synthetic biology are opening the door to unprecedented levels of control over microalgae metabolism, allowing scientists to tailor strains for specific industrial applications, increase productivity, or even introduce new metabolic pathways (Sproles et al., 2021; Goold et al., 2024). Imagine, for example, microalgae engineered to produce biofuels, pharmaceuticals, and bioplastics in a single integrated process, a "biorefinery scenario" (Severo et al., 2019), or strains that can thrive in extreme environments, expanding the geographic scope of microalgae-based industries. It is worth noting that synthetic biology approaches could also enhance the role of microalgae in supporting human space exploration. These advances will provide numerous opportunities for the development of a spectrum of strains designed for habitation on Mars (Mapstone et al., 2022). In addition, the power of artificial intelligence, machine learning, and new computational tools is expected to play a significant role in optimizing growing conditions and predicting yield outcomes with remarkable accuracy, making large-scale production more economically viable (Plouviez et al., 2024).

However, the future will also bring challenges: from scaling up production cost-effectively to navigating regulatory frameworks and public acceptance. Transitioning from a small-scale laboratory to a fully operational, industrial-scale microalgae production complex is no easy task, as it involves overcoming complex technical, economic, and regulatory obstacles (Plouviez et al., 2022; Guieysse & Plouviez, 2024). But with each study, discovery, and innovation, we are moving closer to a world where microalgae fulfill their potential to address the pressing challenges of our time. Microalgae are no longer just a tool for scientists; they are a viable path to a sustainable, bio-based economy that benefits both people and the planet. As our global society seeks sustainable alternatives, microalgae emerge not only as a "green" solution but as a versatile platform of possibilities waiting to be cultivated, refined, and scaled up. The road ahead may be challenging, but the potential for microalgae to transform industries and contribute to a more sustainable future is undeniable.

In this book, we will explore these topics across multiple chapters, each focusing on specific aspects of microalgae cultivation, technology, and applications. From cultivation techniques and crop protection strategies to socio-economic considerations and case studies, this edited book aims to provide a comprehensive overview of where the field of microalgae biotechnology is today and where it is going. This introductory chapter serves as an invitation to join us on a timeline through the past, present, and future of microalgae—a journey of exploration, discovery, and innovation with potential far beyond what we can see under the microscope.

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Challenges of Microalgae Production: From the Laboratory to Large Scale



Daniel Nascimento Motta, Vanderson Gabriel Souza Torres, Vanessa Leal de Queiroz Hermino, Siglia Maria Braga Neves, Luis Vitor Vieira de Oliveira, Rosany Piccolotto Carvalho, and Edson Pablo da Silva

Abstract The use of microalgae as a source of essential nutrients and compounds with bioactive properties of interest in human food and various industrial segments has been a great challenge to be explored and increasingly used, meeting the desires of consumers for increasingly natural products, acting effectively in reducing the percentage of synthetics in various formulations such as food, pharmaceutical, and/or chemical industries. Although the great benefits of the consumption and use of microalgae are well known, their production, cultivation, and marketing systems are still incipient. Given these changes, the great challenge is the control of production at the laboratory level and the systematization of the scaling process using environments and means of control of growth and production, which can influence the quality of the microalgae and the synthesis of its nutrients. Parameters such as temperature, pH, nutrients, and lighting need to be carefully monitored and controlled to ensure healthy microalgae growth. As well as microbiological contamination, which is a significant challenge, especially in open systems, the presence of bacteria, fungi, and other microalgae can compete with the desired microalgae, reducing not only the crop yield but also the bioactive compounds of interest present in the microalgae. Determining the optimal harvest point is also a major challenge that can effectively influence these desirable traits. Regulations and permits for large-scale production of microalgae should be subject to environmental and food safety regulations. Obtaining the necessary licenses and permits to operate a large-

D. N. Motta · V. L. de Queiroz Hermino · E. P. da Silva (✉)
Amazon Biobusiness Center, Manaus, Brazil

Postgraduate Program in Biotechnology – PPGBIOTEC/UFAM/BRAZIL, Manaus, Brazil
e-mail: edsonsilva@fuea.org.br

V. G. S. Torres
Amazon Biobusiness Center, Manaus, Brazil

Paulista State University – UNESP/BRAZIL, São Paulo, Brazil

S. M. B. Neves · L. V. V. de Oliveira
Amazon Biobusiness Center, Manaus, Brazil

R. P. Carvalho
Postgraduate Program in Biotechnology – PPGBIOTEC/UFAM/BRAZIL, Manaus, Brazil

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scale production facility can be a time-consuming and costly process. Overcoming these challenges requires ongoing research and development in various areas, including molecular biology, process engineering, biotechnology, and economics. As technology advances and new solutions are developed, large-scale microalgae production has the potential to become a major source of renewable biomass, with applications in food, feed, biofuels, and chemicals.

Keywords Microalgae production · Large-scale cultivation · Renewable biomass · Biofuels · Microalgae harvesting · Contamination control

1 Introduction

In this chapter, we will address some of the main challenges regarding scaling microalgae production, emphasizing some points that we think are crucial in this process and that we have been working on in recent years. Parameters such as temperature, pH, nutrients, and adequate photoperiod need to be carefully monitored and controlled to ensure the healthy growth of microalgae, avoiding the appearance of strange odors, which may be related to the consumption of unsaturated fatty acids, generating the appearance of undesirable odors and which often make the entire process unfeasible. Effective production and harvesting methods are needed to separate microalgae from culture water profitably and economically. In this sense, the use of alternative sources of nutrients can be an interesting strategy to reduce production costs. The high energy cost can be an obstacle to the economic viability of large-scale production, requiring structural adaptations within the production site in order to obtain a greater natural light source during the photoperiod in the production process. The development of cultivation systems that minimize the use of water and allow efficient recycling of cultivation water is fundamental for the sustainability of large-scale production, always respecting the recommended standards and regulations in order to maintain the safety and security of the product. As biotechnological processes advance and new solutions are developed in the production process and a greater understanding of the physiology of microalgae, large-scale production of microalgae has a great chance of becoming an important source of nutrients and inputs with diverse applications, in extremely varied economic sectors such as food, pharmaceuticals, textiles, biofuels, and chemicals.

2 Cultivation Control

The large-scale production of microalgae is a promising source of renewable biomass with applications in several areas, such as food, feed, biofuels, and chemicals. However, controlling large-scale microalgae cultivation presents significant challenges that need to be overcome to ensure the efficiency and economic viability of these systems (Borowitzka, 2020). One of the main challenges is maintaining ideal

Table 1 Some parameters and preventive corrections in large-scale microalgae cultivation

Monitored Parameters	Sensors	Control option/ Monitoring method	Acceptable range	References
pH	Optical pH sensor	CO_2 Injection	7–10	Nwoba et al. (2022)
Temperature	Temperature Sensor/Temperature Controller	Water spraying; Heat exchanger; Shading	15–35 °C	Nwoba et al. (2022)
Luminosity	Brightness sensor	Cell density; Design of the photobioreactor or raceway; Mixing	10–250 μM/m^2/s (ideal);0–2000 μM/m^2/s (real)	Masojídek et al. (2021)
CO_2	Meters	CO_2 feed	>0.15%	Havlik et al. (2022)

growing conditions on a larger scale. In the laboratory, microalgae can be grown under controlled, optimized conditions to promote maximum growth. However, when expanding production to an industrial scale, complications arise related to the homogeneity and stability of growing conditions. Variations in environmental conditions, such as temperature, pH, and light intensity, can significantly affect the growth of microalgae and, consequently, the productivity of the system (Dębowski et al., 2023). This fact can also lead to the appearance of undesirable contaminants, making the production process unviable. Ma and Hu (2023) report that microbiological contamination is a constant challenge in large-scale microalgae cultivation systems. The presence of bacteria, fungi, and other unwanted microalgae can compete with the desired species, reducing the cultivation yield and compromising the quality of the final product. Controlling and mitigating contamination requires effective monitoring and prevention strategies, as well as cultivation systems designed to minimize the risk of infection. In this sense, controlling effective harvesting methods is essential to separate microalgae from culture water economically and sustainably. Many conventional harvesting methods are expensive, energy-intensive, and can result in significant biomass losses. Therefore, developing more efficient and cost-effective harvesting techniques is crucial to making large-scale production of microalgae more viable (Ruiz et al., 2022). Table 1 shows the parameters used in microalgae cultivation, which highlights the sensor control options, monitoring method, and acceptable range.

3 Contamination

Microbiological contamination represents a significant challenge in the cultivation of microalgae on a large scale, which can compromise the productivity and quality of the final product. This phenomenon occurs due to the presence of unwanted

organisms, such as bacteria, fungi, and other species of microalgae, which compete for nutrients, space, and light, harming the growth and development of the desired microalgae (Novoveská et al., 2023). Even small amounts of contaminants can multiply rapidly under favorable conditions, leading to suppression of the growth of cultivated microalgae. Among the main contaminants, we can find some bacterial groups mainly linked to deficient hygienic processes such as *Escherichia coli, Salmonella* sp., and *Staphylococcus*, among others. Furthermore, contamination can occur through water supplies, ambient air, non-sterilized growing equipment and materials, and even external agents such as birds and insects (Iamtham & Sornchai, 2022). Controlling microbiological contamination requires the implementation of preventative measures and rigorous monitoring strategies. This includes sterilizing equipment and materials before use, filtering and treating culture water to remove unwanted organisms, and maintaining environmental conditions that favor the growth of cultured microalgae over contaminants. Furthermore, it is important to develop and maintain adequate hygiene practices and ensure adequate segregation between different strains of microalgae to prevent the spread of contamination between crops (Abiusi et al., 2022). Microbiological contamination can have significant consequences in large-scale microalgae cultivation, resulting in production losses and additional costs associated with cleaning and disinfecting cultivation systems. Therefore, it is essential to invest in research and development of technologies and cultivation practices that minimize the risk of contamination and promote the sustainable production of microalgae on a large scale.

4 Harvest Efficiency

Harvest efficiency is a crucial aspect of large-scale microalgae cultivation, but it faces significant challenges that can affect the economic and environmental viability of production systems. Efficient harvesting of microalgae is essential to separate cells from culture waters, concentrate, and process them for subsequent use in various sectors, such as biofuels, food, feed, and pharmaceuticals (Kumar et al., 2024). One of the main difficulties in harvesting microalgae on a large scale is the selection of methods that are effective, economical, and environmentally sustainable. Many of the conventional harvesting methods, such as centrifugation, filtration, and sedimentation, are effective on a small scale, but face scalability and cost challenges in larger production systems. Furthermore, these methods often consume large amounts of energy, water, and chemical reagents, contributing to the operational and environmental costs of the harvesting process (Thiviyanathan et al., 2024). Another challenge is the need to adapt harvesting methods to different microalgae species and cultivation conditions. Not all harvesting methods are suitable for all microalgae species, and cultivation conditions, such as cell density, medium viscosity, and the presence of contaminants, can affect the efficiency and selectivity of harvesting methods (Long et al., 2024).

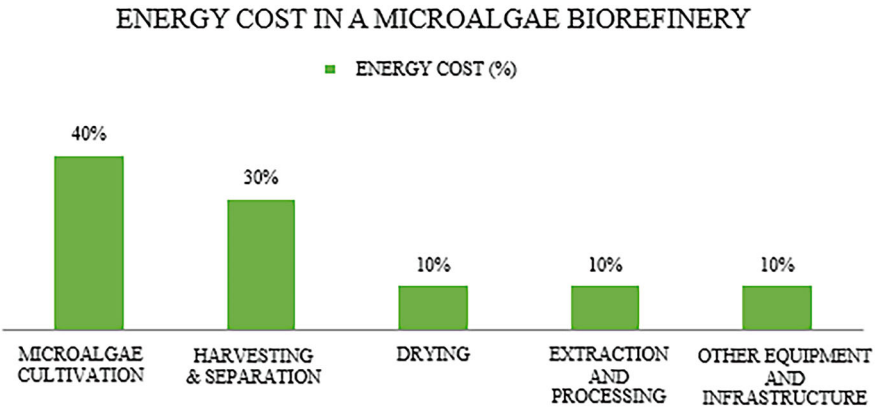
Furthermore, harvesting microalgae on a large scale can be complicated by the presence of contaminants and impurities in the cultivation medium, which can make cell separation difficult and reduce the quality of the final product. The presence of suspended solids, organic materials, and unwanted microorganisms can increase the viscosity of the medium and obstruct harvesting equipment, compromising the efficiency of the process (Mishra et al., 2023). Overcoming these challenges entails the development and implementation of innovative technologies and engineering solutions that improve the efficiency, selectivity, and sustainability of large-scale microalgae harvesting methods. This includes the use of integrated approaches, such as combinations of physical, chemical, and biological methods, as well as the development of row cropping systems that can be easily integrated into existing cropping systems (Pei et al., 2022). In summary, microalgae harvesting efficiency is essential for successful large-scale production, but they face significant challenges related to the scalability, cost, selectivity, and sustainability of harvesting methods. Overcoming these challenges will require collaboration between scientists, engineers, and entrepreneurs to develop and elucidate innovative solutions that promote sustainable large-scale microalgae production.

5 Energy Cost

Energy cost plays a crucial role in the microalgae cultivation process, and its effective management is essential to ensure the economic and environmental viability of large-scale production. The cultivation of microalgae requires energy for several steps of the process, including artificial lighting in photobioreactor systems, aeration and agitation of culture media, and the operation of harvesting and processing equipment (Khor et al., 2022). Energy cost plays a crucial role in the microalgae cultivation process, and its effective management is essential to ensure the economic and environmental viability of large-scale production. The cultivation of microalgae requires energy for several steps of the process, including artificial lighting in photobioreactor systems, aeration and agitation of culture media, and the operation of harvesting and processing equipment (Khor et al., 2022). Another challenge is efficient energy management during the cultivation process. Aeration and agitation are necessary to maintain the homogeneity of the culture medium and provide oxygen to microalgae during growth. However, these processes are energy-consuming, and optimizing energy consumption without compromising the productivity and quality of cultivated microalgae is an important consideration for large-scale producers (Singh & Mishra, 2023).

Additionally, energy costs are also associated with operating harvesting and processing equipment. Harvesting methods such as centrifugation, filtration, and drying often require the use of electrical or thermal energy, contributing to total production costs. Selecting harvesting and processing methods that minimize energy consumption while ensuring the efficiency and quality of the final product is critical to optimizing production costs (Zhao et al., 2023). This may include developing and

implementing more energy-efficient farming systems, utilizing renewable energy sources such as solar or wind power, and adopting energy management practices that minimize waste and maximize efficiency (Souza et al., 2023). The graph below shows the energy cost of producing microalgae in a biorefinery.



6 Technology Scalability

Technology scalability is a crucial aspect in transitioning microalgae cultivation from the laboratory to large-scale production. Although microalgae cultivation techniques can be effective on a small scale, replicating these methods on a commercial scale presents several challenges that need to be overcome to ensure the success and economic viability of large-scale production systems (Hosseini et al., 2024). Many of the cultivation techniques developed in the laboratory are optimized for small volumes and may not be directly applicable to commercial systems. For example, small-scale photobioreactor systems and cultivation tanks may not be viable on a large scale due to cost, efficiency, and logistics issues (Castañeda et al., 2023). Maintaining ideal growing conditions, such as temperature, pH, lighting, and aeration, in large culture volumes can be challenging and requires advanced automation and control systems. Lack of ability to consistently control these parameters can result in variations in the growth and quality of cultivated microalgae, affecting the productivity and profitability of the system (Min et al., 2022).

It is important to say that not all microalgae strains are susceptible to scaling processes; therefore, the selection and improvement of microalgae strains that are suitable for large-scale production are also important when wanting to implement this process. Furthermore, the development of genetically modified strains to maximize biomass yield and optimize desired traits can also be complicated by the need to meet safety and regulatory requirements (Alavianghavanini et al., 2024). Science

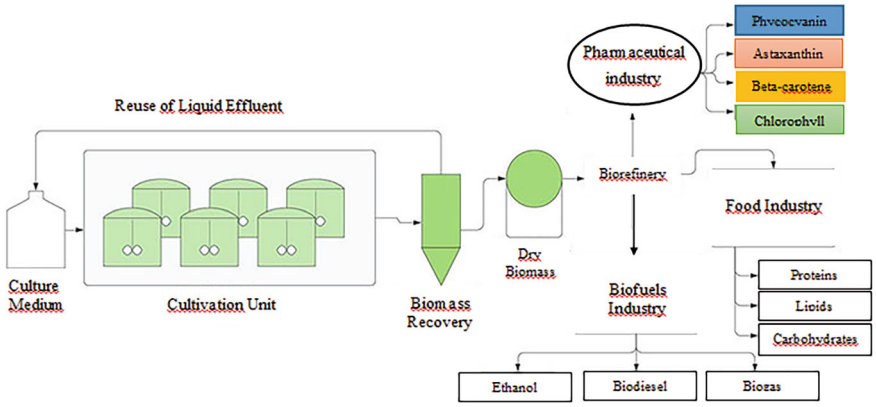


Fig. 1 Schematic of a biorefinery to produce microalgae biomass on a large scale. (Source: Guimarães, 2020)

will always play an important role in all development processes, allowing the development and programming of new technologies and cultivation practices that are adaptable and scalable for large-scale production of microalgae. Furthermore, it is important to create partnerships and collaborations between academic institutions, industry, and governments to share knowledge, resources, and experience and advance the large-scale microalgae industry (Pandey et al., 2024). Given this, technology scalability represents a significant challenge in large-scale microalgae production, but it also offers opportunities for innovation and growth. Overcoming these challenges will require investment in research and development, collaboration between different stakeholders, and a commitment to the sustainability and efficiency of the large-scale microalgae cultivation process. The Fig. 1 above shows the operation of a biorefinery to produce microalgae where the scalability process was applied.

7 Saving Water and Nutrients

One of the main challenges associated with saving water in large-scale microalgae cultivation is minimizing cultivation water consumption. Cultivating microalgae requires large volumes of water to provide a conducive environment for cell growth. However, water scarcity in many regions of the world makes it imperative to develop farming practices that minimize water use and promote the recycling and reuse of farming water (Tang et al., 2023). The efficient use of water and nutrients is essential to ensure the environmental and economic sustainability of microalgae production systems, as well as to minimize negative impacts on the environment (Farooq et al., 2015). According to Yang et al. (2015), nutrients such as nitrogen, phosphorus, and micronutrients are essential for the healthy growth of microalgae, but their excessive

use can lead to eutrophication and water pollution. Therefore, it is important to develop nutrient management strategies that minimize waste and maximize the efficient use of available resources. Chaudry et al. (2024) will say that another challenge is the selection and optimization of culture media that provide the nutrients necessary for the growth of microalgae, without compromising the quality of the final product. Many conventional growing media contain synthetic nutrients that can be expensive and environmentally harmful. Therefore, it is important to explore more sustainable alternatives, such as using organic waste or treated wastewater as nutrient sources for microalgae cultivation. The concentration of nutrients and contaminants in cultured water can increase over time, requiring more complex treatment systems to ensure water quality and prevent the accumulation of pollutants (Jwa et al., 2023). In this sense, the recycling and reuse of nutrients and water from cultivation has been facing technical and logistical challenges in large-scale production systems. The use of agro-industrial by-products rich in nutrients can also be an interesting alternative as a source of essential nutrients for the metabolism of microalgae, this can greatly impact the cost of production and bring viable and profitable alternatives to the market. This includes research into new nutrient sources, developing more water- and nutrient-efficient farming methods, and implementing farming water recycling and reuse systems (Pugazhendhi et al., 2020).

8 Regulations and Permissions

One of the main challenges in large-scale microalgae production is the need to comply with environmental and food safety regulations. The cultivation of microalgae can have environmental impacts, such as the use of water and nutrients, the release of effluents, and the potential introduction of exotic species. Therefore, microalgae producers must ensure compliance with local, state, and federal regulations related to environmental protection and conservation of natural resources (Gao et al., 2024). Although microalgae offer promising opportunities as a sustainable source of biomass, their large-scale production faces a number of challenges related to regulatory compliance and obtaining authorizations (Syed et al., 2024), in Europe, it is on the regulations for new foods (Reg. EU 2015/2283) and in Brazil Ordinance No. 2914, of December 12, 2011. This includes compliance with hygiene standards, quality control, and traceability throughout the production chain. Microalgae producers must ensure that their cultivation, harvesting, processing, and storage processes comply with relevant regulations to ensure the safety and quality of final products (Ayub et al., 2024). Another important aspect is obtaining the necessary permits and licenses to operate large-scale microalgae cultivation facilities. This may involve obtaining environmental permits, water use permits, effluent discharge permits, zoning permits, and construction approvals. The process of obtaining permits can be complex and time-consuming, and microalgae producers must be prepared to face challenges such as environmental impact assessment, public consultation, and local community opposition (Adamu Ugya et al., 2023). Furthermore,

evolving regulations can pose additional challenges for large-scale microalgae producers. Changes in government policies, industry standards, and labeling requirements can affect the operation and costs of microalgae cultivation facilities. As a result, manufacturers must stay abreast of the latest regulations and adapt their processes as necessary to ensure ongoing compliance (Gurreri et al., 2023) and overcome these challenges and prompt a proactive and collaborative approach between producers, regulators, scientists, and stakeholders. It is essential to establish dialogue and cooperation to develop policies and regulations that promote the sustainable growth of the microalgae industry, while ensuring environmental protection, food safety, and public well-being. In summary, regulations and permits represent significant challenges in large-scale microalgae production, but they are also critical to ensure the sustainability and safety of production systems. Overcoming these challenges will require collaboration, transparency, and a commitment to complying with applicable regulations and standards.

9 Final Considerations

In conclusion, large-scale production of microalgae presents significant challenges, from cultivation control to necessary regulations and permits. However, with continued research and development in diverse areas such as biology, engineering, and economics, these challenges can be overcome. As new solutions are developed, large-scale production of microalgae has the potential to become a crucial source of renewable biomass, with applications across multiple industries including food, feed, biofuels, and chemicals. This sustainable approach can significantly contribute to mitigating the environmental and energy challenges faced by society today, promoting a more sustainable and resilient future.

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Cultivation Conditions and Operating Modes in Large-Scale Microalgae Processes



Chukwuemeka Samson Ahamefule, Chidimma Osilo, John N. Idenyi, Innocent O. Ogbonna, and James C. Ogbonna

Abstract The quest for large-scale production of microalgae biomass as sustainable complements or replacement of some critical raw materials and/or processes in food, energy, agriculture, pharmaceutical, medical and nutraceutical industries has been quite enormous in recent times. In order to produce microalgae biomass and metabolites at competitive prices, several potentially efficient photobioreactors have been developed. However, their potentials can only be realized by selection of appropriate operation modes and culture systems, and optimization of the culture conditions. Operation modes such as batch, fed-batch, semi-continuous, and continuous processes have been investigated for large-scale cultivation of some important freshwater and marine microalgae including several species of *Chlorella* and *Desmodesmus, Spirulina platensis, Isochrysis galbana, Dunaliella salina,* and *Phaeodactylum tricornutum*. The choice of the operation mode depends on the species, the photobioreactor configuration, especially their light supply efficiencies, as well as culture conditions. Although photoautotrophic cultures are extensively used due to their simplicity and reduced risk of contamination, their productivities and standing biomass concentrations are usually very low. However, many species of microalgae can metabolize many organic carbon sources in the presence of light (mixotrophic metabolism) or in dark (heterotrophic metabolism). Thus, mixotrophic

C. S. Ahamefule
Department of Microbiology, University of Nigeria, Nsukka, Nigeria

C. Osilo
Department of Applied Microbiology, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria

J. N. Idenyi
West Virginia State University, Institute, WV, USA

I. O. Ogbonna
Federal University of Agriculture, Makurdi, Nigeria

J. C. Ogbonna (✉)
Department of Microbiology, University of Nigeria, Nsukka, Nigeria

State University of Medical and Applied Sciences, Nsukka, Enugu State, Nigeria
e-mail: james.ogbonna@unn.edu.ng

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and heterotrophic culture systems have been developed for high-density cultures of many species of microalgae. With these culture systems, very simple bioreactors can be used to achieve high cell densities with high productivities. However, the risk of contamination by fast-growing heterotrophic microorganisms is very high while accumulation of light-induced metabolites is very low. These limitations can be overcome by a combination of culture systems. For examples, sequential heterotrophic-photoautotrophic system, sequential heterotrophic-mixotrophic system, and sequential mixotrophic-photoautotrophic system can be used to obtain high cell densities with high concentrations of light-induced metabolites. Furthermore, cyclic photoautotrophic-heterotrophic system and cyclic mixotrophic-heterotrophic system can be used to achieve continuous cell growth under day-night (light-dark) cycles. In all these production modes and culture systems, there is a need to optimize culture conditions such as light intensity and distribution, pH, media components and concentrations, salinity, temperature, mixing and aeration gas composition. The optimal conditions of these variables depend on the species and the target metabolites. In this chapter, the various production modes and culture systems are discussed with an emphasis on their comparative advantages and disadvantages. The effects of various physical (light, temperature, mixing), chemical (nutrient types and concentrations, pH, salinity, aeration gas) and biological (contamination, morphology and size of the cells, as well as light absorption capacity of the cells) conditions on cell growth and productivity in large-scale microalgae cultures are also discussed.

Keywords Microalgae · Photobioreactor · Culture systems · Large scale · Biomass productivity

1 Introduction

The applications of microalgae keep expanding across various fields, including food/feed, energy, agriculture, medical, and pharmaceutical industries. Successful use of microalgal biomass and/or their enormous metabolites in these industries requires cost-effective large-scale cultivation. Adequately selecting the appropriate bioreactor operation modes—batch, fed-batch, semi-continuous, or continuous modes—and culture systems to suit specific microalgal strains and metabolite productions is sine qua non in achieving optima microalgal productivities. Furthermore, the exploitation of organic waste substrates in large-scale microalgal cultivation under heterotrophic or mixotrophic conditions has high potentials for practical applications. These waste substrates could also be valorized under sequential or cyclic conditions such as the sequential heterotrophic-photoautotrophic or sequential heterotrophic-mixotrophic culture conditions as well as in cyclic heterotrophic-photoautotrophic or cyclic heterotrophic-mixotrophic culture conditions. All these operation modes and culture systems have their specific advantages and disadvantages.

The optimization of physical, chemical, and biological conditions is crucial in any chosen bioreactor operation mode and system to achieve maximal biomass and/or metabolite productivities. Different microalgal species need varying light quality, intensity, and photoperiods, as well as temperature and mixing regimen requirements for optimal growth and production of essential bioactive compounds. Similarly, the type and concentrations of various nutrients, pH, salinity, and aeration gas concentration should be optimized as much as possible for increased productivity. Furthermore, contaminations should be avoided as competing organisms adversely affect microalgal productivity in most cases. Other biological conditions such as cell morphology, and sizes of antenna and microalgal cells should be given critical considerations as they affect photosynthetic efficiency and response to hydrodynamic stress. All these conditions have been thoroughly reviewed in this chapter toward achieving optima microalgal productivities. Specific examples of several microalgae such as different strains of *Chlorella, Desmodesmus*, as well as *Dunaliella salina, Haematococcus pluvialis,* and *Nannochloropsis oculata* have also been used to illustrate these systems.

2 Bioreactor Operation Modes for Large-Scale Microalgae Cultivation

Microalgae can be cultivated using several bioreactor operation modes. These include batch, fed-batch, semi-continuous, and continuous cultures.

2.1 Batch Culture

Microalgae cultivation using batch culture method has become a widely used technique which involves inoculating nutrient-rich liquid medium with a relatively low concentration of microalgal cells at the beginning of a batch culture (Henley, 2019). In this method, microalgae can grow in a closed system under changing environmental conditions (Sen et al., 2020). Microalgae typically follow a sequential pattern of growth in batch cultures defined by distinct phases: the initial phase consists of slow growth as the cells acclimate to the new culture medium, followed by log or exponential growth phase marked by accelerated cell replication, and then a stationary phase caused by the formation of toxic by-products or nutrient limitation. In the final phase, the viability of cells steadily declines as a result of increasingly toxic conditions in the culture medium (Finkel, 2006; Lund et al., 2000).

A growth curve in batch culture can be used as an ideal model for studying basic microorganisms, microalgal physiology inclusive, and the effects of various genetic changes (Veselovsky et al., 2022). It offers a detailed explanation of how growth rates are affected by environmental and genetic factors. It is important to note that a

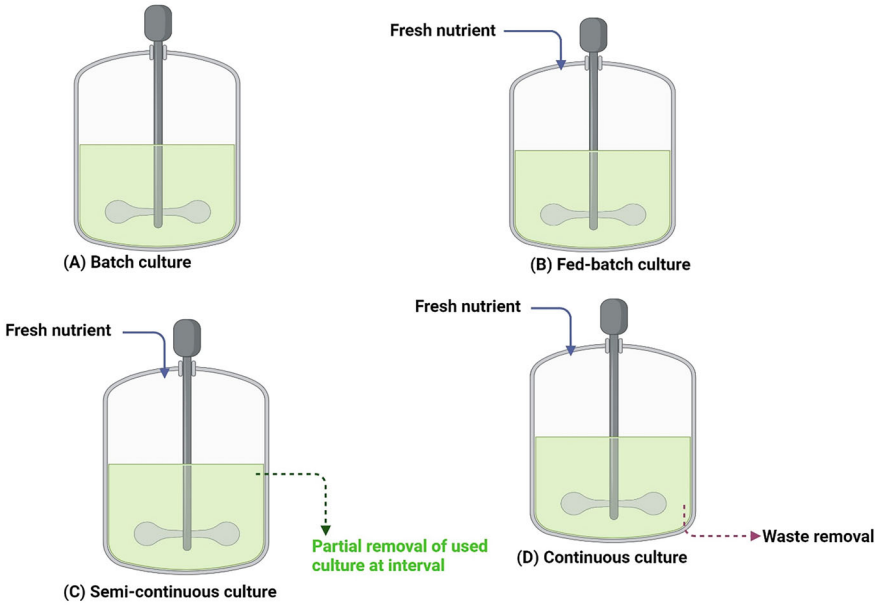


Fig. 1 Bioreactor operation modes for microalgae cultivation (**a**). Batch culture: batch cultivation does not involve the addition of nutrients or medium extraction; (**b**) fed-batch culture: in fed-batch systems, nutrients are continuously or intermittently fed to the system, but there is no removal of the product so that the broth volume continues to increase; (**c**) semi-continuous: in this system, nutrients are continuously fed to the system while culture broth is occasionally removed; (**d**) continuous culture: nutrients are continuously fed to the system and culture broth is continuously removed at the same rate so that a steady state is achieved

batch culture is a closed system (Fig. 1a), which means that no additional nutrients are added once the fermentation has begun, and no waste products are disposed of until the process has been completed. Because batch cultures are relatively easy to establish and operate, they require less equipment and technical abilities than continuous cultures, which are more complex.

Even though batch cultures are simple, they have limitations, primarily in terms of scalability and stability over prolonged periods of time. The culture conditions often change when microalgae take up nutrients, resulting in conditions less than optimal for growth and product production. This may have an impact on experimental consistency and accuracy, particularly in research that is geared toward large-scale production. Due to its ease of operation and ability to provide insights into microbial growth and metabolism, batch culture remains a fundamental technique in biotechnology.

2.1.1 Conditions Under Which Batch Culture Is Recommended

Generally, batch culture should be used for experiments in which maintaining consistency and control over experimental conditions is crucial, but only for a limited period of time. It is widely used in laboratories for maintaining collections of cultures of microalgae that serve as the foundation or reference cultures for a variety of laboratory-based studies. Its simple design allows for easy replication of conditions and results, which is essential for comparative studies, such as evaluating genetic variation effects on algal growth and metabolism (Sen et al., 2005).

In addition to providing a controlled and predictable environment for microalgae growth, batch cultures are also ideal for educational purposes. With this technique, variables are minimized, making it easier for beginners to understand algal growth and their physiological processes. Compared to continuous cultures, batch cultures employ less labor and are more economically feasible for short-term studies since nutrients and by-products are not continuously added or removed (Rafa et al., 2021).

In laboratory studies, batch cultures are used to test the efficacy of novel nutrient formulations or environmental light conditions on algal growth and performance. By providing a controlled environment, scientists can determine the impact of a single variable on algal physiology without the influence of external factors. This is particularly useful in preliminary studies intended to screen for optimal conditions before scaling up to more complex systems like continuous or semi-continuous fermentation.

However, batch culture is not appropriate for steady production over a prolonged period of time or in commercial applications where high production levels are desirable. For these reasons, the depletion of nutrients and buildup of waste that occur in batch cultures can adversely affect productivity and profitability of the operation. This requires cultivation methods that ensure sustained growth and higher biomass production over extended periods while replenishing nutrients and removing wastes continuously.

2.1.2 Description of Batch Culture

Batch culture involves preparing a medium enriched with all essential nutrients and inoculating it with microalgal cells at a specific concentration. This closed system can be properly insulated to avoid contamination and to maintain a sterile environment, which is essential to obtaining accurate results. At first, microalgae are in a lag phase, adjusting to a new culture medium, and their growth is slow as they initiate the physiological mechanisms necessary to grow.

Following the lag phase is the log growth phase, which is characterized by a rapid increase in cell division and population size. This phase is characterized by the high availability of nutrients and suitable growth conditions, such as appropriate light, temperature, and pH. The biomass of the culture increases significantly during this

phase, often doubling over a short period of time, depending on the species and the culture conditions.

After a certain period of time, as the nutrients in the medium become less and less abundant, the culture starts to experience limiting conditions, which is a sign that the culture will enter into its stationary phase. A plateau in biomass accumulation occurs in this phase, where the cell growth rate equals the death rate. The decrease in growth can be attributed to both nutrient depletion, mostly of essential elements, such as nitrogen and phosphorus, and the buildup of waste products, such as free radicals produced by oxygen and organic compounds.

Finally, when the culture conditions become increasingly toxic due to increased nutrient depletion and accumulation of metabolic waste, the microalgae enter a phase of death. At this point, cell death exceeds cell growth. Culture overall condition deteriorates, and total biomass may decrease as dead and decaying cells increase.

It is essential to monitor the growth phases throughout the batch culture process to evaluate and track the overall culture condition. The pH levels, oxygen levels, concentration of nutrients, and density of the cells are all measured at regular intervals. Data such as these provide valuable insight into the progress of a culture, and they are essential in order to determine the optimal time to harvest the biomass in order to extract bio-products or to use the biomass as a starting material for subsequent cultures, depending on the applications for which the biomass will be utilized.

2.1.3 Advantages and Disadvantages of Batch Culture

Advantages

1. **Simple to operate:** Batch cultures are relatively simple to operate, making them accessible for educational purposes and professional research. The system usually requires only standard laboratory equipment and minimal ongoing adjustments once the culture is initiated.
2. **Ease of controlling experimental conditions:** It is possible to design an experiment with a high degree of control over the experimental environment, including the initial concentration of nutrients, the period of exposure to various conditions, and many other variables. In experiments aimed at investigating the effects of specific conditions on microalgal growth or physiological functions, this control is crucial.
3. **Economic efficiency:** The fact that batch cultures are less expensive and can be carried out without continuous nutrient addition or complex monitoring equipment makes them ideal for low-budget projects or pilot experiments.
4. **Analyses are easy to conduct:** As a result of the varied growth phases in batch culture, it is possible to sample and analyze biomass at various phases of microalgae cultivation, which allows researchers to examine metabolic changes over time.

5. **Standardization:** A batch culture provides a standardized approach to scientific investigations, which can be readily reproduced or scaled up based on initial findings, making it possible for more complex studies to be developed in the future.

Disadvantages

1. **Short growth time:** As there are limited nutrients available in the medium, microalgae can grow only for a limited period of time until these resources are exhausted. Consequently, the experiment period is shortened and the amount of biomass produced may be limited.
2. **Low output:** Comparatively, batch cultures generally generate lower biomass because nutrients are eventually depleted, which affect growth in contrast to continuous cultures, where nutrients are continuously added and by-products are continuously collected.
3. **Changes in culture conditions:** A significant change in the culture conditions occurs as a result of the formation of metabolic wastes and the depletion of nutrients during the course of the cultivation. As a result of these changes, the physiological processes of the microalgae can be altered, which makes it challenging to establish whether observed changes are a result of experimental factors or of changes in the culture environment.
4. **Scalability issues:** It is without question that batch cultures are ideal for small-scale studies, but scaling them up to commercial quantities is challenging due to the challenges associated with sustaining the optimal conditions across large volumes of culture.
5. **End-phase risk:** There can be cellular breakdown and leakage of intracellular products during the death phase, which can result in difficulty in recovering and purifying target chemical products from microalgal biomass.

2.1.4 Specific Examples of Microalgae and Results Using Batch Culture

Typically, batch culture is the most popular method of growing microalgae. Each species of microalgae exhibits unique growth characteristics and responses. A variety of microalgae can be grown in batch cultures under different conditions, and the following examples demonstrate how they perform:

1. ***Picochlorum oklahomense***: When grown in batch culture, this species exhibits what is known as a standard linear growth pattern, with an initial growth rate of nearly 0.89 per day, which indicates a rapid initial growth. After few days, the microalgae growth rate significantly decreases, demonstrating the effects of nutrient depletion on the growth performance characteristics of the microalgae (Henley, 2019).
2. ***Dunaliella salina***: In batch cultures, *Dunaliella* is known for the production of beta-carotene and exhibits rapid growth at the beginning of its growth. A rapid decline in growth rate is observed between the stages of nutrient depletion to the

point where secondary bioactive compounds (beta-carotene) are produced as the culture approaches nutrient depletion (Rafa et al., 2021).

3. ***Chlorella vulgaris***: This *Chlorella* species is commonly used in pharmaceutical and biotech applications as it grows quickly in the log phase. However, due to nutrient restrictions, the culture finally attains a stationary phase, which inhibits biomass accumulation and the quality of useful molecules like proteins and lipids that can be extracted (Sen et al., 2005).
4. ***Spirulina* (*Arthrospira*) *platensis***: It undergoes a rapid growth phase that is short in batch cultures, making it often cultured for its nutritional value. High protein content makes it susceptible to nitrogen exhaustion, which substantially slows biomass accumulation (Delrue et al., 2017).
5. ***Haematococcus pluvialis***: This alga is known for its high astaxanthin content. It shows a distinct adaptation phase when cultured in batch as it adapts to environmental stress, followed by a logarithmic increase in growth, and a rapid decline when nutrients run out, resulting in astaxanthin formation (Fitriana et al., 2021).
6. ***Nannochloropsis oculata***: There is a lot of interest in *Nannochloropsis* for its oil content, and its growth pattern is characterized by a classic batch culture pattern with a distinct exponential phase. Under nutrient deprivation, especially nitrogen limitation, lipid synthesis is frequently promoted during the decline phase (Ye et al., 2024).
7. ***Phaeodactylum tricornutum***: A diatom from this species has been used in studies regarding the bioprocessing of silica. In batch cultures, it exhibits a rapid response to the silica limitation that is critical for the synthesis of a cell wall and has a direct influence on its growth potential and structural stability (Ding et al., 2023).
8. ***Isochrysis galbana***: This marine alga plays a vital role in aquaculture nutrition, and it shows rapid growth in batch cultures. But when there is no consistent nutrient supply, fatty acid production and other metabolites can be affected, reducing its ability to play a crucial role in marine food chains (Matos et al., 2019; Mishra et al., 2019; Casini et al., 2021).
9. ***Tetraselmis suecica***: *Tetraselmis* is a commonly used shellfish aquaculture species that displays a fast growth rate when grown in batch culture. However, like other species, it declines quickly when the nutrients in the culture are utilized, which impacts the nutritional profile if cultivation is prolonged (Paterson et al., 2023).
10. ***Porphyridium cruentum***: In batch cultures of this red alga, it normally starts out slowly as it becomes accustomed to the growing conditions. However, after a few days the growth rate rapidly increases before slowing down once nutrients, especially phosphorus, run out in the cultures (Asgharpour et al., 2015).

This observation illustrates the variety of growth dynamics and physiological processes as they apply to different species of algae when they are cultured in batch mode, illustrating the differences that exist between them. Batch culture operations require precise timing and control according to each species' unique characteristics.

2.2 *Fed-Batch Culture*

A fed-batch culture technique is a complex culture technique that improves production and efficiency by transforming conventional batch culture mode. Fresh nutrients are systematically added to the bioreactor during the culture in controlled amounts, and as the culture volume increases, the amount of nutrients are gradually increased (Fig. 1b). Fed-batch culture is semi-open, which implies that, despite the system being mostly closed, new substrates or nutrients may be introduced periodically or continuously into the reactor without removing the existing growth medium or biomass (Fields et al., 2018).

In the course of cultivation, nutrients are continually added to ensure that optimal growth conditions are maintained. Simple batch cultures characterized by peaks and troughs in nutrient concentrations can be avoided by controlling the nutrient flow (Ji et al., 2015). Cells can grow at a higher density in these controlled environments because nutrients are constantly available throughout the growth phase, preventing nutrient depletion from stressing them.

Fed-batch culture also extends the active phase of cell metabolism, increasing the production of the desired product. The extended growth phase especially benefits products produced during the latter stages of cell growth, since these products yield more. By controlling the timing and amount of nutrients added, metabolic pathways within cells can be targeted and adjusted to maximize the production of a product or alter its characteristics (Henley, 2019; Kumar et al., 2014). Because of its efficiency and stability, this method is more productive than traditional batch processes. As a result, not only are yields increased, but the quality of the final product is also improved.

2.2.1 Conditions Under Which Fed-Batch Culture Is Recommended

The fed-batch culture is particularly beneficial to both productivity and process stability in a number of industrial and research scenarios. By gradually adding nutrients, this method prevents toxic buildup that can impede cell growth and product formation at high substrate concentrations. As a result of avoiding substrate inhibition, fed-batch culture maintains a more favorable environment for microbial or cellular growth.

Moreover, fed-batch techniques are beneficial to cultures that are susceptible to nutrient shock. By gradually introducing nutrients, cells will be able to adapt to the new environment without the stress caused by sudden high concentrations of nutrients, which normally occurs in batch cultures. In this way, growth rates are more stable and cultures are able to be more productive and healthy.

When the production phase needs to be extended beyond the capacity of standard batch processes, fed-batch culture is also a suitable option. When a culture is continually provided with essential nutrients, the active metabolic phase can be

extended, stimulating the yield of biomass or other desired products, such as enzymes, and pharmaceuticals.

Fed-batch cultures provide a viable alternative when continuous cultures are not feasible as a result of economic or operational constraints. Continuous systems require a greater level of control over environmental conditions than discontinuous systems, which require costly and complex infrastructure to maintain constant conditions.

2.2.2 Description of the Process of Fed-Batch Culture

A fed-batch culture process is a commonly used method for the cultivation of microalgae that optimizes biomass productivity and controls nutrient supply. An initial step in cultivation involves the preparation of a bioreactor. It is essential that all equipment be sterilized to prevent contamination by other microorganisms. The initial concentration of sterile culture medium is introduced into the bioreactor. Microalgae can grow in the medium after inoculation using the new nutrients. A healthy, robust algal population depends on this phase. In contrast to batch culture, in fed-batch culture, nutrients are continuously or periodically added as the growth of the culture progresses. This method helps to avoid nutrient exhaustion and the formation of waste products that could prevent growth (Brignoli et al., 2020). The feed rate can be constant, but if it is desired to maintain a constant nutrient concentration, the feed rate is made a function of the growth rate.

It is possible that the feeding strategy will change according to the growth conditions of the microalgal species and specific metabolites of interest. To maintain optimal growth conditions, pH, temperature, dissolved oxygen, and light intensity are closely monitored and regulated throughout cultivation (Figueroa-Torres et al., 2022). Several nutrient concentrations are monitored regularly, particularly nitrogen and phosphorus, in order to determine when and how to feed. The microalgae are harvested once the density of the culture reaches its maximum or after a specified period of cultivation.

2.2.3 Advantages and Disadvantages of Fed-Batch Culture

Advantages

1. **Maintaining nutrient concentration:** As nutrients are added intermittently or continuously, the levels of nutrient depletion and toxic accumulation are minimized. Over time, this helps sustain cell growth and productivity.
2. **Increased product yields:** The fed-batch culture method can achieve a higher cell density as well as a higher product output by regulating the environmental conditions. Those involved in the production of pharmaceuticals, chemicals, and biologics will greatly benefit from this process.

3. **Decreased by-product formation:** In fed-batch processes, the substrate concentration is minimized, thereby reducing the formation of unwanted by-products that are more common in other culture methods where nutrient concentrations vary greatly.
4. **Adaptability**: Depending on the organism's growth kinetics and metabolism, the feeding strategy can be adjusted, optimizing the production process.
5. **Large-scale production**: Due to their scalability and adaptability, fed-batch techniques are ideal for commercial productions.

Disadvantages

1. **Complexity**: Fed-batch culture management is more complex than managing a batch culture. Nutrient feeds and other environmental conditions will also require careful monitoring and control in order to achieve the best results.
2. **Possibility of contamination:** There is an increased risk of contamination since the culture system is subject to addition of nutrients. The process requires special aseptic techniques and can have an impact on the production costs.
3. **Costs of operations:** Nutrient addition can increase the operational costs compared to batch cultures that require less monitoring equipment, sensors, and control systems to prevent excess nutrients.
4. **Optimization of processes:** It is imperative that fed-batch processes are carefully optimized for each organism and product, which can be time-consuming and require an in-depth understanding of the metabolism of each organism.
5. **Inhibited by-product build up:** If not adequately managed, some metabolic products may build up to toxic levels in spite of efforts to control them.

2.2.4 Specific Examples of Microalgae and Results Using Fed-Batch Culture

1. ***Chlorella vulgaris***: There has been extensive research on the potential of *C. vulgaris* for the production of biodiesel, particularly under the controlled conditions of fed-batch cultivation. A study was reported to develop a two-stage fed-batch process in which initial growth was supported by glucose, followed by additional crude glycerol to increase lipid accumulation. This approach yielded lipid concentration (1663.02 mg L^{-1}) and a lipid content (36.39%) indicating that lipids from *C. vulgaris* are effective for making biodiesel (Sun et al., 2014).
2. ***Haematococcus pluvialis***: The cultivation of *H. pluvialis*, particularly for enhancing astaxanthin production, often involves two steps. It is first grown under favorable conditions in bioreactors or photobioreactors (PBRs) to increase biomass, and then stressed in raceway ponds in which nitrogen is limited and salinity or iron concentrations are manipulated to increase astaxanthin production. High yields of astaxanthin are often obtained through this method of balancing growth and astaxanthin production (An et al., 2024).

3. ***Nannochloropsis oculata***: *N. oculata* is an important source of high-value products such as eicosapentaenoic acid (EPA) when cultivated using fed-batch and semi-continuous regimens. Fed-batch tubular reactors (FBTRs) achieved high cell densities, while semi-continuous reactors (SCRs) improved specific growth rates and lipid production when used with algal medium (AM). This implies that combining these approaches would increase the production of omega-3 fatty acids and other metabolites in *N. oculata* (Martínez-Macías et al., 2018).

2.3 Semi-continuous Culture

Semi-continuous microalgal culture refers to a mode of cultivation that provides optimal growth conditions for algae, extending their productive phase without the need to establish new cultures frequently. This method involves regular harvesting of the culture, followed by the addition of an equivalent volume of fresh medium (Fig. 1c). With this method, batch and continuous cultures are balanced in a way that maximizes the benefits of both (Chisti, 2007; Posten & Schaub, 2009).

Semi-continuous culture involves systematically removing a certain percentage of the culture broth followed by replenishing it with fresh nutrient medium on a regular basis—either daily or at other predetermined intervals. During these harvesting and replacement cycles, algae are controlled to grow at a steady pace, which in turn enhances overall productivity and capacity for biomass production. Moreover, it prevents the culture from entering the stationary phase, which is a common occurrence in batch cultures where nutrient exhaustion and waste build up can hinder the growth of the organism (Han et al., 2013).

Specifically, this type of cultivation is beneficial for commercial microalgae production, where consistency is key to biomass yield and metabolite production. As a result, steady-state algal growth can be achieved, and the volume and frequency of harvesting can be adjusted in order to achieve the ideal levels of growth. To maximize the production of specific compounds, such as lipids, proteins, or pigments, this precise adjustment is vital (Davis et al., 2015).

The use of semi-continuous cultures also reduces the risks connected with restarting cultures from scratch, such as contamination and the fluctuation of growth factors. Additionally, it has the advantage of offering a more judicious use of resources, since continuous utilization of tools and facilities has the potential to lead to a reduction in operating costs compared to batch operations (Tachihana et al., 2020).

Semi-continuous systems can maintain optimal growth conditions by closely monitoring and adjusting conditions such as lighting, temperature, and pH. A high-quality algal output is often required in the pharmaceutical and food industries, and this control is integral to achieving this.

2.3.1 Conditions Under Which Semi-continuous Culture Is Recommended

For microalgae cultivation under certain conditions that seek to maximize productivity as well as maintain sustainability, semi-continuous culture is recommended. This technique is useful in the following circumstances:

1. **High biomass production:** A semi-continuous culture is highly capable of maintaining high biomass concentrations over a long period of time, which makes it an efficient method of producing large quantities of biomass.
2. **Production stability:** In this culture mode, stable environmental conditions are maintained which are conducive to a higher yield and superior quality of microalgae products.
3. **Efficiency**: The culture is periodically collected and replenished with fresh nutrients, which minimizes the demand of resources such as water and nutrients compared to batch cultures.
4. **Contaminant control:** By preventing metabolic waste accumulation, it lowers the possibility of contamination. This is important for pharmaceutical and food-grade cultures.

2.3.2 Description of the Process of Semi-continuous Culture

Design and Inoculation Microalgae are cultivated by establishing a cultivation tank or photobioreactor in which they multiply and yield products. This system is initially inoculated with a small volume of microalgae culture, which serves as the starting population (Narala et al., 2016).

Growth Pattern Microalgae cells grow and multiply under controlled environmental conditions (such as light, temperature, CO_2 supply, and nutrient availability). In order to avoid contamination and maintain optimal growth conditions, the system remains closed during this phase.

Incubation The culture is continuously monitored for various parameters throughout the growth phase, such as cell density, pH, oxygen levels, and nutrient concentrations. This monitoring helps in evaluating the health and growth rate of microalgal cultures.

Harvesting A certain percentage of the culture is harvested whenever the cell density reaches a particular level (around the stationary phase of growth). For example, around 25% or 50% of the culture volume is removed in this process (Reichert et al., 2006). The microalgal biomass in the collected volume is then separated and concentrated.

Nutrient Replenishment The remaining culture in the bioreactor is then replenished with new nutrients after harvesting. In this medium, essential nutrients

are provided and the culture is diluted to prevent the loss of resources and accumulation of metabolic waste products, which can hinder the growth of the culture.

Renewal of Growth In the wake of replenishment, the microalgal culture restarts its growth until the culture density attains a level that suggests yet another harvest is appropriate. By repeating this cycle of harvesting and replenishment, conditions can be created in which microalgae can be produced semi-continuously.

Optimization Bioreactor conditions, including nutrient ratios, illumination, and harvesting schedules, may ultimately be modified in response to experimental results in order to achieve maximum yield and production efficiency.

This approach is remarkably effective as it integrates the benefits of batch culture (high yield per cycle) with those of continuous culture (steady-state operation and ongoing production), making it appropriate for commercial algae cultivation.

2.3.3 Advantages and Disadvantages of Semi-continuous Culture

Advantages

1. **Increased productivity**: Maintaining optimal growth conditions and high cell density can be achieved by regularly removing a portion of the culture and replacing it with fresh medium.
2. **Ecological efficiency**: In comparison to batch cultures, it can reduce waste and be more efficient at utilizing nutrients.
3. **Creating a stable culture:** Semi-continuous culture condition ensures stable conditions for culture, thus minimizing the negative effects related to changes in environmental conditions.

Disadvantages

1. **Complexity of operations:** The operation may become more complex as nutrient levels and biomass need to be monitored and controlled more precisely.
2. **Higher risk of contamination:** Open culture systems that run continuously may be more likely to become contaminated with other microorganisms over the long term.
3. **Partial dependency:** Culture success is greatly affected by the timing and ratio of harvest to replenishment, which makes the optimization of harvest to replenishment procedures crucial.

2.3.4 Specific Examples of Microalgae and Results with Semi-continuous Culture

1. ***Chlorella vulgaris***: In semi-continuous cultures, regularly harvesting and replenishing can result in stable yields of biomass, as well as increased lipid

productivity, which is crucial to the production of biofuel as a source of energy. As compared to batch cultures, studies have shown that continuous growth rates and lipid accumulation are higher (Zhang et al., 2014).

2. ***Spirulina* (*Arthrospira*) *platensis***: A semi-continuous cultivation process produces *Spirulina* with a high protein content that is consistent throughout. An optimal growth environment is maintained in order to ensure a uniform distribution of nutrients and cells (Karemore et al., 2020).
3. ***Nannochloropsis salina***: *Nannochloropsis* cultures that are semi-continuous perform better in terms of EPA production and overall biomass production. A design like this provides optimal light penetration as well as nutrient recycling, which are essential for the synthesis of fatty acids (Safafar et al., 2016).
4. ***Haematococcus pluvialis***: Astaxanthin accumulation can be enhanced in semi-continuous cultures under stress conditions. The production of astaxanthin increases when harvesting and nutrient replenishment are regulated (Khazi et al., 2021).
5. ***Dunaliella salina***: In semi-continuous systems, beta-carotene accumulation can be sustained under stress conditions. Regular dilution with fresh medium can prevent many risks associated with elevated cell density, including nutrient loss and self-shading of microalgae culture (Hosseini Tafreshi & Shariati, 2009).

2.4 Continuous Culture

Continuous microalgal culture grows microalgae continuously in a controlled environment with fresh medium being supplied at the same rate as algae-containing culture fluid is removed (Fig. 1d). Over time, this method optimizes the biomass and metabolite production by maintaining algae in a steady state of growth (López-Hernández et al., 2020; Reyes et al., 2022).

It is important to maintain equilibrium between biomass production and nutrient supply in a continuous culture system. Keeping this balance ensures the microalgae are steadily growing exponentially as opposed to getting stuck in the stationary phase usually associated with batch cultures. A continuous feed of fresh nutrients and removal of culture at a pace that minimizes overpopulation and nutrient deficiency ensures continued high production level (Barbera et al., 2019).

2.4.1 Conditions Under Which Continuous Culture Is Recommended

Under specific conditions of constant environment and high productivity, continuous microalgae culture may be recommended. Continuous culture is particularly beneficial in the following applications:

1. **Biomass is in high demand:** It is ideal to use continuous culture when there is a high and steady demand for algal biomass, such as when biofuels,

pharmaceuticals, or nutritional supplements are being produced on a large scale. Biomass is consistently generated without the fluctuations associated with batch operations.
2. **Synthesis of specific biochemicals:** The continuous cultivation of algae can maintain them in optimal growth phases if specific metabolites such as pigments and fatty acids are targeted. Metabolite production during specific growth stages can benefit from this technique.
3. **Studies and commercial applications**: The continuous culture system provides an effective way to assess the effects of fixed variables independently of cultural influences, which is useful when studying biochemical responses or designing commercial strategies.
4. **Long-term cultivation at low cost:** A continuous culture can be a more cost-effective option when microalgae are being grown on a commercial scale over a long period of time. A process that uses this method can minimize the amount of waiting time that is associated with preparing and establishing new batches, and can use nutrients and carbon dioxide in a more effective way.
5. **Production stability:** With continuous culture systems, conditions such as light intensity, temperature, pH, and nutrient levels can be adjusted finely during cultivation. The stable control of these parameters can increase predictability and uniformity in growth rates as well as product quality.
6. **Efficiency of space and resources:** It is possible to utilize these systems in a more efficient manner and save space and resources. Since they maintain a high density and continuously remove excess cells, they utilize nutrients and light more efficiently than batch cultures, improving light penetration and reducing nutrient competition.

2.4.2 Description of the Process of Continuous Culture

By continuously supplying nutrients and removing parts of the culture, continuous culture of microalgae maintains a constant growth phase (Reyes et al., 2022). It aims to maintain a steady culture, where cell growth rate equals cell loss rate, resulting in an even yield (Curves et al., 2005).

In general, continuous culture begins with the installation of a cultivation system, such as a photobioreactor. Microalgae culture is used to inoculate the system, which serves as a seed for the system to grow on. In addition to maintaining controlled lighting, temperature, pH, and carbon dioxide levels, microalgae are also cultivated under controlled environmental conditions. Optimal growth and productivity depend on these parameters.

Continually or at regular intervals, nutrients are supplied to the culture in order to prevent depletion. For microalgae to grow properly, nutrients such as nitrogen, phosphorus, and other essential minerals and vitamins must be provided. It is important to maintain stable growing conditions throughout the cultivation process by monitoring and adjusting the environmental conditions as necessary. This includes regulating the duration of light exposure, temperature, and gas exchange.

It is necessary to continuously or periodically remove some of the culture to prevent the culture from reaching a stationary phase, where growth ceases due to the depletion of nutrients or accumulation of waste. A fresh medium is added to achieve a balance (Matteau et al., 2015).

The culture that is removed from the cell are processed and harvested in order to produce the desired products, such as biomass, lipids, or specific biomolecules. Microalgae growth rates and system designs determine the rate of removal.

In order for the culture to be maintained at an optimal density for growth and production, the dilution rate is adjusted regularly. This is useful in order to avoid overly dense cultures that can result in nutrient shortages within the culture as well as a limitation in light availability (Zhang et al., 2004).

In continuous culture systems that are advanced, sensors and control systems monitor and adjust parameters such as nutrient levels, pH, and temperature continuously to maximize productivity and optimize conditions (Peebo & Neubauer, 2018).

2.4.3 Advantages and Disadvantages of Continuous Culture

Advantages

1. **Steady-state conditions**: The continuous culture of microalgae allows the algae to remain in a constant state of growth, allowing consistent quality and quantity of biomass to be generated.
2. **Increased productivity:** In continuous culture systems, algae continue to grow exponentially, so productivity per unit volume is higher than in batch cultures because there is less downtime waiting for cultures to grow.
3. **Efficient resource use**: The use of continuous systems can cut waste and reduce input costs by maximizing nutrients and light.
4. **Controlled growth environment**: By precisely controlling factors such as light, temperature, pH, and nutrient concentrations, better growth conditions can be achieved, potentially resulting in greater yields.
5. **Reduction in contamination risks**: The separation between the system and the environment greatly reduces the possibility of contamination, which is crucial in the pharmaceutical production process.
6. **Ease of scale-up:** It is typically easier to scale up continuous cultures rather than batch cultures since they operate under stable environmental conditions that are maintained throughout the process of scaling up.

Disadvantages

1. **Expenses and complexity:** Continuous culture systems are generally more complex and costly to establish and maintain than batch culture systems. It is necessary to monitor and regulate continuous systems to maintain stable environmental conditions.

2. **Risk of washout:** There is a risk of washout if the culture does not grow at the same rate as it is removed, resulting in a diluted culture after the culture is removed.
3. **Steady-state maintenance**: It may be challenging to achieve and maintain a steady state, particularly when environmental conditions are variable, as precise control of growth conditions is required.
4. **System stability**: Even minor fluctuations in conditions can have a significant impact on the culture over the course of a long-term operation. Hence, for long-term operations to be stable, continuous monitoring is necessary.
5. **Risk of selection pressure:** It is likely that continuous microbe culture will result in selection pressures that over time will change the microalgal population. In some cases, this can lead to the dominance of population groups that are not necessarily optimal for the production of desired products.

2.4.4 Specific Examples of Microalgae and Results Using Continuous Culture

1. ***Phaeodactylum tricornutum***: A continuous culture provides a way of maintaining high cell densities and lipid accumulation over time, which is beneficial for the production of biodiesel. The technique allows for continuous extraction of lipids and the optimization of growth conditions in order to optimize lipid profiles suitable for use in fuel production (Branco-Vieira et al., 2018).
2. ***Haematococcus pluvialis***: It is more challenging to produce continuous astaxanthin under controlled stress (e.g., nutrient limitation, high light) because it requires stimulating conditions. Through the design of the system, cells will be exposed to induced conditions very often, increasing astaxanthin yields (An et al., 2024).

3 Microalgae Culture Systems

Different culture systems may be used in the cultivation of microalgae depending on a number of reasons. Some of the commonly used systems are photoautotrophic, heterotrophic, and mixotrophic cultures. Others include sequential heterotrophic-photoautotrophic culture, sequential heterotrophic-mixotrophic culture, sequential mixotrophic-photoautotrophic culture, cyclic heterotrophic-photoautotrophic culture, and cyclic heterotrophic-mixotrophic culture systems.

3.1 Photoautotrophic Culture

Microalgae that use energy from photosynthesis to grow are called photoautotrophic microalgae. In the context of photosynthetic metabolism, autotrophy refers to the

reduction of carbon dioxide to carbohydrates. Cultivation of microalgae requiring light energy, carbon dioxide, water, and nutrients to yield a biomass rich in protein, sugars, and lipids is called photoautotrophic cultivation. It is a process whereby complex organic compounds are made via photosynthesis where simple inorganic molecules including CO_2 and water in the presence of chlorophyll, using the energy of the light, are utilized to build food. Photoautotrophic cultivation is the commonest culture modes for microalgae probably due to its simplicity and low contamination risks (Jareonsin & Pumas, 2021). A very common photoautotrophic cultivation of microalgae is the open pond system, whereby microalgae utilize atmospheric carbon dioxide and sunlight energy for the synthesis of macromolecules.

3.1.1 Conditions Under Which Photoautotrophic Cultivation of Microalgae Is Recommended

There are some conditions under which photoautotrophic cultivation is recommended. In algal cultivation system where there is adequate availability of light, carbon dioxide, and inorganic nutrients, photoautotrophic cultivation of algae will be permissible as most algae are photosynthetic in nature. Similarly, there are instances where the desired cultivation product is light dependent. Under such situation, photoautotrophic culture is recommended. Besides, great majority of microalgae are obligate photoautotrophs and will only progress their life cycle in the presence of light.

3.1.2 Description of Photoautotrophic Cultivation System

Photoautotrophic cultivation involving the assimilation of CO_2 as sole carbon source by microorganisms with the help of NAD(P)H, ATP, and some key enzymes—ribulose bisphosphate carboxylase and phosphoribulokinase. For photoautotrophic cultivation of microalgae, simple mineral salts medium (like BG 11 medium), with guaranteed equitable carbon to nitrogen (C/N) ratio, phosphorous, and trace elements, is required. This is sterilized and used in the presence of available light energy and CO_2 as the carbon source.

3.1.3 General Advantages and Disadvantages of Photoautotrophic Cultivation System

The major advantages of photoautotrophic cultivation are simplicity and reduced risk of contamination. Low cost of light and carbon dioxide has also propelled the utilization of this culture strategy by many researchers. Productivities and standing biomass concentrations are however usually very low under this culture mode. When open pond is used for the photoautotrophic culture, cost effectiveness is an advantage; however, there is low standing biomass concentration probably owing to

limited supply of optimal sunlight and carbon dioxide and night biomass loss (Nagarajan et al., 2018; Ogbonna et al., 1999).

There is also increased risk of contaminations in open pond photoautotrophic cultivation system. In addition, in autotrophic cultures, photobioreactors, and open ponds alike, there may be technical challenges of light penetration during large-scale culture, and this becomes a critical drawback to these cultivation strategies (Ogbonna & Tanaka, 2000; Brennan & Owende, 2010). Furthermore, circumventing the cost of construction, working, and maintenance of a photobioreactor for mass production of microalgal cells has been a complex challenge limiting commercialization. Introduction of proper light intensity and CO_2 needed for photoautotrophic cultures is also critical in this culture strategy as that could impact on the overall cost of production.

3.1.4 Microalgae Cultivation Using Photoautotrophic Cultivation System

Some microalgae such as several species of *Chlorella* and *Desmodesmus* have been demonstrated to produce biomass and other metabolites like lipids and carotenoids using photoautotrophic cultivation system. Some of these are presented in Table 1.

3.1.5 Future Prospects of Using Photoautotrophic Culture

The future prospect with the use of any cultivation strategy is the need to improve the productivity of the microalgae and increase the value of the biomass produced. To improve the biomass and lipid productivities, it has been identified that going through the algae's natural life cycle and metabolic mechanisms will be of essence (Je & Yamaoka, 2022).

3.2 Heterotrophic Culture System

There are some microalgae with the intrinsic ability to grow in the absence of light energy. They can grow on organic carbon through respiratory or fermentative processes. This type of culture in which organic carbon source is used for the growth of microalgae in the absence of radiant energy is called heterotrophic culture.

3.2.1 Description of Heterotrophic Culture System

For heterotrophic cultivation, simple mineral salts medium is supplemented with organic carbon sources. Glucose, glycerol, and acetate are some of the notable organic carbon sources used for heterotrophic cultivation of microalgae. Other

Table 1 Cultivation of microalgae using photoautotrophic cultivation system

Strain	Culture system	Culture conditions	Biomass productivity (g-biomass/L.h)	Metabolite production	Reference
Chlorella minutissima	Autotrophic	Guillard f/2, CO_2	62.5 mg/L/d	5.72 mg/L/d lipid productivity	Amaral et al. (2020)
Desmodesmus subspicatus LC172266	Photoautotrophic	BG11 medium, CO_2, light intensity 3500 lux, 12 h light: 12 h dark	0.27 ± 0.01 g/L/day	77.50 ± 2.20 mg/L/d of lipid productivity	Ogbonna et al. (2021a)
Dictyosphaerium sp. LC172264	Photoautotrophic	Bold basal medium, CO_2. 12 h sunlight (intensity 2000 lux at 28 °C)	0.238 g/L	15.32% lipid content/ CDW	Ogbonna et al. (2021b)
Chlorella minutissima	Autotrophic	Guillard f/2, CO_2	62.5 mg/L/d	5.72 mg/L/d lipid productivity	Amaral et al. (2020)
Chlorella pyrenoidosa 2738	Autotrophic	Fog's media CO_2	5 g/L/13d	410 mg/L/d lipid productivity	Nawkarkar et al. (2019)
Desmodesmus subspicatus LC172266	Autotrophic	BG 11 medium, CO_2 with urea supplementation	0.109 g/L/d	2.0 mg/L carotenoids	Eze et al. (2022)
Chlorella lewinii	Autotrophic	BG 11 medium, CO_2, with urea supplementation	0.11 g/L/d	1.848 mg/L carotenoid	Eze et al. (2022)
Desmodesmus armatus	Autotrophic	BG 11 medium, CO_2, with urea supplementation	0.091 g/L/d	1.095 mg/L carotenoid	Eze et al. (2022)
Dictyosphaerium sp. LC172264	Autotrophic	BG 11 medium, CO_2. With urea supplementation	0.075 g/L/d	1.62 mg/L carotenoid	Eze et al. (2022)
Chlorella sorokiniana AM-02	Autotrophic	BBM CO_2	2.4 g/L	3.45 g/L lipid content	Ziganshina et al. (2022)
Desmodesmus subspicatus LC172266	Autotrophic	BBM CO_2 (unoptimized)	0.217 g/L	15.86% lipid content/ DCW	Ogbonna et al. (2019)
Desmodesmus armatus	Autotrophic	BBM CO_2	21.3 mg/L/d	12.41% lipid content/ DCW	Okpozu et al. (2019)

(continued)

Table 1 (continued)

Strain	Culture system	Culture conditions	Biomass productivity (g-biomass/L.h)	Metabolite production	Reference
Chlorella vulgaris NIOCCV	Autotrophic	Wastewater + CO_2	264 mg/L/d	100.54 mg/L/d lipid productivity	Jain et al. (2019)
Chlorella vulgaris MBFJNU-1	Autotrophic	Wastewater + CO_2	478.5 mg/L/d	9.1 mg/L/d lipid productivity	Xie et al. (2020)

components of the culture medium such as nitrogen, phosphorous, and trace elements are required. The medium is sterilized, inoculated with a seed culture, and incubated in the dark in the absence of inorganic carbon source. In heterotrophic microalgal cultivation system, energy is produced via oxidation of organic carbon substrate in a process known as respiration. Furthermore, the carbon intermediates of heterotrophs are transformed into main metabolic pathways, replacing photosynthetically produced molecules (Morales-Sánchez et al., 2015).

3.2.2 General Advantages and Disadvantages of Heterotrophic Culture System

There are many advantages of heterotrophic cultivation over photoautotrophic modes. One of these advantages is the elimination of light requirements which enables the use of innovative bioreactors free from design constraints. Others include ease of scale-up, more FDA-approved standards and protocol for industrial fermenters, and the ability to achieve higher cell densities (Rasala & Mayfield, 2015). In heterotrophic cultures, as high as 50–100 g/L of cell dry weight can be achieved, which is higher than that of photoautotrophs with a maximum yield of 30 g/L of cell dry weight (Perez-Garcia et al., 2011). Besides, the concentrations of *Chlorella* species obtained in heterotrophic cultures are approximately 5.5 times higher than the values obtained in photoautotrophic cultures (Yu et al., 2013). There is also an advantage of gain in cultivation time for scale-up as heterotrophic microalgae take much shorter time than photoautotrophic microalgae. In all, the overall area of space used for heterotrophic cultivation is usually 12 times less than that of photoautotrophic cultures (Barros et al., 2019).

However, heterotrophic cultivation has its own drawbacks as the processes might limit the development of chlorophyll (Klamczynska & Mooney, 2017). In addition, the chances of contamination by fast-growing heterotrophs are very high while accumulation of light-induced metabolites (photochemical products) is very low. Besides, a critical factor that limits the utilization of heterotrophic cultivation strategy is that only a few species of microalgae can grow under this condition, thus, limiting their potential use for large-scale production (Lee, 2001). Furthermore, there is production of carbon dioxide from the respiration of organic carbon, which will increase GHG emission.

3.2.3 Microalgae Cultivation Using Heterotrophic Cultivation System

Several species of microalgae have shown good biomass and lipid productivities using BG 11 medium supplemented with glucose, sucrose, and other simple carbohydrates. The use of different waste substrates and/or wastewaters has also been demonstrated as cheap carbon sources for large-scale microalgal production under heterotrophic conditions (Table 2).

Table 2 Cultivation of microalgae using heterotrophic cultivation system

Strain	Culture system	Culture conditions	Biomass productivity (g-biomass/ L.h)	Lipid production	Reference
Chlorella vulgaris AB MCCS 130	Heterotrophic	BG11 Glucose	265 mg/L/d	118 mg/L/d	Morowvat and Ghasemi (2018)
Desmodesmus subspicatus LC172266	Heterotrophic	Cassava wastewater (unoptimized)	0.63 g/L/d	21.40% DCW	Ogbonna et al. (2019)
Chlorella. vulgaris AB	Heterotrophic	Glucose in BG11 medium	265 mg/L/d	118 mg/L/d lipid	Morowvat and Ghasemi (2018)
Chlorella sp. HS2	Heterotrophic	BG11 glucose	5370 mg/L/d	860 mg/L/d	Kim et al. (2019a)
Dictyosphaerium sp.	Heterotrophic	BG11 glucose	0.23 g/L/d	96.6 mg/L/d	Ogbonna and Ogbonna (2018a)
Dictyosphaerium sp.	Heterotrophic	BG11 glycerol	0.22 g/L/d	60.9 mg/L/d	Ogbonna and Ogbonna (2018a)
Chlorella vulgaris KNUA104	Heterotrophic	Glucose	1.72	30% DCW	Yun et al. (2021)
Desmodesmus armatus	Heterotrophic	Cassava wastewater	52.14 mg/L/ d	20.86% DCW	Okpozu et al. (2019)
Chlorella sorokiniana KNUA122	Heterotrophic	Glucose	3.64	40% DCW	Yun et al. (2021)
Chlorella vulgaris CCALA 256	Heterotrophic	BBM glucose	NA	32.70%	Canelli et al. (2020)
Dictyosphaerium sp. LC172264	Heterotrophic	Cassava wastewater at 28 °C	0.63 g/L	16.54% lipid/ CDW	Ogbonna et al. (2021b)
Chlorella protothecoides	Heterotrophic	Wastewater	10.7 g/L	16.80% DCW	Chen et al. (2019)
Chlorella sorokiniana SAG 211–8 k	Heterotrophic	Wastewater	8.28 g/L	3.61 g/L	Vyas et al. (2022)
Chlorella lewinii SUB3545914	Heterotrophic	BG11 glucose	3.15 g/L	118.8 mg/L/d	Ogbonna and Ogbonna (2018b)
Chlorella lewinii SUB3545914	Heterotrophic	BG11 glycerol	2.03 g/L	47.21 mg/L/d	Ogbonna and

(continued)

Table 2 (continued)

Strain	Culture system	Culture conditions	Biomass productivity (g-biomass/L.h)	Lipid production	Reference
					Ogbonna (2018b)
Chlorella pyrenoidosa	Heterotrophic	BG11 sucrose + yeast	2290 mg/L/10d	124.3 mg/L/d	Kilian et al. (1996)
Chlorella pyrenoidosa FACHB-9 + *Rhodotorula glutinis*	Heterotrophic	BG11 sucrose + yeast	340 mg/L/d	29.70% DCW	Wang et al. (2016a)

3.2.4 Future Prospects of Using Heterotrophic Cultivation System

Future studies on heterotrophic cultivation are warranted to address the issue of contamination, especially where cheap mixed organic carbon sources (e.g., wastewater) are used.

3.3 Mixotrophic Culture System

In some microalgae, photoautotrophic metabolism is integrated with heterotrophic metabolism that oxidizes the reduced carbon sources available in the medium. This type of culture is called mixotrophic culture. In mixotrophic culture, the energy sources are both light energy and organic carbon sources.

3.3.1 Condition Under Which Mixotrophic Cultivation of Microalgae Is Recommended

Mixotrophic culture system is recommended under conditions with variable nutrient availability in the environment. The concurrent metabolism of both the organic and inorganic carbon sources called mixotrophy has been suggested for the survival and high biomass generation by the algae (Heredia-Arroyo et al., 2011).

3.3.2 Description of Mixotrophic Cultivation System

For mixotrophic culture system, an organic carbon source is added to the medium just as we have in heterotrophic cultures but incubated under solar (natural) or electrical (artificial) light. There is simultaneous utilization of CO_2 and organic carbon sources. During mixotrophic metabolism, the algae assimilate organic carbon nutrients and inorganic carbon (CO_2) and simultaneously perform both respiration and photosynthesis (Cheah et al., 2018). For the reduction of carbon under this culture strategy, the energy needs come from organic carbon substrate and radiant energy (Wen et al., 2019). The nature of the carbon substrates, the light intensity, and the photoperiods are important factors that determine the metabolism and certain product formation in mixotrophic cultures (Mondal et al., 2017). Cell biomass and product formation under this culture condition may be affected by the nature of supplementary nutrients in the medium.

In order to make algal production less expensive, cheap substrates are being advocated. As a result, the use of low-cost carbon substrate has been identified as promising for microalgae mixotrophic cultivation (Nguyen et al., 2018). Some microalgae have the ability to move from photoautotrophic to mixotrophic culture mode, and it is an intrinsic capacity of certain taxonomic class and genera (Choi & Lee, 2015). The molecular physiologic mechanism of microalgae growth under mixotrophic condition is not well understood (Vidotti et al., 2020).

3.3.3 General Advantages and Disadvantages of Mixotrophic Cultivation System

A major advantage of mixotrophic culture is that the chemical and the light energy act together to increase cell density. Growth rate and biomass productivity may be several times more than those in photoautotrophic cultures. However, in most cases, metabolic product synthesis is usually dependent on the quality and quantity of the organic carbon substrates (Choi & Lee, 2015). Similarly, there are reports of reduction of the photosynthetic efficiency in mixotrophic cultivation (Cecchin et al., 2018). This might be a major disadvantage because reduction of photosynthetic efficiency might limit the application of algae in greenhouse gas (GHG) mitigation. In addition, the requirements for light, organic carbon, carbon dioxide, and oxygen could be additional source of cost. Energy conversion efficiency is low relative to heterotrophy, irrespective of improved energy economics compared to photoautotrophic growth (Yang et al., 2000). Carbon dioxide is still released under mixotrophic culture system despite its photosynthetic fixation (Perez-Garcia et al., 2011; Yang et al., 2000).

3.3.4 Microalgae Cultivation Using Mixotrophic Cultivation System

Several strains of *Chlorella* have been demonstrated to grow well, yielding good biomass and lipid under mixotrophic conditions where different organic carbons were used. Wastewater has been shown to be efficient carbon source in the production of lipids for biodiesel and other applications in mixotrophic cultures (Table 3).

3.3.5 Future Prospects of Using Mixotrophic Cultivation System

Future research relating to mixotrophy would entail identifying and studying the genes involved in mixotrophy to enable the development of strains with higher performance in terms of biomass and other product yields.

3.4 Exploitation of Heterotrophic and Photoautotrophic Metabolic Activities in Microalgae

As outlined above, mixotrophic, heterotrophic, and photoautotrophic cultures have advantages and disadvantages and can be combined to complement each other. For example, although heterotrophic cultures can be used to achieve high cell densities, production of light-induced metabolites require light. Thus, sequential heterotrophic-photoautotrophic culture systems where algae cells are cultivated heterotrophically to a very high cell density after which the cells are transferred to photoautotrophic condition for the accumulation of photoproducts as in α-tocopherol production can be used (Ogbonna et al., 1999). Similarly, sequential heterotrophic-mixotrophic culture system can be used to overcome the limitations of heterotrophic and mixotrophic cultures. In this case, the microalgae are cultivated under heterotrophic conditions to achieve high cell density and thereafter transferred to a mixotrophic condition for the accumulation of light-induced metabolites and further high cell density. Where the presence of organic carbon source in mixotrophic culture suppresses production of some metabolites, sequential mixotrophic-photoautotrophic culture system can be used. In this system, mixotrophic culture is used to grow the cells to very high density and the condition switched to photoautotrophic condition for the accumulation of the desired products.

Night biomass loss is a major problem in photoautotrophic cultures when sunlight is used as the sole energy source. At night, not only that the cells will not grow but dark respiration leads to loss in their body weights. This problem can be solved by cyclic photoautotrophic-heterotrophic cultures. In this case, the cells are cultivated photoautotrophically during the day and at night predetermined quantity of organic carbon is added so that the cells grow heterotrophically. This cycle is repeated until the desired cell concentration is achieved. In this system, both the heterotrophic and photoautotrophic metabolites are accumulated. This system can also be used even

Table 3 Cultivation of microalgae using mixotrophic cultivation system

Strain	Culture system	Culture conditions	Biomass concentration (g/L) or productivity (g-biomass/L.h)	Lipid content (%) or productivity(mg/L/d)	Reference
Chlorella. vulgaris KNUA104	Mixotrophic	Glucose in BG11 medium	2.98 mg/L/d	68.80% DCW	Yun et al. (2021)
Dictyosphaerium sp.	Mixotrophic	BG11 glucose	0.25 g/L/d	115.8 mg/L/d	Ogbonna and Ogbonna (2018a)
Dictyosphaerium sp.	Mixotrophic	BG11 glycerol	0.23 g/L/d	69.0 mg/L/d	Ogbonna and Ogbonna (2018a)
Chlorella sorokiniana KNUA122	Mixotrophic	BG11 glucose	4.73	40% DCW	Yun et al. (2021)
Desmodesmus subspicatus LC172266	Mixotrophic	Cassava wastewater (unoptimized)	1.042 g/L	24.70% lipid/DCW	Ogbonna et al. (2019)
Chlorella vulgaris strain UTEX 2714	Mixotrophic	TAP glucose	6.1 g/L	383 mg/L/d	Ward and Rehmann (2019)
Desmodesmus armatus	Mixotrophic	Cassava wastewater	70.88 mg/L/d	21.91% lipid/DCW	Okpozu et al. (2019)
Chlorella sorokiniana 211-32	Mixotrophic	Acetate	1390 mg/L/d	193.37 mg/L/d	León-Vaz et al. (2019)
Chlorella pyrenoidosa (FACHB-9)	Mixotrophic	BG11 acetate	40 mg/L/d	13.48 mg/L/d	Liu et al. (2018)
Chlorella pyrenoidosa	Mixotrophic	Glycerol	1.28 g/L	30.76% DCW	Rana and Prajapati (2021)
Chlorella vulgaris UTEX 395	Mixotrophic	BBM wastewater	3.44 g/L	141 mg/L/d	Arora and Philippidis (2021)
Chlorella sp. GY-H4	Mixotrophic	Wastewater	6.9 g/L	1.8 g/L	Wang et al. (2020)
Chlorella sorokiniana CY-1	Mixotrophic	Wastewater	409 mg/L/d	14.43% DCW	Cheah et al. (2018)
Dictyosphaerium sp. LC172264	Mixotrophic	Cassava wastewater at 28 °C	1.022 g/L	24.53% CDW	Ogbonna et al. (2021b)
Chlorella vulgaris	Mixotrophic	Wastewater			

			3.25 g/L	25.40% DCW	Saranya and Shanthakumar (2019)
Chlorella vulgaris (NRMCF0128)	Mixotrophic	Wastewater	60.1 mg/L/d	20.8 mg/L/d	Afam et al. (2022)
Chlorella pyrenoidosa	Mixotrophic	Wastewater	2.84	9.30%	
P. pringsheimii (VIT_SDSS)	Mixotrophic	Wastewater	56.5	17.5	
Chlorella sp. UJ-3	Mixotrophic	Wastewater	NA	25.40% DCW	Huo et al. (2018)
Chlorella sp.	Mixotrophic	Wastewater	288.84 mg/L/d	104.89 mg/L/d	Zhu et al. (2017)

when electric light is used and the ratio of light and dark periods can be adjusted depending on the strain and desired metabolites. Different ratios such as 12 h dark: 12 h light, 10 h dark: 14 h light, 8 h dark: 16 h light, and 6 h dark: 18 h light have been investigated (Cheirsilp & Torpee, 2012). When the organic carbon source is present throughout the culture but the culture is alternatingly exposed to light and dark, the system is called cyclic mixotrophic-heterotrophic culture.

4 Factors Influencing Large-Scale Microalgae Production

Several factors usually influence the cultivation and production of microalgae in large scale. These can be grouped into physical, chemical, and biological culture conditions.

4.1 Physical Culture Conditions That Affect Large-Scale Microalgae Production

Several physical conditions affect microalgal growth and productivity. It is important to consider and optimize these conditions in large-scale production in order to have enhanced biomass production. Some of these physical conditions include light intensity, quality (i.e., wavelength of light) and duration, temperature, and mixing. The optima physical conditions usually differ among microalgal species. These conditions could also differ between microalgal biomass and metabolite productions. Generally, optima temperature, light intensity, and duration for some microalgae species are 20–30 °C, 2000–4000 lux, 12:12 or 16:8 h (light-dark cycles), respectively (Gaurav et al., 2024; Zhu, 2015).

4.1.1 The Effects of Light on Microalgae Growth and Productivity

Microalgae can be grown photoautotrophically or mixotrophically in the presence of light. They use light energy as a necessary component for photosynthesis and the synthesis of energy-bound molecules. Therefore, light plays a crucial role in the growth and production of microalgae (Schulze et al., 2020; Abdur Razzak et al., 2023). Light intensity and quality (i.e., wavelength), as well as photoperiods and light distribution in photobioreactors (PBRs) greatly affect microalgal growth and biomass production. Optimizing these conditions is quintessential in microalgal cultivation (Abdur Razzak et al., 2023). Researchers have reported that light intensity and quality required for optima microalgal growth and biomass production are usually not the same in the production of some essential metabolites, such as lipids and pigments, in the same algal strains. For example, the light intensity required for optimal

growth of most microalgae lies in the range of 26–400 μmol photons m^{-2} s^{-1} while that for optimal lipid productivity is between 60 and 700 μmol photons m^{-2} s^{-1}. However, some microalgae attain maximum growth and lipid productivities outside the above ranges (Abdur Razzak et al., 2023; Maltsev et al., 2021).

Sunlight is a cheap source of light energy that could be harnessed in microalgae large-scale production. However, the challenge of controlling the sunlight intensity, quality, and photoperiods coupled with the associated heating-up of cultures, especially in closed PBRs, makes this natural light source difficult to exploit in commercial microalgal production. Notwithstanding, sunlight is still used in open PBRs in large-scale microalgal production. Continuous high light intensity either from the sun or from other artificial light sources could lead to photoinhibition while low light intensity might limit optimal growth and biomass production, as well as limit the synthesis of desired metabolites in some microalgal species. Therefore, the depth of culture and adequate light distribution (which can be achieved by proper mixing) could help to prevent obvious variation of light intensities in large-scale cultivation (Schulze et al., 2020; Abdur Razzak et al., 2023; Abu-Ghosh et al., 2016).

Another way to address photoinhibition, stratification in light distributions, and the cost of continuous lighting in microalgae cultivation is the adoption of flashing light techniques. Flashing light is a system in microalgal biotechnology whereby intermittent high light intensity/flashes emission is coupled with alternating dark periods as against the conventional continuous lighting (Schulze et al., 2020; Abu-Ghosh et al., 2016; Schulze et al., 2017). Several publications have reported that flashing lights can significantly increase microalgal growth and biomass compared to continuous lights while some others have indicated that continuous lights still give better microalgal growth than flashing light (Schulze et al., 2017, 2020; Fathurrahman et al., 2013; Olivieri et al., 2021). However, many research works are still on going on the applications of flashing lights in the cultivation of microalgae.

Different kinds of light — white, red, blue, green, etc. — emit different qualities of light. Several kinds of microalgae grow in different light wavelengths or ranges. Microalga may grow in a particular wavelength; however, obtaining maximal growth rate may necessitate cultivating such alga in another wavelength or a mix of wavelengths which is optimum for growth (Mishra et al., 2019; Maltsev et al., 2021; Kwan et al., 2021). Table 4 gives a catalog of some microalgae with the various light intensities, wavelengths, and photoperiods for optimum growth. A number of the given microalgal species grew optimally to produce good biomass when cultivated in red, blue, green, or white lights or a mix of two of the lights. For example, *Haematococcus pluvialis* and *Isochrysis galbana* grew optimally in a mix of red and blue lights with different mixing ratios (Kwan et al., 2021; Domínguez et al., 2019; Che et al., 2019). The applications of light emitting diodes (LEDs) in microalgal biotechnology have greatly simplified the process of getting different light wavelengths or a mixture of them in order to obtain optimal light conditions for growing microalgae and producing their metabolites (Che et al., 2019; Baidya et al., 2021; Habibi & Sibi, 2019; Kim et al., 2019b).

Table 4 Optima light intensities, wavelengths, and photoperiods for maximal microalgae growth

Class	Microalgae	Optima light intensity (μmol photons m^{-2} s^{-1})	Optima light wavelength (nm)	Light: dark cycle	References
Trebouxiophyceae	*Scenedesmus* (*Tetradesmus*) *obliquus*	150, 420, 180–540	620–750 (red light)	22:2; 24:0	Maltsev et al. (2021), Habibi and Sibi (2019), Sforza et al. (2014), Nzayisenga et al. (2020), Ho et al. (2012), Sartori et al. (2022), and Krzemińska et al. (2014)
	Scenedesmus quadricauda	500	660	1:1 h	Fettah et al. (2022)
	Chlorella vulgaris	105.41–175.68, 150, 200	620–750 (red light)	12:12	Kim et al. (2019b), Nzayisenga et al. (2020), Atta et al. (2013), Lee et al. (2011), Kendirlioglu and Kadri (2017)
	Chlorella ellipsoidea	200	475 (blue light)	12:12	Baidya et al. (2021)
	Botryococcus braunii	100, 850	620–750 (red light)	16:8; 24:0	Krzemińska et al. (2014), Baba et al. (2012), Ruangsomboon (2012), Sakamoto et al. (2012), and Acuapan-Hernandez et al. (2017)
Cyanophyceae	*Arthrospira (Spirulina) platensis*	60, 166	620–750 (red light)	24:00; 18:06	Jung et al. (2022), Godoy Danesi et al. (2011), Niangoran et al. (2021), and Soletto et al. (2008)
	Phormidium sp.	160, 100	380–750 (white light)		Hotos (2021) and Zampieri et al. (2023)
	Synechococcus sp.	500, 660	630; 680		Włodarczyk et al. (2020) and Bernstein et al. (2014)
	Dunaliella salina	1000	620–750 (red light)	12:12	Kendirlioglu and Kadri (2017), Xu et al. (2018), Sui and Harvey (2021), and Xu et al. (2016)
	Desmodesmus sp.	600; 5000 lux	689	18:6	Ogbonna et al. (2021a), Xie et al. (2013a), and Wang et al. (2022)
	Anabaena torulosa	25, 120; 2000 lux	495–570 (green light)	18:6	Schagerl and Müller (2006), Hotos (2023), and Salem et al. (2019)
Bacillariophyceae		60–112, 496		16:8	

	Phaeodactylum tricornutum				Remmers et al. (2017), Ova Ozcan and Ovez (2020), and Chauton et al. (2013)
	Skeletonema costatum	40, 50	450–495 (blue light)	16:8	Miao et al. (2012) and Li et al. (2021a)
Porphyridiophyceae	*Porphyridium purpureum*	70, 100	495–570 (green light)		Coward et al. (2016) and Sosa-Hernández et al. (2019)
Cryptophyceae	*Rhodomonas* sp.	50	400–600 (blue/green light)		Latsos et al. (2021)
	Neochloris oleoabundans	136	380–750 (white light)	12:12	Loera-Quezada et al. (2011), Esteves et al. (2020), and De Winter et al. (2017)
Coccolithophyceae	*Isochrysis galbana*	325, 400	465 and 640 (mixed blue and red light)	18:6; 12:12	Mishra et al. (2019), Kwan et al. (2021), Che et al. (2019), and Yoshioka et al. (2012)
Chlorodendrophyceae	*Tetraselmis* sp.	133	465 (blue light)	24:0	Kwan et al. (2021), Yoshioka et al. (2012), Dammak et al. (2016), Teo et al. (2014), and Fakhri et al., (2015)
Eustigmatophyceae	*Nannochloropsis* (*Microchloropsis*) *salina*	26–55, 250	430/680 (blue/red light)	24:0	Maltsev et al. (2021), Fakhri et al. (2015), Van Wagenen et al. (2012), Yan and Zheng (2013), and Shirazian et al. (2022)
Chlorophyceae	*Haematococcus pluvialis*	260, 2500	689/430 [red/blue (2:1) mixed light]	12:12	Domínguez et al. (2019), García-Malea et al. (2006), Evens et al. (2008), and Li et al. (2020)

4.1.2 The Effects of Temperature on Microalgae Growth and Productivity

Microalgae can grow at different temperatures. These microalgae include psychrophiles and psychrotrophs, mesophiles, as well as thermotrophs. A good number of characterized microalgae with industrial relevance can grow optimally within mesophilic temperatures—20 to 30 °C (Gao et al., 2023; Singh & Singh, 2015). Temperature is a very important factor in the growth and production of essential metabolites by microalgae. Although some microalgae species can grow and tolerate some level of temperature ranges and fluctuations, very high or low temperatures (out of the optimum range) could lead to growth inhibition and death of cells (Abdur Razzak et al., 2023; Gao et al., 2023; Pessi et al., 2022). In some cases, the temperatures required for optimum growth differ from those needed to produce some essential products like carotenoids, tryglycerols, polyunsaturated fatty acids, etc. The temperatures for optimal growth of *Synechococcus* sp. and *Scenedesmus acutus* are 38 and 30 °C, respectively, while the optimal production of carotenoids or lipids by these microalgae is achieved at 50 or 15/38 °C, respectively (Table 5) (Włodarczyk et al., 2020; El-Sheekh et al., 2017; Castro-Tapia et al., 2022).

Table 5 Optima temperature for microalgae growth and productivities

Microalgae	Optima temperature (°C)		References
	Growth	Metabolites	
Chlorella vulgaris	25–30	Lipids: 25; carotenoids: 30	Ma et al. (2014) and Yadav et al. (2020)
B. braunii	25–30	Lipids: 25; exopolysaccharide: 26	Ruangsomboon (2012), Al-Hothaly et al. (2016), and García-Cubero et al. (2018)
Scenedesmus sp.	20	Lipids: 20; carotenoids: 30	Xin et al. (2011) and Přibyl et al. (2015)
Haematococcus pluvialis	20, 27	Astaxanthin: 20, 28	Evens et al. (2008) and Giannelli et al. (2015)
Anabaena sp.	28–32	Carotenoids: 23	Kłodawska et al. (2019) and Nalewajko and Murphy (2001)
Arthrospira (Spirulina) platensis	30	Lipids: 30; β-carotene: 30; phycocyanin: 25	Godoy Danesi et al. (2011) and Hamidi et al. (2023)
Phormidium sp.	45	Phycocyanin: 45	Zampieri et al. (2023)
Synechococcus sp.	38	Carotenoids: 50	Włodarczyk et al. (2020)
Phaeodactylum tricornutum	21, 25	Lipids: 25	Ova Ozcan and Ovez (2020) and Zhao et al. (2020)
Skeletonema costatum	25	Eicosapentaenoic acid: 15	Li et al. (2021a) and Blanchemain and Grizeau (1999)
Scenedesmus acutus	30	Lipids: 15, 38	El-Sheekh et al. (2017) and Castro-Tapia et al. (2022)
Chlamydomonas reinhardtii	20–32	Carotenoids: 15; lipids: 30	Li et al. (2021b), Supakorn et al. (2021), and Xie et al. (2013b)
Schizochytrium sp.	20–30	Lipids: 34	Chi et al. (2022) and Hu et al. (2021)

Temperature could be used as a very important stressor/stimulant for the production of some useful metabolites by microalgae. Therefore, switching of temperatures after achieving a desired microalgal growth/biomass to lower or higher temperatures has been reported to increase lipid and carotenoid syntheses, as well as some other metabolites (Han et al., 2016; Morales-Sánchez et al., 2020; Paliwal et al., 2017). Temperature control in closed and computerized PBRs is not a big deal as they have inbuilt sensors to moderate temperature fluctuations. The challenge of thermal stratification within large PBRs with mixing/agitations is also minimal as adequate mixing facilitates proper heat distribution. However, in reactors without temperature sensors and provisions for mixing (like some open ponds), adequate temperature control could be a big challenge. In such reactors, microalgal cultures are usually left to adapt to variations between day and night temperatures. Change in season that results in drastic temperature changes would therefore limit the cultivation of some microalgal strains all year-round in such bioreactors. However, techniques such as shading, spraying water, submerging the culture in water, and others could be applied for cooling in such PBRs (Novoveská et al., 2023; Benner et al., 2022; Parsy et al., 2021; Wang et al., 2012).

4.1.3 The Effects of Mixing on Microalgae Growth and Productivity

Mixing is another condition that is very crucial in the cultivation of microalgae especially in large-scale productions. It will be practically impossible to maintain average distributions of light and temperature, dissolved carbon dioxide and oxygen, as well as nutrients and pH in a large-scale PBR without adequate mixing and agitation. Therefore, mixing prevents gradients in light, temperature, pH, and nutrients in PBRs. It facilitates homogeneous mass transfer and prevents sedimentation of microalgal cells (Abdur Razzak et al., 2023; Wang et al., 2012; Tong et al., 2020; Carvalho et al., 2006). Depending on the sensitivity of microalgae species, the kind of cultivation system, and the scale of production, mixing in algal biotechnology could be achieved through stirring by pumping, aeration, different mechanical agitations, or a combination of them (Wang et al., 2012; Tong et al., 2020).

Appropriate mixing aids in boosting microalgae growth and/or the production of desired metabolites as it helps to maintain the cultivation optimal conditions across the PBR. However, not all microalgae strains can tolerate some kinds of turbulent mixing from mechanical agitators and bubble break-up. These could lead to very high velocity and high hydrodynamic stress which becomes counterproductive in the growth and metabolites' productions by microalgae (Abdur Razzak et al., 2023; Wang et al., 2012; Carvalho et al., 2006). Excessive shear stress could affect microalgal mechanical properties and lead to some damages on their cells and/or eventual lysis. These therefore can result in decreased cell viability and growth rate, as well as increased mortality (Wang & Lan, 2018; Ali, 2018). It is very important to

consider the kind of mixing regimen to be applied in cultivation juxtaposing it to the strains of microalgae cultures, PBR, needed metabolites, among other factors.

Three major mixing/agitation systems can be applied in microalgae biotechnology. These include pneumatic, mechanical, or both. Mechanical mixing involves agitating PBRs with systems that convert mechanical energy to kinetic energy to facilitate the movement of fluid. Impellers such as blades, propellers, or turbines are used in mechanical agitations depending on the viscosity of the culture. On the other hand, pneumatic mixing is the type of agitation which is promoted by the injection of gas (such as carbon dioxide) into the PBR. Spargers—devices which aid the uniform distributions of bubbles in adequate sizes—that are usually located at the bottom of PBRs are essential devices in pneumatic mixing. Examples of pneumatically agitated PBRs include bubble column, internal loop airlift, external loop airlift, flat plate with and without risers, among others (Abdur Razzak et al., 2023; de Souza Kirnev et al., 2022).

4.2 Chemical Culture Conditions That Affect Large-Scale Microalgae Production

Several culture parameters including salinity, pH, available nutrients, intensity of light, CO_2, and dissolved oxygen concentration can impact the growth of microalgae, giving a refined biomass productivity when optimized (Chowdury et al., 2020). Although the culture conditions have been shown to vary with different species, successful growth of the microalgae, especially in large scale, requires specific growth factors.

4.2.1 Effects of Media Components and Concentrations on Microalgae Growth and Productivity

Media composition of the culture greatly affects the growth rate and the final concentrations of microalgae biomass as well as accumulation of metabolites such as lipids, proteins, antioxidants, and vitamins. Sufficient biomass production requires all the integral nutrients in the growth medium (Panahi et al., 2019). A culture medium should contain carbon, nitrogen, phosphorus, potassium, calcium, magnesium, some microelements, as well as vitamins (de Carvalho et al., 2018). A major constituent of the algal medium is carbon, which consists of about 50% and can be supplied either organically as peptone or acetic acid which can be utilized by certain microalgae or inorganically as carbon dioxide obtained from the atmosphere (Cysewski & Todd, 2003).

Nitrogen is another important nutrient in the medium, added in the form of nitrate (NO_3^-), ammonium salts, or other organic forms such as urea, and consists about 10% of the biomass. Nitrogen shortage in the medium can give rise to increase in

carotenoids and production of lipids, decrease in chlorophylls, as well as polysaccharide build up (Widjaja et al., 2009). The influence of limited nitrogen was studied in antioxidant production by three microalgae species—*Tetraselmis suecica, Phaeodactylum tricornutum*, and *Chlorella vulgaris*. It was observed that chlorophyll content was reduced significantly in nitrogen-limited culture (Goiris et al., 2015). Phosphorous is another vital element usually included in the medium in the form of orthophosphate (PO_4^{3-}) and is involved in the transmission of energy and in DNA anabolism (Cysewski & Todd, 2003). It is also needed for amino acids and phospholipid synthesis (Morales et al., 2018). The lack of nitrogen and phosphorus leads to a reduction in cellular concentrations of photosynthetic pigments and cellular proteins which have a negative effect on the microalgae viability. This explains why microalgae response to limiting conditions of nitrogen and phosphorous is lipids and carbohydrate accumulations (Cakmak et al., 2012; Pancha et al., 2014).

Magnesium is a vital nutrient which holds a fundamental place in molecules of chlorophyll and is required for microalgae growth and photosynthetic activity (Ayed et al., 2015). Other elements like potassium, calcium, sodium, and iron are also important. Micronutrients are usually provided by commercial salts. They can also be obtained from the water used in preparing the media, which could be untreated wastewater containing several micronutrients needed for cultivation including boron, copper, zinc, manganese, and molybdenum required in low quantities (de Carvalho et al., 2018; Cysewski & Todd, 2003).

Formulation of appropriate media is highly essential for increased growth rate and microalgal biomass productivity (Danquah et al., 2010). A number of studies rely on modifying already established media like the blue green-11 (BG-11), which composed majorly of $NaNO_3$ and K_2HPO_4 as sources of nitrogen and phosphorus

Table 6 Culture medium for the growth of different microalgae species

Microalgae specie	Culture media	Reference
Cyanobacteria such as *Nostoc* sp.	BG-11	Ahlgren et al. (1992)
Neochloris oleoabundans	MBM with an excess of phosphate	Wang and Lan (2011)
Spirulina sp.	Modified Zarrouk medium (MZM), also with a moderate excess of phosphate	Schoepp et al. (2014)
Chlorella vulgaris, Tetraselmis chuii, and *Isochrysis galbana*	Bold's Basal Medium (BBM); BBM + ammonium chloride; Conway medium; and Conway medium + silica + ammonium chloride	Haris et al. (2022)
Scenedesmus sp.	Modified Detmer's Medium (MDM) contained in 1 L: 1 g KNO_3, 0.25 g K_2HPO_4, 0.25 g $MgSO_4{\cdot}7H_2O$, 0.1 g NaCl, 0.01 g $CaCl_2{\cdot}2H_2O$, 1 mL of Fe solution, and 1 mL of A5 solution	Elloumi et al. (2020)
Chlorella vulgaris	Modified BG-11 growth medium containing 17.6 mM nitrogen	Almutairi et al. (2021)

(Table 6) (Panahi et al., 2019). For instance, BG-11 was modified by adding an additional nitrate for the production of lipids by *Scenedesmus* sp. (Xia et al., 2013). Also, a research by Crofcheck et al. (2012) showed that a modification of M-8 medium by replacing the nitrogen source, KNO_3, with urea—which is considered more cost effective—increased the growth of *Chlorella vulgaris* and can lead to economical large-scale cultivation.

The source and composition of nutrients for scale-up cultivation should be put into consideration since the use of laboratory culture nutrients are expensive for large-sale production of algal biomass (Borowitzka & Vonshak, 2017). Different carbon sources (fructose, glucose, maltose, sucrose, and starch) tested at varying concentrations enhanced the growth rate of *Scenedesmus obliquus*, from an initial concentration to 20 g L^{-1}, but the growth rate decreased with increasing carbohydrate concentrations. Glucose gave the highest growth rate of 1.31 day^{-1} at 20 g L^{-1} and 0.56 day^{-1} at 50 g L^{-1} (Sibi, 2015). Interestingly, some of these carbon sources could be obtained from waste substrates.

Maintaining the optimal concentrations of nutrients can be a problem, especially in large-scale culture of industrial algae (Borowitzka & Vonshak, 2017).

4.2.2 Effect of pH on Microalgae Growth and Productivity

One of the major factors affecting the division of algae in terrestrial and aquatic ecosystems is the pH (Gaysina, 2024). pH is also an important environmental condition in the cultivation of microalgae as it determines the accessibility and solubility of CO_2 and other nutrients with a major effect on the metabolism of microalgae (Qiu et al., 2017).

The pH tolerance level of algae is broad, although the extent of pH survival in several group representatives varies (Gaysina, 2024). A research carried out by Qiu et al. (2017) showed that *Chlorella sorokiniana* gave high biomass productivity at a pH range of 6.5–8. Another study by Khalil et al. (2010) showed growth of *Dunaliella bardawil* at a pH range of 4–9, while *Chlorella ellipsoidea* grew at a pH range of 4–10.

Growth and development of microalgae as well as biomass production, production of metabolites like lipids, carbohydrates, and proteins are all influenced by the value of pH, due to its effect on enzymatic activities, cell physiology, metabolism, and balance of inorganic carbon (Nurafifah et al., 2023; Almutairi & Toulibah, 2017). Microalgae species are considered to have optimal pH for the generation of biomass and lipids, and is considered strain specific (Table 7) (Khalil et al., 2010; Moheimani, 2013).

Varying pH range could be created by fluctuations that occur night and day via respiration and photosynthesis, especially in sunlight-illuminated PBRs without pH control. The use of CO_2 and photosynthesis increases the pH level during the day while respiration that occurs at night overturns the process by lowering the pH levels (Bartley et al., 2014). The gradual increase of the pH of microalgae cultures through

Table 7 Optimal pH for the growth of different microalgae species

Microalgae	Optimal pH	Reference
Euglena sp.	Showed highest growth rate and biomass production at pH 3.5	Nurafifah et al. (2023)
C. Sorokiniana	At a pH range of 6.5–8, highest biomass productivity was recorded	Qiu et al. (2017)
Nannochloropsis salina	Maximum growth rate was observed at pH 8 and 9	Bartley et al. (2014)
Auxenochlorella pyrenoidosa	The optimal pH for the production of biomass and lipid was observed at 7	D'Mello and Chemburkar (2018)
Chlorella sorokiniana	At a pH of 10, sufficient growth was observed both indoors and outdoors without additional CO_2	Vadlamani et al. (2017)
Chlorella sp.	Highest specific growth rate, biomass production, and productivity were attained at an optimal pH of 8	Nouri et al. (2021)
Vischeria magna	The cells grew at a pH range of 3.5–11. There was maximal production of zoospores at pH 3.5 and increased volume of cell in the acidic and alkaline pH range	Gaysina (2024)
Pseudococcomyxa simplex	The cells grew at a pH range of 4–11.5, with the algae morphology nearly same as that of the control variant	

the day is due to inorganic carbon uptake by the microalgae. This high pH restricts the CO_2 available thereby inhibiting the cell growth (Qiu et al., 2017). An increase in pH increases the concentration of carbonate and decreases bicarbonate and molecular CO_2. On the other hand, at a neutral pH, CO_2 consumed through photosynthesis is replaced by bicarbonate, which is the major carbon source at neutral pH (Elisabeth et al., 2021).

The pH can be controlled using three methods which include, acid/base addition, buffer addition, and CO_2 injection. The last two are more often used for the cultivation of algae. The use of buffer is a drawback due to its effects on the metabolism of microalgae, which is still not fully understood and is considered unrealistic for large-scale cultivation due to its expensive nature (Moheimani, 2013; Bartley et al., 2014). A research conducted by Qiu et al. (2017) showed pH 6 to be the most favorable for the growth of *C. sorokiniana* DOE1412 and the accumulation of lipids when manipulating the pH using CO_2 injection. At large scale, pH 8 was less expensive in terms of demand for CO_2 despite the lower growth rate.

The slow alterations in pH can be monitored to determine the optimum cultivation and phase of harvest, identify bioavailable nutrients based on their chemical dissociation dynamics, and establish adverse conditions to stop the growth of fecal coliform bacteria (Beltrán-Rocha et al., 2021; Beltrán-Rocha et al., 2024). Cultivation of algae at a high pH can also lead to the control of undesired microbial contaminants, since contamination is still a major challenge in algae cultivation (Bartley et al., 2014; Das et al., 2011). Some of the limitations of algae production in large scale can be surmounted by using extreme alkaliphilic microalgae (Canon-Rubio et al., 2016; Vadlamani et al., 2017).

4.2.3 Effects of Salinity on Microalgae Growth and Productivity

Very high salinity affects microbes adversely, especially microalgae, by inhibiting growth and increasing protein decomposition through the production of pigments and oils, thereby altering the accumulation of certain bioactive substrates (Almutairi et al., 2021). Salinity directly affects the microalgal biomass productivity and it is considered a growth-limiting factor although its effect can vary from one species and strain to another (Ishika et al., 2017) (Table 8). Optimal salinity of 2.12 g/L was reported for a strain of *Chlorella vulgaris* (Daneshvar et al., 2018) while another strain had enhanced growth at 32 g/L NaCl after long adaptation (Almutairi et al., 2021).

A good number of freshwater algal strains exhibit limited growth and survival in increased salt environments and are not capable of surviving such salinity threshold. However, some freshwater microalgae have been shown to grow well at marginal saline conditions (Almutairi et al., 2021; Shetty et al., 2019). Some other halophiles have been reported to adapt and grow well in very high salinity conditions different

Table 8 Optimal salinity for the growth of different microalgae species

Microalgae species	Optimal salinity	Reference
Amphidinium carterae	Highest performance was observed at a salinity of 20 ppt	Hotos and Avramidou (2021)
Asteromonas gracilis	Best productivity was at a salinity of 100 ppt, although it is capable of growing pretty well at 40 and 60 ppt	
Tetraselmis chuii	The optimum salinity was 40 ppt with the best culture medium in respect to elevated cell density, carbohydrate, and protein content	Haris et al. (2022)
Isochrysis galbana	Salinity of 24 ppt was the best as regards the carbohydrate content and high cell density	
Chlorella vulgaris	Culture medium with a salinity of 10 ppt was the best for elevated density of the cell and lipid content	
Scenedesmus sp.	Growth and tolerance within a class of salt concentrations from 5 to 20 g/L. Highest pigment production at 10 g/L probably due to strain adaptation	Elloumi et al. (2020)
Mixed culture with more of *C. vulgaris* (27%) and *Scenedesmus armatus* (39%)	Highest and optimal salinity for lipid production was at 50 mM while the highest cell growth was observed at 12 mM with a decline as salinity increased	Ermis and Altinbas (2020)
C. vulgaris	The optimal salinity was at a low NaCl concentration of 2.125 g/L and further increase led to a decrease in biomass	Daneshvar et al. (2018)

from the source of isolation. For example, Abomohra et al. (2020a) observed that a halophilic *Dunaliella salina* KSA-HS022 isolated from a lagoon of hyper salinity with an average salinity of 45.3% grew at an increased salinity of up to 250%.

Based on tolerance to salt, microalgae can be either halophilic or halotolerant. All these algae usually produce metabolites for defense against salt damage and equilibrating the environmental osmotic pressure (Abomohra et al., 2020b; Ahamefule et al., 2021). High salt concentration in the culture medium causes stress on microalgae through osmotic and ionic stress. Water shortage as a result of high salt concentration can lead to high osmotic stress which then negatively affects the uptake of other mineral nutrients causing ionic imbalance (Almutairi et al., 2021; Ashraf & Harris, 2004). Various valuable secondary metabolites with several health, food, and industrial applications can be produced by algae when exposed to stress conditions like salinity. These metabolites include carotenoids, vitamins, polyunsaturated fatty acids, anticancer agents, etc. (Elloumi et al., 2020; Skjånes et al., 2013). Chu et al. (2011) demonstrated that increasing salt concentration up to 30 g/L resulted in optimum carotenoid accumulation by *Scenedesmus* sp.

Microalgae have developed many adaptation strategies under salt stress environments through responses such as alterations in physiological, morphological, and biochemical processes (Hiremath & Mathad, 2010). Accumulation of lipids is usually aided in the process of resisting osmotic stress that occurs as a result of saline stress (Zhu et al., 2016). For example, Ji et al. (2018) recorded a very high lipid content of 32.26% for *Scenedesmus obliquus*, which was 2.52-fold higher at a salt concentration of 0.20 M than that produced without the addition NaCl.

Salt stress also involves limiting competing or invasive microbes in microalgae cultivation which is highly necessary especially when the culture medium is unsterile wastewater (Ermis & Altinbas, 2020).

4.2.4 Effects of Aeration Gas on Microalgae Growth and Productivity

At high concentrations of CO_2 and high irradiance, increased biomass densities encourage the build up of oxygen particularly in closed thin PBRs which could be a challenge for scale-up (da Silva & Reis, 2015). Microalgae utilize CO_2 and give off oxygen throughout photoautotrophic growth. The accumulation of oxygen in high concentrations impacts negatively on biomass productivity by inhibiting the microalgal growth (Kazbar et al., 2019). Raso et al. (2012) reported a reduction in the specific growth rate (56% loss) of *Nannochloropsis* sp. with a concentration of oxygen that ranged from 75% to 250% of air saturation.

Therefore, an efficient degassing/air stripping system is highly required in closed PBRs to evacuate accumulated dissolved oxygen from the microalgae cultivation (Kazbar et al., 2019; Sforza et al., 2020).

Microalgae optimal concentration of CO_2 and tolerance level are usually strain dependent, although a majority of microalgae can grow efficiently at 2% CO_2 (Morales et al., 2018; Solovchenko & Khozin-Goldberg, 2013). One of the core challenges in increasing the rate of CO_2 fixation is the buildup of oxygen in the

microalgae cultivation systems (Morales et al., 2018). Inhibition of microalgae growth at high concentration of dissolved oxygen occurs as a result of three major processes: photoinhibition, Mehler reaction also known as water-water cycle, and photorespiration (Kliphuis et al., 2011). Generation of reactive oxygen species capable of damaging cellular components due to exposure of microalgae to high light intensities for a long duration results in the occurrence of photoinhibition (Torzillo & Vonshak, 2013). The excess energy generated is dissipated through Mehler reaction, which shows that high concentration of dissolved oxygen can take part in this reaction (Kazbar et al., 2019).

Photorespiration involves the utilization of oxygen and CO_2 generation in the presence of light under unequal ratio of CO_2 and oxygen as a result of oxygenase reaction catalyzed by ribulose-1,5-bisphosphate carboxylase-oxygenase (rubisco) (Sforza et al., 2020; Moroney et al., 2013; Bauwe et al., 2012). The reactive oxygen species exerts oxidative stress because of the elevated concentration of oxygen in competition with CO_2 for rubisco required for fixation of CO_2 thereby increasing photorespiration (Morales et al., 2018). Sousa et al. (2013) reported a loss in biomass for *Neochloris oleoabundans*, under sub-saturating light conditions, as a result of photorespiration activity. This observation was based on the fact that below saturating light, photoinhibition is ruled out and photorespiration is the sole reason for photosynthesis rate reduction (Kliphuis et al., 2011).

In another research, the loss of biomass productivity of *N. oleoanbudans* and *Chlamydomonas reinhardtii* due to the effect of dissolved oxygen concentration at high light intensities was attributed to a combination of photoinhibition and photorespiration (Kliphuis et al., 2011; Costache et al., 2013). Although the two mechanisms are different, the injury that photoinhibition causes is considered irreparable while the photorespiration may possibly be deactivated leading to a decrease in the oxygen:carbon dioxide ratio (Sforza et al., 2020).

The influence of O_2-dependent photorespiration on microalgae photosynthetic growth can be reduced through the maintenance of high dissolved concentration of carbon in the culture system. Proper maintenance, usually by CO_2 injection, is required for sufficient dissolved carbon concentration in the culture medium (Kazbar et al., 2019). Reduction of the poisoning effect of CO_2 and increase of the productivity of CO_2 fixation are achieved by applying flue gas that contains high concentrations of CO_2 which improves the capacity of CO_2-tolerant microalgae (Wei et al., 2019).

Since high CO_2 concentrations can be inhibitory to the growth of microalgae and biofixation of CO_2, the utilization of gas mixtures with elevated CO_2 content can elevate the microalgae's specific growth rate (Chunzhuk et al., 2023; Chiu et al., 2008; Aslam et al., 2017). A research by Aslam et al. (2017), carried out with flue gases from a coal-fired power plant, was used in the cultivation of very productive strains of *Scenedesmus dimorphus* NT8c, *Chlorella vulgaris*, and *Limnothrix planctonica.* This demonstrated that little quantities of CO_2 added to the gas-air mixture gave a biomass productivity that is triple that cultivated in just air at limited CO_2 content.

The optimal cultivation conditions and the highest CO_2 concentrations in gas mixture that support high microalgae productivity should be known for efficient CO_2 biofixation (Chunzhuk et al., 2023). Increasing the efficiency of aeration of gas and mixing are fundamental for microalgae large-scale outdoor cultivation system and can be achieved by using a mixture of flue gas and CO_2 for aeration. This will provide sufficient CO_2 for improved growth of microalgae at a lower cost (Kuo et al., 2021).

4.3 Biological Factors That Affect Large-Scale Microalgae Production

Some of the biological factors that affect large-scale microalgae production include contaminations by other microorganisms, cell morphology and size, antenna sizes, and standing biomass concentration.

4.3.1 Effects of Contaminations by Other Microorganisms on Microalgae Growth and Productivity

Microbial contaminations could be a big challenge in microalgae cultivation, especially in large-scale productions in open PBRs. Microalgae cultures could be contaminated by bacteria, fungi, viruses, predatory micro-zooplanktons, and competitive microalgae (Hoeger et al., 2022; Montemezzani et al., 2015; Wang et al., 2016b). Some of these microorganisms could compete directly or indirectly with the microalgae for nutrients and/or light. They could also inactivate, attack, and/or lyse microalgal cells using different mechanisms including enzymatic actions, direct grazing, parasitism, etc. (Hoeger et al., 2022; Montemezzani et al., 2015; Wang et al., 2016b; Lena et al., 2021). These eventually lead to high mortality, and reduced growth rate and metabolite production by the microalgal strains.

Notwithstanding the negative effects of microbial contaminations on microalgae productivities, recent studies have demonstrated that some "microbial contaminants" or microbiome of algae do have very important mutualistic/synergistic functions (Hoeger et al., 2022; Lena et al., 2021; Yao, 2018). For example, some of the microorganisms produced growth-stimulating substances that enhanced the growth of microalgae. Some others helped in the breakdown and proper up take of complex substrates by microalgae while other microbes, such as yeasts, are used as sources of carbon dioxide for microalgae in co-cultures. Some researchers have shown that the growth and productivities of microalgae are better enhanced when they are co-cultured with some bacteria and fungi (Hoeger et al., 2022; Zeng et al., 2018; Kumsiri et al., 2018).

4.3.2 Effects of Cell Morphology and Size on Microalgae Growth and Productivity

Light energy is absorbed by the microalgal pigments and converted to high energy state when excited. These pigments release energy which leads to the occurrence of photochemical transformation when they return to their original state (Senge et al., 2014). Alterations in the morphology and size of the microalgal cells have been observed under several light wavelengths. For example, microalgae such as *Chlorella* sp. (Kommareddy et al., 2013), *Dunaliella salina* (Nwoba et al., 2021), and *C. reinhardtii* (Oldenhof et al., 2004) have displayed larger cell sizes in blue light when compared to the red light. Furthermore, the arrangement pattern of microalgal cells could also affect the light absorption. Hoeniges et al. (2020) reported that the average light spectral mass absorption of *B. braunii* cells was reduced as a result of the arrangement of the cell colonies which could be due to joint shading amid the cells.

Activities such as aeration, mixing, circulation, and pumping which help facilitate heat and mass transfer, as well as the distribution of light, could generate hydrodynamic stress experienced during microalgae cultivation (Wang & Lan, 2018). The morphology and size of the microbe contribute significantly to the sensitivity of the cells to hydrodynamic stress. The size of the cell contributes to the damage caused by hydrodynamic stress. Cells of similar sizes as the microeddies have a higher possibility of damage from hydrodynamic stress since the microeddies are capable of transmitting turbulent forces to the cells (Wang & Lan, 2018; Hadiyanto et al., 2013; Camacho et al., 2001). The microeddy length generated by the paddle wheel should be larger than the size of the microalgal cell to avoid damage (Berdalet et al., 2007; Camacho et al., 2000).

Cell morphology has been observed to affect susceptibility of microalgae to hydrodynamic stress. Filamentous microalgae show more susceptibility to hydrodynamic stress than unicellular organisms due to increased stress causing the breakage of filament leading to a decrease in viability (Wang & Lan, 2018). Microalgae that grow in short chains are also considered less tolerant as a result of breakage of the chains by stress (Michels & Van Der, 2015). However, *S. obliquus* and *S. dimorphus* which grow in colonies and break apart into individual cells during mixing permit increased access to nutrients and light thereby leading to increased growth (Wang & Lan, 2018).

The damage to microalgal cell walls further exposes them to the environment, which could hasten the death and lysis of the microalgae. These therefore lead to increased mortality and decreased productivity. To overcome this, the effect of turbulence on the damage to microalgal cells must be investigated using the cell wall mechanical properties such as wall deformation and von Misses stress (Ali, 2018).

4.3.3 Effect of Antenna Sizes on Microalgae Growth and Productivity

The major challenge in the cultivation of microalgae which limits the production of biomass is low thermodynamic photosynthesis efficiency that ranges from 1% to 4% as well as maintaining a high efficiency at high light intensity to which algae are usually exposed to (Wu et al., 2020; De Mooij et al., 2014). Light intensity is a fundamental abiotic factor governing the biomass productivity of microalgae. Microalgae can utilize light energy at very high efficiency but with elevated light intensities, the efficiency goes down as a result of oversaturation leading to wasteful dispersion of excess light energy in the form of heat and fluorescence via generation of damaging reactive oxygen species (Wu et al., 2020; De Mooij et al., 2014; Kumar et al., 2021).

The surface layers of microalgae culture readily reach saturation as a result of their enormous optical density hence leading to photoinhibition while there is limited light in the inner layers. This non-uniform distribution of light leads to low productivity (Cazzaniga et al., 2014). Because of the high pigment optical density, self-shading causes a quick light attenuation close to or lower than the point of compensation between respiration and photosynthesis in majority of the culture (Formighieri et al., 2012). As a result of the over-saturating intensity of sunlight, there could be challenge cultivating microalgae under outdoor conditions at elevated photosynthetic efficiency. Therefore, to accomplish economically favorable production of biomass on a large scale, sufficient photosynthetic efficiencies are compulsory (Kumar et al., 2021; Zemke et al., 2013).

The antenna system acts as light harvesters, regulates the use of light energy, and protects against reactive oxygen species. Light-harvesting antennas located in photosynthetic organisms are photosynthetic pigment-binding proteins capable of capturing light energy to aid oxygenic photosynthesis (Kumar et al., 2021; Green & Durnford., 1996). Chlorophyll b accounts for almost half of the chlorophyll in the peripheral light-harvesting antennas but not present in the photosynthetic centers. Therefore, the decrease or lack of chlorophyll b has the ability to decrease the stability of the light-harvesting antenna proteins leading to degradation and deductions in the evident optical cross section of the light-harvesting antennas (Hoober et al., 2007; Friedland et al., 2019).

Reducing the size of light-harvesting antenna is observed as an efficient means of enhancing the efficiency of photosynthesis and general productivity of biomass in the microalgae cultures since they absorb lower light per cell which makes the conversion of solar energy to biochemical energy happen at high efficiency (Kumar et al., 2021). A study by Perrine et al. (2012) observed smaller optimized light-harvesting antenna sizes for *Chlamydomonas* with a growth rate 40% greater than the wild-type algae grown in stimulated pond environments.

Microalgal strains with reduced/truncated light-harvesting chlorophyll antennas have shown to significantly reduce over absorption and subsequent lavish dispersion of excess light, decrease photodamage and photoinhibition of photosynthesis, and lessen mutual cell shading that decreases productivity in microalgae cells situated at

the bottom of the culture water column (Mitra & Melis, 2008). A study by Cazzaniga et al. (2014) showed an important improvement in the light to biomass conversion efficiency of *C. sorokiniana* under mass culture conditions with low chlorophyll mutant.

In another research, De Mooij et al. (2014) observed a lower light efficiency with no increase in productivity using mass culture conditions for *C. reinhardtii* antenna size mutant cultures in comparison to the wild type, which could be due to reduced mutant fitness, reduced size antenna cultures caused by elevated light intensity making the reduced antenna probably susceptible to photodamage. This could be that smaller light-harvesting antennas are more susceptible to photodamage compared to the large antenna, which contradicts the fact that deductions in light-harvesting antenna size are normally required to reduce damage from high light stress due to a decline in the efficiency of light capture (Wu et al., 2020). Also, some pigment-lacking mutants had no improvements in productivities of the biomass in both laboratory cultures and outdoor microalgae ponds despite exhibiting a truncated antenna size and elevated light saturation (Huesemann et al., 2009). The manner of the antenna reduction might be significant in deciding the extent to which the photosynthetic efficiency is improved (Ort et al., 2010).

5 Conclusions

About 2 kg dry weight of oleaginous algae (of 40–50% lipid content) is needed to produce 1 L of biodiesel. In Nigeria alone, there is a daily consumption of about 14 million liters of diesel. If microalgae must be used as a sustainable and cost-effective substitute to fossil diesel and for the production of other essential metabolites, large area of land is required and the productivity must be increased significantly. This requires development of efficient photobioreactors, selection of best operation modes and culture systems, as well as process optimization in terms of physical, chemical, and biological factors that influence growth and productivities. Therefore, more inter-disciplinary research works are needed in these aspects of achieving efficient large-scale microalgae cultivation for various applications.

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Mathematical Modeling and Simulation Applied to Microalgae



José C. M. Pires

Abstract Microalgal-based processes are increasingly valued for their applications in environmental protection, bioenergy production, and food manufacturing. The cultivation stage is crucial, as it heavily influences the final product's cost, thus impacting the commercial feasibility of microalgae in various industries. A deep understanding of how microalgae thrive under different environmental conditions is vital for optimizing biomass yield. This chapter offers an extensive review of key models that relate microalgal growth with chemical and physical environmental factors, providing critical insights for improving cultivation efficiency and productivity.

The reviewed models are categorically grouped based on the specific environmental factor under analysis and their underlying conceptual frameworks. The inclusion of multiple-factor modeling adds depth to our understanding, offering a comprehensive perspective on the intricate relationships governing microalgal growth. These models can be effectively integrated with hydrodynamics simulations through Computational Fluid Dynamics (CFD).

CFD has emerged as a compelling tool for assessing the variability of culture variables in diverse photobioreactor (PBR) geometries. This approach eliminates the need for expensive and time-consuming experimental work and contributes to a more efficient and streamlined research process. The coupling of hydrodynamics simulation with diverse models linking microalgal growth to environmental factors establishes a holistic framework for the complete and robust performance simulation of various PBR designs.

By synergistically employing CFD and diverse growth models, researchers can gain valuable insights into the complex dynamics governing microalgal-based processes. This integrated approach enhances our understanding of microalgal

J. C. M. Pires (✉)
LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, Porto, Portugal

ALiCE – Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr Roberto Frias, Porto, Portugal
e-mail: jcpires@fe.up.pt

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growth in varying environmental conditions and paves the way for more informed and strategic decision-making in the design and optimization of PBRs. As microalgae continue to emerge as a pivotal player in sustainable solutions, this chapter contributes to advancing the knowledge base and methodologies crucial for harnessing their full potential across a spectrum of industrial applications.

Keywords Computational fluid dynamics · Environmental factors · Growth modeling · Microalgal-based processes · Photobioreactor optimization

1 Introduction

Microalgae play essential roles in global ecosystems. Their rapid growth, often doubling biomass concentrations within hours, highlights their significant economic potential. Furthermore, cultivating these organisms provides environmental advantages like carbon dioxide sequestration, biomass generation, and nutrient absorption from diverse wastewater sources (Gonçalves et al., 2016b; Su, 2021; Sutherland & Ralph, 2019). Due to their diverse chemical compositions, microalgae find applications across industries including human and animal nutrition, cosmetics, pharmaceuticals, and biofuels (Borowitzka, 2013; Levasseur et al., 2020; Novoveská et al., 2019).

Achieving industrial-scale application of microalgal technology requires a comprehensive understanding of several fundamental phenomena, with the optimization of photobioreactor (PBR) design being paramount. A successful PBR design fosters optimal conditions for microalgal growth by leveraging knowledge of biological reaction stoichiometry, mass and energy balances, and the kinetics of cell growth and nutrient consumption. Additionally, the optimization process is complicated by numerous biotic and abiotic factors, including light supply, temperature, nutrient concentrations, pH, and the presence of toxins or predators. These factors must be managed to enhance productivity and ensure the viability of large-scale microalgal cultivation (Mohsenpour et al., 2021; Nwoba et al., 2019; Peter et al., 2022). While conventional experimental methods offer insights, they consume considerable time and resources.

Mathematical modeling and process simulation emerge as indispensable tools, facilitating the selection of optimal cultivation conditions and bioreactor designs. The exploration of bioreactor modeling and simulation enables to (Salgado & Pires, 2023): (i) elucidate the dynamic behavior of key production variables; (ii) reveal underlying mechanisms; (iii) predict system parameters and their sensitivities; (iv) establish process variable limits; (v) design experiments; (vi) assess costs; and (vii) facilitate process scale-up, optimization, and control. Computational Fluid Dynamics (CFD) is an important simulation tool to describe hydrodynamics, heat, and mass transfer phenomena and to optimize PBR designs (Bitog et al., 2014; Ranganathan et al., 2022). CFD elucidates complex fluid flow phenomena within PBRs, aiding in the identification of optimal configurations and operational parameters to enhance microalgal growth and productivity.

Through a comprehensive overview of mathematical models pertaining to microalgal growth kinetics and their responses to chemical and physical environmental factors, alongside recent research findings on PBR design optimization using these models and CFD simulations, this chapter aims to offer valuable insights into advancing microalgal-based processes. By synthesizing theoretical frameworks with practical applications, this chapter seeks to contribute to the ongoing efforts aimed at joining the full potential of microalgae in sustainable biotechnological processes.

2 Environmental Factors Impacting Microalgae Growth

Autotrophic microalgae cultivation is intricately shaped by a spectrum of environmental variables, encompassing light availability and distribution, temperature fluctuations, pH levels, nutrient quality and quantity, and dissolved oxygen and CO_2 concentrations. The presence of harmful substances, notably heavy metals, also plays a crucial role. These parameters significantly impact the growth rate and overall health of microalgae, necessitating careful monitoring and optimization to achieve optimal biomass production. The growth rate of microalgae can be described using first-order reaction kinetics, where the biomass mass concentration (X, in $g{\cdot}L^{-1}$) changes over time (t, d) as follows:

$$\frac{dX}{dt} = \mu X \tag{1}$$

Here, μ represents the cell-specific growth rate (in d^{-1}), which can be further modeled by incorporating various environmental factors. According to Jayaraman and Rhinehart (2015):

$$\mu = \mu_{max} \prod_{i=a}^{b} \phi_i \tag{2}$$

In this equation, μ_{max} is the maximum specific growth rate, and ϕ_i are mathematical factors representing the effects of each environmental parameter (such as nutrients light) from a to b. This model suggests that each factor acts independently to influence the growth rate, characterizing it as an "uncoupled" model.

Alternatively, Liebig's law of the minimum offers a different perspective, suggesting that the growth rate is limited by the scarcest resource, the parameter exerting the most detrimental effect on growth (Tang & Riley, 2021). The specific growth rate can be calculated through:

$$\mu = \mu_{max} \min\left[\phi_i, \phi_j, \ldots\right] \tag{3}$$

This approach can sometimes provide a more accurate depiction of reality, especially in environments where one particular factor significantly limits growth.

Subsequent sections will comprehensively examine crucial culture variables, accompanied by the principal equations describing the associated phenomena. By considering these factors and using appropriate models, researchers can better understand and optimize the conditions for microalgal growth, enhancing productivity and efficiency in various applications.

2.1 Light Supply

Sunlight serves as Earth's primary energy source, and autotrophic microalgae, similar to terrestrial plants, utilize light energy through photosynthesis. Therefore, light stands as a critical factor in microalgae cultivation (Darvehei et al., 2018a; Esteves et al., 2024b). While solar radiation encompasses a wide wavelength spectrum at ground level, microalgae predominantly utilize visible light (400–700 nm). This specific band, termed Photosynthetically Active Radiation (PAR), represents approximately 43% of total solar radiation (Ooms et al., 2016).

The intensity and quality of solar radiation vary depending on geographical location, weather conditions, time of day, and season. In tropical regions, the intensity of sunlight is generally higher and more consistent throughout the year compared to temperate regions. Cloud cover, atmospheric pollutants, and seasonal changes can significantly affect sunlight availability, influencing photosynthesis efficiency in microalgae cultures.

Considerable focus has been placed on characterizing the incident radiation intensity (I_0). Nevertheless, the depth of the culture medium directly impacts light penetration. Conventionally, this light attenuation process is elucidated by the Beer–Lambert law, which articulates that light intensity diminishes exponentially with the distance traveled (s, in cm) and the concentration of light-absorbing entities (c, such as biomass in $g{\cdot}L^{-1}$), influenced by a specific absorption coefficient (β, in $L{\cdot}g^{-1}{\cdot}cm^{-1}$).

$$I(s) = I_0 e^{-\beta cs} \tag{4}$$

However, this equation typically proves to be inadequate for microalgal cultures due to its omission of light scattering, a significant factor contributing to the attenuation of light intensity. In microalgal environments, light scattering arises from the presence of cells and, if applicable, air bubbles introduced through aeration. Consequently, more sophisticated models are needed to accurately describe light distribution within PBRs.

Improved models for light attenuation in microalgal cultures often incorporate empirical adjustments to account for both absorption and scattering effects due to the presence of microalgal cells, depending on their concentration. Esteves et al. (2024a)

conducted a comparative analysis of various kinetic models for microalgal growth, emphasizing the combined influence of light intensity and biomass concentration. Their study utilized experimental data and literature findings specific to *Chlorella vulgaris*, evaluating nine kinetic models. The Bannister and Grima models exhibited the most accurate fitting to experimental data. Certain models identified cultivation conditions that could potentially induce photoinhibition. Validation against independent datasets confirmed that the Bannister and Grima models offered superior predictive capabilities. These models represent valuable tools for forecasting microalgal growth and optimizing operational parameters, thereby minimizing the necessity for time-intensive and expensive experimentation.

Furthermore, artificial lighting systems, such as LEDs, can be used to supplement natural sunlight, particularly in indoor or controlled environment cultivation. These systems allow for precise control over light intensity and spectral quality, which can be tailored to the specific needs of different microalgal species to maximize growth and productivity (Borella et al., 2021, 2022, 2023).

In summary, while sunlight is the primary energy source for microalgae, understanding and managing the factors affecting light availability and distribution are essential for optimizing microalgal cultivation systems. This includes considering geographical and climatic conditions, culture medium depth, and the use of supplementary artificial lighting to ensure optimal photosynthetic performance.

2.2 Temperature

Temperature plays a pivotal role in microalgal cultures by influencing metabolic rates, enzymatic activities, and the structural integrity of essential components (Béchet et al., 2013, 2017; Darvehei et al., 2018b; Gonçalves et al., 2016a; Ras et al., 2013). Microalgae exhibit adaptation across a broad spectrum of temperatures, categorizing their tolerance into psychrophilic, mesophilic, and thermophilic groups (Chen & Berns, 1980). Mesophilic microalgae, thriving between 20 and 25 °C, are particularly significant due to their compatibility with ambient temperatures. Below 16 °C, growth slows, while temperatures above 35 °C can be lethal for some species.

Temperature correction models are used to adjust growth rates. James and Boriah (2010) proposed a simple model:

$$\phi_T = e^{-K\left(T - T_{0pt}\right)^2} \tag{5}$$

where T is the temperature, T_{opt} is the optimal value for microalgal growth, and K is a model parameter. The growth rate decreases with deviations from T_{opt}.

More complex models by Yan and Hunt (1999) and Bernard and Remond (2012) use cardinal temperatures with biological significance:

$$\mu_{max} = \mu_{opt} \frac{(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})\left[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{max} - 2T)\right]} \quad (6)$$

$$\mu_{max} = \mu_{opt}\left(\frac{T_{max} - T}{T_{max} - T_{opt}}\right)\left(\frac{T}{T_{max}}\right)^{\frac{T_{opt}}{T_{max} - T_{opt}}} \quad (7)$$

where T_{min} and T_{max} are the minimum and maximum growth temperatures. These models provide valuable insights for optimizing microalgal cultivation by adjusting growth parameters based on temperature variations.

2.3 Medium pH

The pH of the medium exerts a profound influence on microalgal cultures, affecting nutrient availability and uptake dynamics. pH governs the chemical equilibrium among carbon species (CO_2, HCO_3^-, and CO_3^{2-}), the precipitation of phosphates, the volatilization of ammonia, and the solubility of trace elements, all of which impact nutrient accessibility (Beltrán-Rocha et al., 2024; Mohsenpour et al., 2021). Furthermore, pH sensitivity varies among microalgal species, influencing community composition in mixed cultures. Optimal pH typically ranges between 7 and 9, with an ideal window typically identified between 8.2 and 8.7 (Alavianghavanini et al., 2024; Pahazri et al., 2016).

In high-density cultures, pH tends to increase, sometimes reaching up to 9, which can be corrected by aerating with CO_2. The effect of pH on growth can be modeled using the following equation (Bekirogullari et al., 2020):

$$\phi_{pH} = \left(\frac{K_{pH}}{K_{pH} + 10^{|pH_{opt} - pH|} - 1}\right) \quad (8)$$

where K_{pH} is the half-velocity constant and pH_{opt} is the optimal value for microalgal growth.

Controlling pH is essential for maintaining nutrient bioavailability and preventing adverse chemical reactions that can hinder microalgal growth. Effective pH management ensures optimal photosynthetic efficiency and biomass productivity in microalgal cultures.

2.4 Availability of Nutrients

Inorganic carbon and nitrogen compounds play indispensable roles in microalgal growth, acting as potential growth-limiting factors when not sufficiently saturated (Bekirogullari et al., 2020). The Monod and Droop models have conventionally served as frameworks for elucidating nutrient dependencies in microalgae:

$$\phi_s = \frac{S}{K_s + S} \tag{9}$$

$$\phi_s = 1 - \frac{q_{s,0}}{q_s} \tag{10}$$

where S is the extracellular substrate concentration, K_s is the half-velocity constant, q_s is the intracellular nutrient concentration, and $q_{s,0}$ is the minimum nutrient quota for growth. The Droop model, although requiring an additional mass balance for nutrient quota, is advantageous for microalgae.

Different models may be applied depending on the nutrient in question. For instance, Ranganathan et al. (2017) used the Monod model for both nitrogen and inorganic carbon, while Shriwastav et al. (2017) applied the Droop model for nitrogen and phosphorus and the Monod model for inorganic carbon.

Nutrient concentration tracking over time or space necessitates mass balances, incorporating major inlets, outlets, consumption, and generation terms. When a nutrient is closely linked to cell growth, its specific uptake rate (ρ_s) is often proportional to the growth rate (μ):

$$\rho_s = \frac{\mu}{Y_{x/s}} \tag{11}$$

where $Y_{x/s}$ is the cell yield per unit substrate consumed. This relationship helps in optimizing nutrient management for enhancing microalgal productivity.

2.5 Mass Transfer of CO_2 and O_2

Ensuring adequate nutrient supply to the bioreactor is crucial for maintaining nutrient availability, with particular emphasis on CO_2 supply through sparging. However, this process can encounter challenges due to limitations in gas–liquid mass transfer. The rate of volumetric CO_2 mass transfer (N_{CO2}, mol·m^{-3}·s^{-1}) is expressed as:

$$N_{CO_2} = K_L a(C^* - C) \tag{12}$$

Here, K_L represents the overall mass transfer coefficient (typically approximated as the liquid-side coefficient, k_L), a denotes the interfacial area per unit volume ($m^2 \cdot m^{-3}$), and C and C^* are the CO_2 concentrations in the medium and at equilibrium with the gas phase, respectively. Enhancing K_{La} improves mass transfer, achievable by increasing liquid velocity (mixing) or reducing bubble size. Various methods, such as the dynamic gassing-out method, can determine this parameter, and empirical correlations can express its dependence on operating parameters, though these correlations are often specific to a reactor geometry (Rezvani & Rostami, 2023).

While CO_2 needs to be supplied for microalgae growth, O_2, produced via photosynthesis, must be efficiently removed to avoid negative effects on cultivation. Oxygen accumulation can decrease Rubisco activity, reducing the cell-specific growth rate (Weber & Bar-Even, 2019). The stoichiometry of photosynthesis suggests a 1:1 molar ratio between O_2 production and CO_2 consumption:

$$\rho_{O_2} = -\rho_{CO2} \tag{13}$$

This equation is valid on a molar basis, not a mass basis. Oxygen consumption during aerobic respiration complicates the O_2 model, but CO_2 release during this process impacts the overall mass balance (Béchet et al., 2018). Oxygen release follows a similar equation to CO_2, with care taken to account for the opposite direction of mass transfer, particularly during daylight when photosynthesis leads to oxygen accumulation in the medium.

3 Design Considerations in Bioreactors

Microalgal cultures can be carried out in either open ponds or enclosed systems (Costa et al., 2019; Kirnev et al., 2020; Xu et al., 2009). Open ponds are widely adopted in commercial settings due to their lower initial investment and operational expenses. However, they frequently exhibit lower biomass productivity compared to enclosed systems because they are influenced by local weather conditions and vulnerable to contamination by predators.

In contrast, closed systems offer greater control over culture parameters, resulting in higher biomass productivity (Xu et al., 2009). Additionally, they mitigate water loss through evaporation and minimize CO_2 loss to the atmosphere. Closed systems come in various configurations, each with its unique advantages and challenges.

Air-lift reactors, characterized by their cyclic circulation pattern, demonstrate significant potential for industrial processes due to their ability to ensure low and uniform hydrodynamic shear distribution. However, the channeling of the medium in these reactors can limit light penetration, resulting in large dark zones within the PBR.

In contrast, tubular reactors offer versatility in outdoor cultivation settings, providing extensive illuminated surface area due to their arrangement in vertical,

horizontal, or inclined orientations. Vertical tubular reactors enhance CO_2 transfer efficiency by extending contact time between gas and liquid phases, albeit at the cost of increased air pumping expenses. Horizontal configurations maximize light exposure but may require additional mechanisms for efficient mixing and gas exchange.

Flat-plate PBRs offer several advantages over other bioreactors, such as significantly higher cell densities, lower energy consumption, enhanced mass transfer capabilities, absence of dark zones, and superior photosynthetic efficiency. However, effectively removing oxygen produced during photosynthesis remains a challenge. The narrow space between panels could potentially hinder the aeration system, impacting light distribution and exerting shear stress on microalgae, thereby affecting growth rates.

In summary, each PBR design presents unique advantages and challenges, necessitating careful consideration of specific cultivation goals, environmental factors, and operational requirements.

4 Computational Fluid Dynamics in Bioreactor Design

4.1 Software Description

CFD is rooted in principles of fluid dynamics, where fluid behaviors are mathematically described by partial differential equations representing conservation laws for mass, momentum, and energy (Ranganathan et al., 2022). CFD simplifies these complex equations into a set of algebraic equations that are solved numerically, revealing the interactions among velocity, pressure, temperature, and liquid density within a fluid system. Employing mathematical modeling, numerical methods, and specialized software tools (such as solvers and pre- and post-processing utilities), CFD provides a qualitative prediction of fluid dynamics.

CFD models offer substantial advantages, delivering high spatial and temporal resolutions and facilitating the evaluation of various reactor configurations virtually, thereby minimizing the need for extensive experimental work (Gu et al., 2023). One key benefit of CFD is its capability to provide detailed insights that are difficult to obtain experimentally, such as simulating cellular movement within a PBR. However, a notable drawback is the high computational demand, typically limiting simulations to relatively short durations (hundreds of seconds), whereas typical growth cycles in PBRs span days or weeks. Consequently, simulating the entire algal cultivation process from start to finish using CFD alone is unfeasible. To overcome this limitation, fluid dynamics simulations can be integrated with light and growth models to construct comprehensive predictive models of overall process performance. These integrated models are invaluable for optimizing reactor designs and understanding their operational dynamics.

Process engineers and scientists harness CFD models to analyze complex, interconnected systems, reducing reliance on extensive empirical data. These models complement laboratory experiments, aiding in all stages of process development—

Table 1 Recent CFD studies focusing on different PBRs

Photobioreactor	CFD software	Modeled phenomena	Reference
Air-lift	ANSYS FLUENT 14.5	BK: three-state photosynthetic unit growth model; FD: three-phase Eulerian model; LS: radiative transport equation	Gao et al. (2017)
	ANSYS FLUENT 14.5	BK: three-state photosynthetic unit growth model; FD: three-phase Eulerian model; LS – Beer-Lambert law	Gao et al. (2018)
	ANSYS CFX 17.2	FD: Eulerian multiphase approach, SST k-Ω turbulence model	Guler et al. (2020)
Flat-plate	COMSOL 5.2a	FD: Navier–Stokes equations; LS: radiative transfer equation; PT: second law of Newton	Loomba et al. (2018)
	n.p.	BK: a kinetic model for microalgae growth, a function of light intensity; FD: free-surface lattice Boltzmann model; LS: discrete ordinate model; PT: Lagrangian model	Li et al. (2019)
	COMSOL multiphysics 5.3a	BK: logistic model, Aiba and Arrhenius models for the effect of light and temperature on microalgal growth; FD: Euler–Euler method	Ali et al. (2019)
	ANSYS FLUENT 14.0	FD: Eulerian–Eulerian two-phase model, k–ε model; PT: discrete phase model	Wang et al. (2021)
	ANSYS FLUENT 19.2	BK: three-state photosynthetic unit growth model; FD: classical Eulerian–Eulerian approach; LS: variant Beer–Lambert's law	Cho et al. 2021)
Tubular	COMSOL Multiphysics	FD: k-ε model; PT: Newton's law	Gómez-Pérez et al. (2017)
	ANSYS FLUENT	FD: Reynolds stress model; LS: Cornet model; PT: discrete random walk model	Qin and Wu (2019)

BK Biological Kinetics; *FD* Fluid Dynamics; *LS* Light Supply; *n.p.* not provided; *PT* Particle Tracking

from conceptual design to plant operation. In recent years, CFD has found application in modeling various bioreactors (Bitog et al., 2011; Pires et al., 2017; Ranganathan et al., 2022), offering distinct advantages over traditional optimization methods, including cost-effectiveness, reduced workload, and shortened design cycles. Table 1 presents the main CFD studies for PBR modeling.

4.2 Hydrodynamics

Qin et al. (2018) investigated the effects of adding double-inclined ribs to tubular PBRs on the light/dark (L/D) cycle enhancement and energy consumption. This PBR configuration is widely used for microalgal cultivation, and the introduction of

mixers alters the L/D cycle experienced by the microalgae, boosting biomass yield but also increasing energy consumption due to a higher fluid pressure drop. They introduced a new parameter to evaluate L/D cycle enhancement, using it to compare different scenarios modeled with varying numbers of ribs using CFD. The results indicated that the increase in L/D cycle frequency outweighed the rise in energy consumption across a broad range of incident light intensities. The optimal configuration featured two pairs of ribs with a length ratio of 5.

Pozzobon and Perré (2020) utilized CFD to simulate the internal hydrodynamics of a PBR, incorporating marine impeller rotation and the motion of sparged bubbles. A Lagrangian approach tracked the movement of microalgae within the culture vessel. The Beer–Lambert law was employed to calculate illumination across the reactor, allowing the reconstruction of each microalga's light exposure history. These histories were then used in Han's model to predict individual growth rates and photodamage. By aggregating thousands of these trajectories, the overall PBR performance was determined. This methodology was applied to various optical densities (0–4.0) and incident light intensities (0–2000μmol/m^2/s) to create response surfaces for growth rate and photodamage. This analysis proposed an optimal lighting strategy that reduced PBR operation time by 16% compared to traditional methods and helped manage light-induced stress to stimulate secondary metabolite production. However, the model's predictions were not validated with experimental data.

4.3 Mass Transfer

In PBR design, addressing mass transfer issues and optimizing hydraulic retention time (HRT) are essential to enhancing productivity and performance while reducing capital and operating costs. Rezvani and Rostami (2023) compared open and closed cultivation systems for wastewater treatment, highlighting that PBR performance significantly depends on their geometry, hydrodynamic behavior, and mass transfer capacities. The study discussed the features, advantages, and disadvantages of horizontal and vertical PBRs, concluding that vertical designs (e.g., bubble columns) are preferable for large-scale applications due to their high mass transfer rates, short liquid circulation times, and prolonged light/dark cycles.

Critical variables influencing PBR performance encompass medium properties, superficial gas velocity, gas holdup, bubble sizes, shear stress, mixing time, sparger design, and the ratio of inner diameter to height. These factors collectively impact the overall volumetric mass transfer coefficient (K_La), a key kinetic parameter for the transfer of gaseous compounds between the liquid and gas phases. Additionally, HRT has been identified as a vital operational parameter for efficient wastewater treatment in PBRs, underscoring the importance of optimized design and operation to enhance overall efficiency.

Petera et al. (2021) developed a CFD model, considering two important phenomena that influence the dissolved oxygen in the medium: (i) mass transfer in the liquid-gas interface and (ii) photosynthetic production by microalgae. The validation was

performed by experimental data (oxygen concentration) achieved in microalgal cultivation in an 80 m long open thin-layer cascade reactor. The authors also proposed a simplified model to describe the oxygen profile in the reactor, whose results were in agreement with CFD simulations.

4.4 Light Supply

CFD models are increasingly utilized to simulate the complex dynamics within photobioreactors (PBRs), enhancing our understanding of bioprocesses (Gu et al., 2023). Lagrangian particle tracking, in particular, is highly effective as it characterizes the flow field inside the reactor. When integrated with a Monte Carlo light attenuation model and a kinetic growth model, this approach enables comprehensive performance prediction over the entire batch process duration. In this methodology, particles with the same density and size as microalgal cells are incorporated into the model to track their positions within the reactor over time. This allows the construction of the "history" or "lifeline" of individual cells as they move through the reactor. Since the light intensity experienced by a cell is primarily determined by its location, coupling particle tracking with a light attenuation model allows for accurate determination of the light intensity experienced by a cell over time. This information is crucial for calculating key parameters such as the light–dark cycle frequency and the average light intensity.

By including a large number of cells in the model, population-averaged values can be derived, providing a comprehensive understanding of the reactor's overall behavior. The Lagrangian particle tracking approach closely represents actual conditions within the PBR, offering a highly accurate method for quantifying reactor performance. This advanced simulation technique is instrumental in optimizing PBR designs, ensuring more efficient and effective microalgal cultivation. Overall, the integration of Lagrangian particle tracking with light attenuation and kinetic growth models represents a significant advancement in bioprocess simulation, allowing for more precise control and optimization of PBR operations.

Inostroza et al. (2023) optimized the configuration of Thin-Layer PBRs (TLPs) for microalgae production through CFD and experimental data. A TLP of 40 m long, 1.5 m wide, and 1% slope was tested. Light gradients within the cultures were estimated by considering light attenuation and cell movement, finding a low frequency of light exposure. The combination of light regime and photosynthetic models enabled the calculation of the oxygen production rate. Also, the integrated model considering PBR fluid dynamics and photosynthetic rates allowed to optimize the operating conditions. For instance, the recommended biomass concentration was 4 g/L, which maintains adequate light/dark cycle frequency, oxygen production, and dissolved oxygen saturation. Moreover, the oxygen desorption process in the bubble column was considered essential for the success of microalgal cultivation. As expected, the main variable that affects the efficiency of TLPs is the thickness of the culture depth.

4.5 Biological Kinetics

Cho et al. (2023) developed a CFD model integrating hydrodynamics, light transfer, and biokinetics to simulate a pumped-recirculated tubular PBR with a volume of 1 L. The model was validated against a literature case study, showing errors within 10% for fluid velocities and within 24% for bioreaction parameters such as biomass growth, substrate consumption, and biohydrogen production.

Jin et al. (2024) emphasized the necessity of a universal, integrated numerical model for PBR design. Their model, which included hydrodynamics, light transfer, and microalgal growth rate, supported aeration and stirring for mixing using a dynamic mesh. Validation against experimental data from flat plate and stirred PBRs showed a maximum error of 7% in estimating microalgal concentration.

Li et al. (2023) introduced a comprehensive multiphysics model to predict microalgae productivity in concentric-tube internal loop airlift PBRs. This model integrated solar radiation, light attenuation, cell growth kinetics, and hydrodynamics using CFD. It assessed the impact of variables such as plant latitude, shading, PBR spacing, dimensions, downtime, and biomass harvesting thresholds on annual biomass productivity. The authors concluded that: (i) the optimal latitude for maximum productivity is around 15° due to solar radiation intensity and shading effects; (ii) smaller clearances between PBRs improve areal productivity despite increased shading losses; (iii) taller PBRs (2.5 m) yield higher productivity than shorter ones; (iv) larger diameter-to-height ratios benefit individual PBR productivity but reduce volumetric productivity in arrays; (v) optimal biomass concentration threshold for harvesting ranges between 0.4 and 0.7 g/L, varying with downtime duration. However, more precise simulations with real-sun conditions and light distribution inside the PBRs are needed.

5 Research Needs

Developing effective, low-computational-cost approaches for large-scale systems is a significant challenge in PBR modeling. The complex interplay of physicochemical and biological processes in PBRs occurs across multiple scales, each with distinct characteristics, demanding high computational resources. Therefore, coupled modeling approaches that integrate various scales without prohibitive computational costs are essential.

Beyond computational model development, there is considerable potential for enhancing the hydrodynamics, mixing, and mass transfer performance of PBRs. These factors are critical for improving algal growth productivity and overall reactor efficiency. Process intensification techniques, such as optimizing reactor geometry, enhancing gas–liquid mass transfer, and improving light distribution, need further exploration to achieve these improvements.

In summary, future research should focus on:

- Refining optimization strategies for PBR design and operation.
- Developing low-computational-cost, coupled modeling approaches suitable for large-scale PBRs.
- Investigating the effects of shear stress on microalgae growth.
- Enhancing hydrodynamics, mixing, and mass transfer through process intensification techniques.
- Integrating experimental and numerical methods to validate and improve model predictions.

These research directions will contribute to the efficient and sustainable production of microalgae in photobioreactors, supporting their broader application in biotechnology and environmental management.

6 Conclusion

The exploration of microalgal cultivation and its industrial applications highlights the significant economic and environmental potential of microalgae. With their rapid growth rates and diverse biomass applications, microalgae are poised to play a crucial role in various industries, from nutrition and pharmaceuticals to biofuels and wastewater treatment.

To harness this potential, optimizing PBR design is crucial. A deep understanding of biological reaction stoichiometry, mass and energy balances, and growth kinetics is essential. Additionally, the impact of biotic and abiotic factors on microalgal growth necessitates sophisticated optimization strategies. While traditional experimental methods provide valuable insights, they are often resource-intensive and time-consuming.

Mathematical modeling and process simulation offer effective alternatives, enabling the prediction and optimization of cultivation conditions and bioreactor designs. These tools help elucidate the dynamic behavior of production variables, predict system parameters, and facilitate scale-up processes. CFD stands out as a particularly powerful simulation tool, enhancing the understanding of fluid dynamics, heat, and mass transfer within PBRs and aiding in the identification of optimal operational parameters.

The comprehensive overview presented in this chapter, encompassing growth kinetics models, environmental factor responses, and PBR design optimizations through CFD simulations, underscores the synergy between theoretical frameworks and practical applications. This synthesis aims to advance microalgal-based processes, contributing significantly to sustainable biotechnological advancements.

By leveraging the insights from mathematical models and simulations, the full potential of microalgae can be realized, promoting their integration into sustainable industrial processes and fostering their role in addressing global environmental and economic challenges.

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Configurations and Design of Bubble Column Photobioreactors for the Production of Protein and Phycocyanin from Microalgae and Cyanobacteria Biomass



Samanta Machado-Cepeda, Rosa M. Rodríguez-Jasso, and Héctor A. Ruiz

Abstract The biorefinery concept, specifically the third generation, arrives to proposing the microalgae and cyanobacteria biomass as a source of high added-value compounds such as proteins, pigments, carbohydrates, and lipids, between others for different industry applications. Within this framework, phycocyanin is a proteic pigment which is already competitive in the market due to its intense blue and fluorescent properties to be utilized in the color engineering also providing great health benefits. In this sense, the protein is directly linked to phycocyanin due to the presence of a blue-colored prosthetic group called phycobilin and this prosthetic group binds to the protein to form the complete phycocyanin. However, it is important to deepen the design to improve the process and thus increase yields in obtaining microalgal biomass. One of the great alternatives to above is the bubble column photobioreactors; these are broadly referred to as "bubbling" because they use a bubbling system to supply gases to the culture and at the same time promote mixing, and the column refers to the vertical shape of the photobioreactor; finally, it is important to highlight that the yields of the compounds obtained from microalgae species depend on inducing stress and adapting certain cultivation strategies. In this way, the objective of this chapter is to compile the most recent information about configurations parameters such as the air flow, temperature, bubble size, and light intensity to the production in industrial scale in bubble column photobioreactors to obtention of phycocyanin and protein from cyanobacterial and microalgal biomass.

Keywords Biorefinery · Bioeconomy · Bioprocess · Pigment · Light intensity

S. Machado-Cepeda · R. M. Rodríguez-Jasso (✉) · H. A. Ruiz (✉)
Biorefinery Group, Food Research Department, School of Chemistry, Universidad Autónoma de Coahuila, Saltillo, Coahuila, Mexico
e-mail: rrodriguezjasso@uadec.edu.mx; hector_ruiz_leza@uadec.edu.mx

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1 Introduction

Nowadays, microalgae have gained more interest from industry due to their versatility to grow in different conditions, for instance, using organic cultures from agroindustrial subproducts, and can minimize associated environmental impacts because they can have lowest water and arable land footprint by recycling atmospheric carbon dioxide which makes them more attractive than vegetable crops and livestock or other sources of high valued-added compounds (Wu et al., 2021; Wang et al., 2021).

Microalgae (eukaryotes) and cyanobacteria (prokaryotes) are photosynthetic organisms that produce biomass with high-value-added compounds, and the way in which they are grown can be similar. To date, more than 15,000 novel compounds are derived from microalgae chemically characterized and used for the biological generation of raw materials (Park et al., 2022).

The phycocyanin is one of the high value-added compounds, and it is very competitive in the market due to its fluorescent properties and pigmentation attractive in different applications, beginning with the food industry, providing it as an additive with health benefits; however, the process of extraction from inside cyanobacteria biomass with a certain purity raises the cost for its achievement in large quantities; this means that its market price depends on their purity, food grade phycocyanin has a price approximately of US $0.13 per milligram, in contrast with reagent or analytical grade can be up to US $15.00 per milligram, specifically, when is partially purified its cost can be US $27 per mg, but when the purity is of 3.5, the cost can reach US $217 per milligram phycocyanin (Hsieh-Lo et al., 2019; Pez-Jaeschke et al., 2021). In the same sense, the demand for other compounds such as proteins has increased, which makes the cyanobacteria very attractive in economic terms. The global protein ingredients price was $38.5 billion in 2020 and is projected an annual growth rate of 10.5% from 2021 to 2028 (https://www.grandviewresearch.com/industry-analysis/protein-ingredients-market).

Phycocyanin is a protein-bound pigment; therefore, it is important to focus on the production of these compounds, and for this purpose, it is necessary to scale up the production. Although laboratory cultures of cyanobacteria are ubiquitous, they are not used in the industry due to the nonlinear scale-up of hydrodynamics, such as mixing time, shear stress, and mass transfer at large-scale, which are significantly different from those of the lab-scale setup (Ding et al., 2016; Banerjee et al., 2020). Thus, the aim of this chapter is to discuss the concepts of different parameters in microalgal and cyanobacterial cultivation that need to be taken into account if the aim is to produce microalgal biomass on an industrial scale for the extraction of high value-added compounds.

2 Microalgae and Cyanobacteria for Third Biorefinery Generation

To take advantage from a laboratory scale to an industrial scale, the third biorefinery does not only offer alternatives to recover, and to fractionate the microalgal biomass to the extraction intracellular microalgae compounds, this concept must be considered a sequence of unit operations since the reproduction of this microorganism along with minimizing waste production and its integration into the circular bioeconomy. Instead, the microalgal biorefinery should be considered linking biomass cultivation, and downstream steps for industrial applications as Fig. 1 shows (Gifuni et al., 2019).

At the same time, it is possible to take advantage of the microalgae metabolism if they are cultivated under stressful conditions, such as nutrient reduction, high light irradiance, extreme pH, or high salinity, temperature, or metal concentration; some antioxidant molecules are synthesized in more proportion by the microalgae and cyanobacteria to protect themself, such as phycobiliproteins, carotenoids, or astaxanthins (Gifuni et al., 2019).

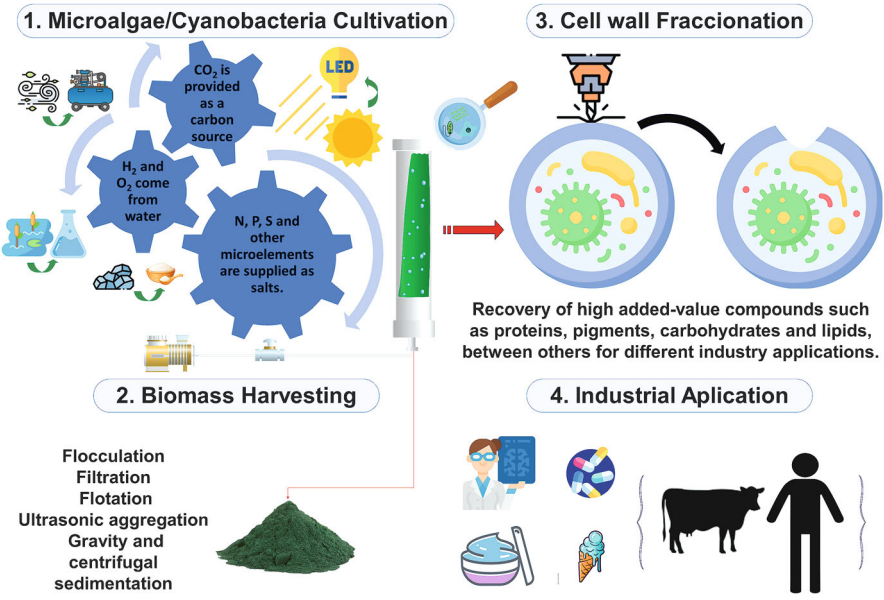


Fig. 1 Concept of third biorefinery generation from microalgae and cyanobacteria

3 Bubble Column Photobioreactors in the Microalgal and Cyanobacterial Biomass Cultivation

There are several types of photobioreactors for microalgae cultivation, each one with specific characteristics and advantages. There are two basic types of open and closed photobioreactors: the "open ponds," which are simple receptacles of the right size and shape, and the "raceways," which are also capable of supplying agitation and mixing, facilitating gas exchange, and even controlling the pH to some extent. They are inexpensive and easy to build; the most common drive device is the "paddle wheel" that manages to keep the crop in suspension with a power expenditure of a few watts per cubic meter. The microalgae suitable for this type of photobioreactors are extremophiles, those capable of surviving in extreme conditions; a typical example is the halophilic microalgae *Dunaliella salina* that grows in saline concentrations of up to 100 g/L, which prevents the proliferation of other species (El-Ardy et al., 2012). Open photobioreactors are large-area systems that are in contact with the atmosphere. These are installations that try to compensate for low productivity at a low cost due to lax or non-existent control of conditions such as pH or temperature. Being open, they are susceptible to invasion by other microorganisms, including microalgae, making them especially suitable for robust, fast-growing species. Closed photobioreactors are systems that allow greater control of growing conditions, typically equipped with systems for agitation, aeration, pH control, heat exchange, addition of nutrients to the medium, and CO_2. Closed photobioreactors can be classified into flat, column, or tubular reactors. Each type of photobioreactor has its own advantages and disadvantages, and its choice depends on the characteristics of the microalgae to be grown, the purpose of the culture, and the environmental conditions of the place where the culture will be carried out (Nosratimovafagh et al., 2022).

Column photobioreactors consist of a cylindrical vertical column, into which gas is introduced from the bottom in a turbulent flow condition. The main problem of these photobioreactors is the scale-up; they are difficult to build in large volume since increasing the diameter increases the proportion of dark volume preventing the passage of light, decreasing productivity. It is also not possible to make them very high since the pressure at the base makes it difficult to bubble and causes hydrodynamic stress; the higher column evaluated has been of 40 m without effect in biomass yields in contrast to increasing the reactor height. It is also proper to affirm that columns should be straight since inclined they lose many of their advantages (Rezvani & Rostami, 2023).

Flat reactors are similar to columns in their principle of agitation and exchange of matter in the same space in which light is captured, but they attempt to solve some of the problems of columns. For example, the optical pitch can be made as thin as you want, it is possible to scale the FBR by increasing its length, without modifying the height of the liquid or the optimal pitch, they can be built slightly inclined, and it is possible to orient them toward the sun, thus maximizing light capture and thus productivity. However, for this reason, microalgae grown in this type of

photobioreactors may be more susceptible to photoinhibition since the light would be directly directed only to one side due to their square geometry, which would also affect the homogenization of temperature compared to column photobioreactors. Finally, tubular photobioreactors are the most sophisticated and specialized, but they are also the most expensive to build. The design distinguishes two parts: loop and degasser. The "loop" is the part in which the capture of solar energy takes place. It is so named because it is a tube arranged in some way that provides a compact shape, requiring elbows and bends. The loop is specifically designed for light harvesting, without having to worry about heat or material exchanges, which allows productivity to be optimized by maximizing photosynthetic efficiency. The degasser is the part in which the exchange of matter takes place, especially the desorption of O_2 and heat exchanges through heat exchangers that can be installed for this purpose (Gifuni et al. 2019).

The majority of the microalgal biomass cultivated in photobioreactors is in a discontinuous mode, but when it is thinking in scale-up, a continuous way of production would improve the yields in comparison with the costs; in this context there is a very important term to take account, hydraulic retention time that determines the accumulation of biomass in a photobioreactor within a residence time. It has been reported that operating a reactor with short hydraulic retention time of 3 days and a moderately high flow rate results in an effective rate of nutrient feeding of the algae in the reactor in economic terms, being enough time to keep the microalgae in their exponential phase after having started it and thus promoting algal growth. In this way, industrial-scale production could be more efficient in a semi-continuous way (Rezvani & Rostami, 2023).

3.1 Evaluation of Different Bubble Column Photobioreactor Configurations

In first place, it is important to identify in a correct way the design of a bubble column photobioreactors watching the differences between each other (see Fig. 2 noticed that the red arrows show the flow direction). All three photobioreactor types have in common that the agitation is performed by un pneumatic upward air flow. The design of the bubble column photobioreactor (see Fig. 2a) is the simplest or, to put it in a better way, it is the starting point for the design of the other two photobioreactors. These photobioreactors consist of a vertical column where the culture medium and microalgae are introduced. Air or carbon dioxide (CO_2) is injected through the bottom to provide agitation and nutrient supply. The bubbles rise along the column, promoting the mixing of the culture medium and the transfer of gases. Its geometry creates a recirculation loop with ascent and descent channels connected to the normal bubble. The advantages are greater mechanical simplicity and a lower shear force gradient, i.e., the changes in fluid velocity are not as marked. Respect to Draft Column (airlift) Photobioreactor (see Fig. 2b) or also known as an

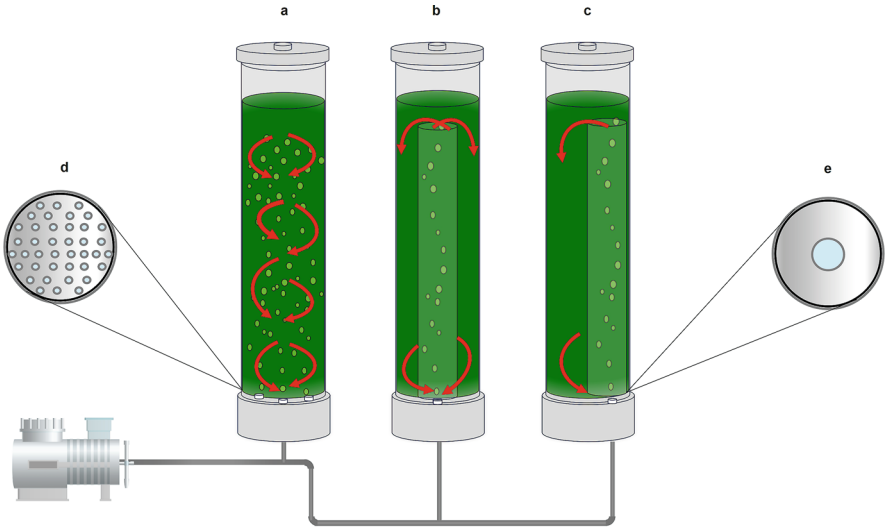


Fig. 2 Differences between column photobioreactors. (**a**) Bubble column photobioreactor, (**b**) draft column (airlfit) photobioreactor, (**c**) split column photobioreactor, (**d**) orifice sparger, (**e**) ring sparger (Adapted and modified from Rezvani and Rostami (2023))

"air-lift" reactor with a suction tube generating a pulling or suction effect to promote the circulation of the liquid, it has the advantage of greater efficiency in the transfer of oxygen, which allows a more uniform distribution of microorganisms and nutrients, improving the efficiency of the crop by avoiding dead zones and promoting greater interaction between water and light; however, it can generate a higher shear force gradient due to the localized application of energy. Finally, the Split Column Photobioreactor (see Fig. 2c) divides the reactor into two sections: one for the growth of microorganisms and the other for the production of oxygen, allowing the conditions in each section to be optimized to maximize productivity, which gives it greater complexity of design and operation by having greater control over factors such as temperature, nutrient concentration, and lighting in each section (Rezvani & Rostami, 2023; Uyar et al., 2024).

All the above designs can have an inlet orifice sparger or ring sparger with equidistant pores (see Fig. 2d, e) (Banerjee et al., 2020). The use of spargers in the production of microalgae biomass is directly related to the mixing time, which is the time required to achieve a certain homogeneity in the culture medium solution with a maximum deviation of 10% from the point of gas injection. Banerjee et al. (2020) compared the effect of the sparger uses in different photobioreactors on biomass production. Table 1 summarizes the yield for different spargers taking into account parameters such as flow rate (mL/min), mixing time (s), shear stress (Pa), kLa (CO_2) (1/h), last one being the transfer mass coefficient of CO_2 as nutrient supply at medium. As it can see, the flow rate is other important factor because it can optimize mixing time and transfer mass coefficient, but at the same time, the coalescence time

Table 1 Effect of sparger in different photobioreactors (PBR) on biomass production (Data from Banerjee et al., 2020)

PBR (sparger)	Flow rate (mL/min)	Mixing time (s)	Shear stress (Pa)	kLa (CO_2) (1/h)	Biomass (g/L)
Airlift (orifice)	600	41 ± 0.03	0.0582 ± 0.03	8.64 ± 0.04	1.130 ± 0.02
Airlift (ring)	600	82 ± 0.02	0.0582 ± 0.01	6.20 ± 0.01	0.865 ± 0.04
Bubble column (orifice)	600	82 ± 0.02	0.0752 ± 0.02	24.84 ± 0.02	1.331 ± 0.01
Bubble column (ring)	600	33 ± 0.03	0.0419 ± 0.02	27.72 ± 0.03	2.012 ± 0.04

decreases as the bubbles grow faster and break more easily (Sanaye Mozaffari Sabet & Golzary, 2022). At the same time, very fast flow rates can generate shear stresses that cause cell damage and inhibit microalgae growth for that reason Banerjee et al. (2020) affirmed that with an aeration of 600 mL/min was obtained the best yields for all the bioreactor configuration, as it cannot be too low to guarantee mixing in the cultivation system but not too high either, flows evaluated ranged from 100 to 800 mL/min. The above experiment used a culture of *N. oleoabundans* UTEX 1185; the working volume of all photobioreactors was of 1.4 L. The bubble column reactor had length and internal diameter of 45.09 cm and 7.50 cm, respectively. The diameter of the ring sparger was 4 cm for the Airlift Reactor because it was positioned inside the draft tube of the airlift reactor, and for the bubble column reactor, the diameter of the ring sparger was of 7.5 cm. Both the spargers had 15 equidistant pores of 0.1 cm diameter. Orifice sparger consisted of 3 cm diameter faucet head. From the above research, it was concluded that ring sparger is better for bubble column photobioreactor and orifice sparger is better for airlift photobioreactor; therefore, the ring sparger must be used in all diameters of the bioreactor because at small does not efficiently distribute the aeration (Banerjee et al., 2020).

In summary, the choice between these types of photobioreactors will depend on the specific objectives of the crop and the desired operating conditions, but in any case, column bioreactors must be made with a height/diameter ratio ranging from 1.5 to 20 to have a good distribution of the biomass and improve its mixing inside the tubular photobioreactor (Pino et al., 2018). However, to optimize the hydrodynamic behavior of the system, it has been recommended to maintain the height/diameter ratio constant at 8.5 (Besagni et al., 2017).

On the other hand, it is important to mention that the microalgae and cyanobacteria have such a high capacity to chemically fix CO_2 in soluble carbonates, such as $NaHCO_3$ and Na_2CO_3 thanks to mass transfer. In addition, gas retention and bubble size as hydrodynamic parameters can influence the light irradiation, which in turn is due to aeration flow to create a homogeneous flow and therefore isothermal conditions within the bubble column, which is discussed in the following sections (Sanaye Mozaffari Sabet & Golzary, 2022).

Ding et al. (2016) stated that as the concentration of carbon dioxide in the inlet stream increases, the diameter and velocity of the bubbles decrease due to increased

Table 2 Response of CO_2 concentration in the inlet flow to bubble size and bubble velocity (Data from Ding et al. 2016)

	3% (V/V)	5% (V/V)	10% (V/V)
Bubble diameter in different inlet CO_2 concentrations (mm)	0.470–0.490	0.445–0.465	0.423–0.440
Bubble velocity in different inlet CO_2 concentrations (m/s)	0.187–0.220	0.167–0.190	0.122–0.148

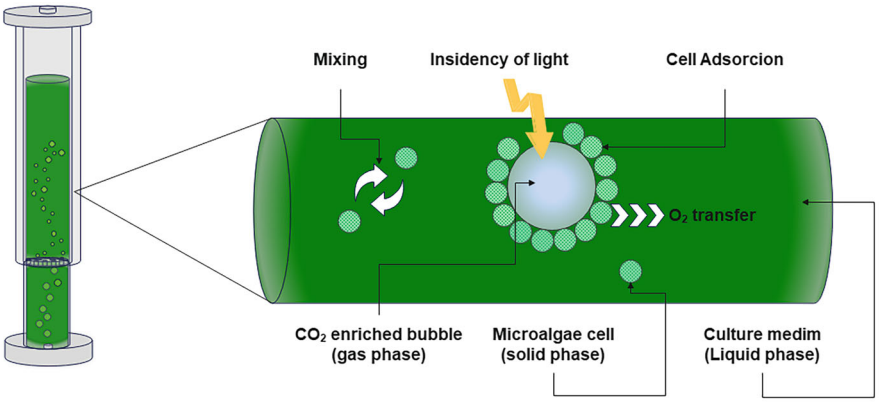


Fig. 3 Interaction within a microalgae or cyanobacteria cultivation system, phases, and their components. (Adapted and modified from Sanaye Mozaffari Sabet and Golzary (2022))

microalgae adsorption, and therefore surface tension also decreases. The orifice size was 20 mm and gas flow rate was 20 mL/min for that research. In other words, the velocity of the bubble decreases as the concentration of the gas-liquid interfacial area and contact time increases (Ding et al., 2016; Sanaye Mozaffari Sabet & Golzary, 2022) (Table 2).

There are many parameters that influence in the microalgal culture to use photobioreactors such as geometry, hydrodynamic behavior, and mass transfer with the propose to reduce inhibitory effects. For that reason, bubble column photobioreactors are preferably used for scaling up applications of microalgae due to high mass transfer, a short liquid circulation time, and a long frequency of light/dark cycle taking more control during the bioprocess (Ding et al., 2016).

3.1.1 Effect of Bubble Size Measurement in Microalgal and Cyanobacterial Biomass Growth

Culture systems in photobioreactors consist of three phases: gas (CO_2 (g) or air and O_2), liquid (culture medium), and solid (microalgae and cyanobacteria cells) (see Fig. 3). Agitation in bubbling photobioreactors is done by injecting CO_2, or air,

which produces bubbles improving the movement of microalgae and cyanobacteria within the photobioreactor, optimizing nutrient mass transfer and exposure to light irradiance, and maintaining a uniform temperature together with pH. In vertical bubble column reactors, the longer residence time of gas supplied to the liquid or culture medium results in smaller bubbles and thus an increase in mass transfer. In this context, bubbles play a very important role as microalgae cells move and adsorb on the bubble surface due to chemotactic migration; that is, the suspended cells tend to be in a place where the concentration of carbon dioxide is high, and this place is the surface of the bubble (Sanaye Mozaffari Sabet & Golzary, 2022).

Medium properties and sparger design are two critical parameters, which could affect bubble size and its distribution in the culture medium. For instance, if the culture medium is more viscous, the turbulence of the flow and the specific interfacial area between the liquid and gas phases will be reduced, thus increasing the average size of the bubbles or the number of them, reducing the overall mass transfer coefficient. On the contrary, when there is a greater presence of carbohydrates in the medium, its ion level increases, decreasing the tendency of the bubbles to coalescence, which results in the reduction of their size because a boundary layer is formed at the gas-liquid interface (Rezvani & Rostami, 2023). The above has to be accounted especially when working with alternative culture media, such as organics cultures from lignocellulosic biomasses.

Therefore, the bubble size directly influences the mass transfer of the nutrients in the medium to the microalgae and cyanobacteria, so it is essential to measure and correlate it. Since there are no bubbles of the same size in the culture, it is necessary to calculate an average diameter assuming that the bubbles are spherical. Although an arithmetic average of bubble size can be estimated, it is recommended to use a statistical average; this parameter was called the average of Sauter mean diameter (d_{32}) defined as the volume of the representative bubble to its surface area, which can be obtained by Eq. (1), where (n_i) is the number of bubbles with diameter (d_i), when the sizes of bubbles are obtained with photographic imaging in a sample (N). The Sauter mean diameter depends non-linearly on the maximum and minimum diameter values, as well as on how the bubble sizes are dispersed, both dependent on the dominant phenomena, either breakup or coalescence (Mohagheghian et al., 2018; Holguín-Salas et al., 2020).

$$d_{32} = \frac{\sum_{i=1}^{N} n_i \times d_i^3}{\sum_{i=1}^{N} n_i \times d_i^2} \tag{1}$$

The rate of gas injection directly influences the size and properties of the bubbles, for example, if the gas velocity is very fast, a greater number of bubbles are generated, but these rupture more quickly resulting in the accumulation of small bubbles, which causes faster coalescence and greater gas retention, and although this means that a higher mass transfer is motivated by a high flow rate, it should be taken into account, as mentioned at the beginning of this section, that a high flow rate also generates shear damage to the cell wall of the microalgae induced by a high shear stress;

therefore, adequate flow values have been found to avoid these damages (Benedetti et al., 2018; Yang et al., 2018; Banerjee et al., 2020). To have a homogeneous bubble flow, the gas injection velocity must be less than 0.04 m/s, as the flow becomes turbulent and the bubble sizes variable with velocities greater than 0.075 m/s and at surface velocities of the gas greater than 0.04 m/s already generate large bubbles decreasing the area for mass transfer (nutrients) (Rezvani & Rostami, 2023). Finally, it should be mentioned that there are parameters that help to understand the behavior of the fluid to have an effective growth of the algae suspension in the culture medium with the aim of increasing its production with an economic interest that is the purpose of a scale-up (Sanaye Mozaffari Sabet & Golzary, 2022).

3.1.2 Effect of Light Intensity in Microalgal and Cyanobacterial Biomass Growth

The light supplied to microalgae and cyanobacteria cultivation system is essential for the process of photosynthesis to occur, i.e., the fixation of CO_2 for the production of oxygen and in turn the reproduction of these microorganisms, which translates into an increase in their biomass. The site of photosynthesis in microalgae is in chloroplasts; it is different from cyanobacteria, which occurs in the cytoplasm, but in both cases through chlorophyll. Chlorophyll stores light energy in the form of energy bands such as ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate), these energy bands are consumed in the dark period to synthesize carbohydrates. All of the above, i.e., the reduction of carbon dioxide to carbohydrates, is called the Calvin cycle. This cycle requires an enzyme called RuBisCO, which is a carboxylase that converts the bicarbonate ions produced in CO_2 fixation into biomass. Therefore, the Calvin cycle includes three stages: carboxylation, reduction, and regeneration. According to previous researchers, blue light (400–500 nm) and red light (600–700 nm) are suitable for microalgae growth; however it must take into account that a high photon flux density can cause damage causing photoinhibition (Sanaye Mozaffari Sabet & Golzary, 2022).

The research of Nzayisenga et al. (2020) reported that in some chlorophyta species such as *Chlorella Vulgaris, Desmodesmus sp.*, *Ettilia Pseudoalveolaris*, and *Scenedesmus Obliquus* an increased light intensity since 50–300 $\mu E/m^2/s$ with a photoperiod of 16 h of light and 8 of dark improved the biomass content up to about 50%. According with the above, there was more remotion of nitrogen and phosphorous content with major light intensity (Nzayisenga et al., 2020). In other study, using red light, it was obtained 1.77 g/L of biomass contrasted with the use of blue light, obtaining 1.59 g/L for *Arthrospira platensis* (Raeisossadati et al., 2019; Portillo et al., 2022).

However, it was found that the synthesis of phycocyanin is mostly stimulated at low light intensity. Chlorophyll cannot absorb enough photons to carry out the basic functions of cellular metabolism; consequently, in order to take better advantage of the low light intensity, more phycobilisomes are synthesized. Under the same premise discussed above, chlorophyll absorbs photons at wavelengths of

400–500 nm, while phycocyanin absorbs at 620 nm, so if crops are exposed to the wavelength equivalent to red light (in the wavelength range where FC absorbs photons), Phycocyanin accumulation increases (Raeisossadati et al., 2019; Morya et al., 2023). In this context, the photobioreactors must be designed in such a way that the microalgae and cyanobacteria can take advantage of as much light as possible because, sometimes, the light from the sun is replaced for artificial light, not only white.

4 Phycocyanin as a High Value-Added Compound

Phycocyanin is a natural blue pigment that is frequently found in cyanobacteria such as *Oscillatoria tenuis* and *Arthrospira platensis*. Wu et al. (2021) reported that this compound is also present in phylum Rhodophyta (*Galdieria sulphuraria)* and Cryptophyte. The phycocyanin participates in various biological effects, such as anti-inflammatory, antioxidant, anti-free radical, antitumor, and antiproliferation activities, and also it has the property to prevent oxidative stress through scavenging reactive oxygen species as well as reactive nitrogen species (Wu et al., 2021).

Since 2013, phycocyanin has been used as a food coloring and authorized by the U.S. Food and Drug Administration (FDA), and in 2014, it was approved for use in the European Union; its principal applications are as nutritional supplements, natural colorants in foods (popsicles, chewing gum, confectionery, wasabi, dairy products, and soft drinks), and pharmaceuticals due to its non-toxic and non-carcinogenic characteristics; it also can be very useful in textiles and cosmetics; these pigments are marketed as anti-cancer, anti-inflammatory, anti-oxidative, anti-viral, hepatoprotective, and neuroprotective agents and as a component for immunological assays (Mysliwa-Kurdziel & Solymosi, 2016; De Morais et al., 2018; Pagels et al., 2019; Morya et al., 2023).

From an application point of view in the food industry, phycocyanin has a great advantage over artificial additives because sometimes these impart an unwanted flavor and color, and also they can interfere with the assimilation of vitamins. For food-grade applications, the level of purity of phycocyanin of 0.7 is enough to include it as a functional food ingredient (Khandual et al., 2021).

The phycocyanin production starts since the capacity of cyanobacteria to produce this compound inside its biomass. There are many ways to cultivate microalgae or cyanobacteria, between them exist photoautotrophic, heterotrophic, and mixotrophic production; the difference between them is the energy and carbon source. In photoautotrophic cultivation, the energy source comes from the light availability and the carbon that these microorganims consume is inorganic. To the contrary of heterotrophic way, where the energy and carbon source has to be organically metabolized without light requirements. And in the mixotrophic production the energy can be assimilated from light or not getting from organic or inorganic carbon source (Perez-Garcia et al., 2015). Currently, the commercial phycocyanin has been obtained from a photoautotrophic production of *Arthrospira platensis* in open

outdoor ponds, putting more attention in the quantity of phycocyanin produced instead its quality. Cyanobacteria have been used in mixotrophic production adding glucose in the culture or fructose obtaining more specific growth rate than the autotrophic or heterotrophic production. However, there is other microalgae species called *Galdieria sulphuraria* that when cultivated in heterotrophic and controlled way in photobioreactors utilizing a variety of carbon sources has more interest in food, nutraceuticals, and pharmaceutical applications since it grows naturally in acidic environments (pH 1–3) and high temperatures (40 °C) and it guarantees the innocuousness of the crop (Morya et al., 2023).

4.1 Phycocyanin Stability Factors

Phycocyanin stability is mainly influenced by the following physical and chemical parameters: temperature, pH, solvent type, biomass/solvent ratio, and biomass form (dried or fresh) in the extraction process (see Fig. 4); the challenge is to increase the mass transfer rates of the internal compounds to the extraction medium by finding the optimum points of these factors without to denaturalized or to affect the purity of the phycocyanin obtained more yields of that high value-added compound (Pez-Jaeschke et al., 2021).

There is no single appropriate method for extraction of phycobiliproteins, from all kinds of cyanobacteria. For the rupture of wall cells for phycocyanin extraction, various techniques have been used such as ultrasound, French press, freeze-thaw, medium, osmosis breaking, abrasives, aqueous extraction, and other

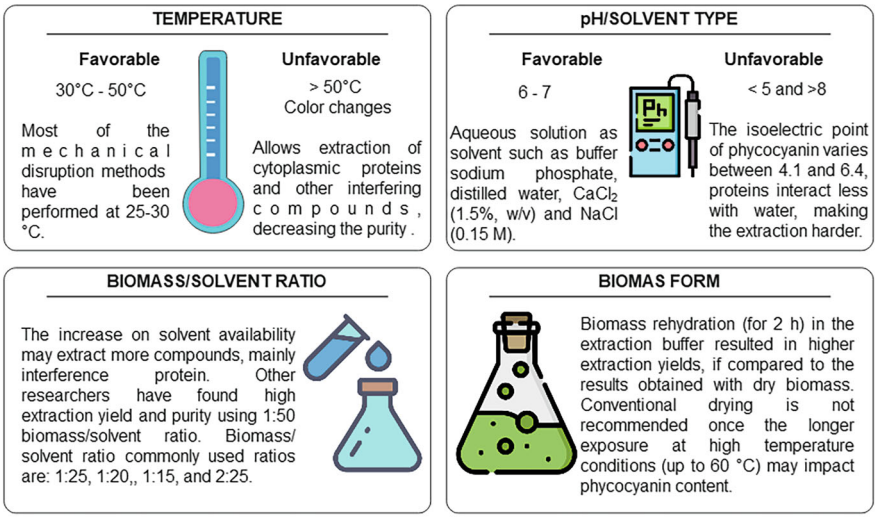


Fig. 4 Factors involved in phycocyanin extraction processes

mechanical methods such as extraction in acid; however, the latter is not a convenient method because it affects the integrity of the thioether bond of the Fluorescent Core Building chromophore with the polypeptide chains. It has been found higher phycocyanin content, 46.65–54.65 mg/g biomass, with water in all methods used compared to other solvents. In the case of phosphate buffer at pH 6, phycocyanin content has been 35.54–37.88 mg/g, and with phosphate buffer at pH 7, phycocyanin content has been 43.13–45.02 mg/g (Khandual et al., 2021).

4.1.1 Freeze-Thawing Method

The methodology reported by Saxena et al. (2022) indicates to take 10 mL of culture medium, to centrifugate, and the cyanobacteria biomass pellet is hydrated with 2 mL of sodium phosphate buffer (0.1 M) with pH between 6.5 and 7.0 in a 15 mL Falcon™' tube and vortexed for 1 min. Then it is kept at −20 °C for cell disruption via the freeze-thawing method; this process was repeated five times. After the freeze-thawing cell disruption period, samples are centrifugate, and aliquots are separated to analyze phycocyanin by spectrophotometric method. The absorbance of the extracts is measuring at wavelengths of 615 nm and 652 nm. The phycocyanin content with this method is around of 48.88 mg/g biomass, with a purity of 0.47 until 172.84 mg/g (Tan et al., 2020; Saxena et al., 2022).

4.1.2 Ultrasonication and Microwave Extraction Method

Ultrasonic and microwave methods are mostly used to break down the cell wall of the cyanobacteria with higher extraction. Microwave heating process uses extraction medium (usually solvent) to extract most of the useful chemical contents out of cyanobacterial biomass. İlter et al. (2018) studied the optimal conditions for classical, ultrasound and microwave extractions of phycocyanin. They reported that for the classical homogenization process was 1.71% biomass/solvent ratio, 6237.66 homogenization rate for 15 min of process; for ultrasound was 1% biomass/solvent ratio, 60% amplitude and 16.23 min for process; and for microwave heating process was 2.34% biomass/solvent ratio, 133.29 W and 165.96 s of extraction time, obtaining the maximum phycocyanin content of 68.12, 98.17, and 29.14 mg/g, respectively (İlter et al., 2018). Other yields of phycocyanin are around 54.65 mg/g from *Arthrospira platensis* biomass using an ultrasonication process (Khandual et al., 2021). However, all of these cell disruption procedures lack specificity as cell debris and other undesirable impurities are also released (Pez-Jaeschke et al., 2021).

4.1.3 Ohmic Heating and Electric Field Extraction Method

Ohmic heating and moderate electric field have advantages such as homogeneity of heating and short extraction time. These technologies are electrical technologies based on the passage of alternate electric current (up to 1 kV/cm) through microalgal and cyanobacteria biomass disposed between two electrodes. This is because the effect heat is generated volumetrically in the biomass, resulting in uniform and fast heating. When the electric field is applied with the purpose of heating, it is called ohmic heating, but when the electric effects (non-thermal) are more important, this technology is named moderate electric field. The non-thermal effects are associated with electroporation which means to promote modifications on cell membrane structure, resulting in changes of membrane permeability, that is attributed to pore formation (aqueous pathways) achieving the phycocyanin extraction into the solvent (Ferreira-Santos et al., 2020; Alkanan et al., 2021).

4.2 Phycocyanin Purity Factors

The phycocyanin purity refers to content of this compound in the final solution after extraction. Its purity determines the potential application depending on the relation of absorbances at 620 and 280 indicating the amount of phycocyanin compared to other proteins such as aromatic amino acids present in the extract obtained. When the absorbances ratio A_{620}/A_{280} is ≥0.7, or between 0.7 and 3.9, or ≥ 4.0 the phycocyanin is for Food Grade (enough pure to be used as food constituents), Reagent Grade (enough pure to show reliable results in fluorescence experiment), or Analytical Grade (used as a standard), respectively (Koyande et al., 2019; Hsieh-Lo et al., 2019; Amarante et al., 2020; Ashaolu et al., 2021; Khandual et al., 2021; Pez-Jaeschke et al., 2021). These absorbances are used because the maximum absorption of phycocyanin is at 620 nm and for aromatic amino acids are measured at 280 nm. Ion-exchange chromatography, ammonium sulfate precipitation method which consists of separating proteins by altering their solubility in the presence of a high salt concentration, and two-phase aqueous extraction that consists of stirring the aqueous phase with an organic solvent that is immiscible in water but related to the phycocyanin molecule in order to separate both phases are considered between other purification technics. Two-phase aqueous extraction with ion-exchange chromatography may result in highly pure phycocyanin with A620/A280 value 6.69, which is considered to be the purest form of phycocyanin (Morya et al., 2023).

4.3 Phycocyanin in the Industry Applications

Phycocyanin has been widely utilized in cosmetics, such as lipstick, eyeliner, nail polish, or eye shadow (Wu et al., 2021). Nowadays, this pigment is deeply used in food industry; however, phycocyanin has anti-bacterial property, and therefore, it must be considering this aspect when the food has its natural flora; also it is important to decide the correct way of incorporation of the matrix foods because phycocyanin is unstable in high light, is insoluble in acidic conditions (pH $\leq$ 3), and suffer, a denaturation process above 45 °C; and not all process in the food industry is suitable to use phycocyanin (Morya et al., 2023).

On the other hand, phycocyanin has high molar extinction coefficient, which let it absorb more incident light, giving it a good fluorescent property applied in various molecular and cell detection techniques such as fluorescent microscopy, histochemistry, flow cytometry, fluorescence-activated cell sorting and fluorescent assay. Similar applications are done by attaching bio-specific recognition molecules to genetically stabilized phycocyanin proteins to use them as bio-specific fluorescent probes (Morya et al., 2023).

The use of this high-value compound has grown by leaps and bounds, reaching to synthesize using phycocyanin proteins to inhibit the growth of Gram-positive and Gram-negative bacteria which are considered medically important microorganisms. The in vivo studies also showed that silver-nanoparticles formed using phycocyanin are capable of reducing tumors in mice (Morya et al., 2023).

5 Relation Between Protein and Phycocyanin

The cyanobacteria have photosystems which constitute the light-harvesting antenna complexes to produce chlorophyll (liposoluble pigment); they also have components of a protein macromolecular complex called phycobilisomes to produce of phycobiliproteins (water-soluble pigment). This complex is formed based on protein component called apoprotein and a non-protein component that is a covalently bound phycobilin or phycocyanobilin serving as the chromophores. Both components are connected with a thioester bond; they do not have a metal atom in their structure; their arrangement around the phycobilisomes allows them to create a chain of energy transfer between them until they reach chlorophyll, giving them the ability to photosynthesize at depths where chlorophyll would not be able to absorb light, making more efficient the photosynthesis process. Phycobiliproteins comprise about 80% of the dry weight of the phycobilisomes, and the remaining 20% is made up of non-pigmented proteins, which are responsible for binding and stabilizing the phycobilisomes, and depending on the species, the protein proportion from cyanobacteria biomass can be between 6% and 70% of its dry weight, and phycocyanin extracted from cyanobacteria in the industrial setup method may contain

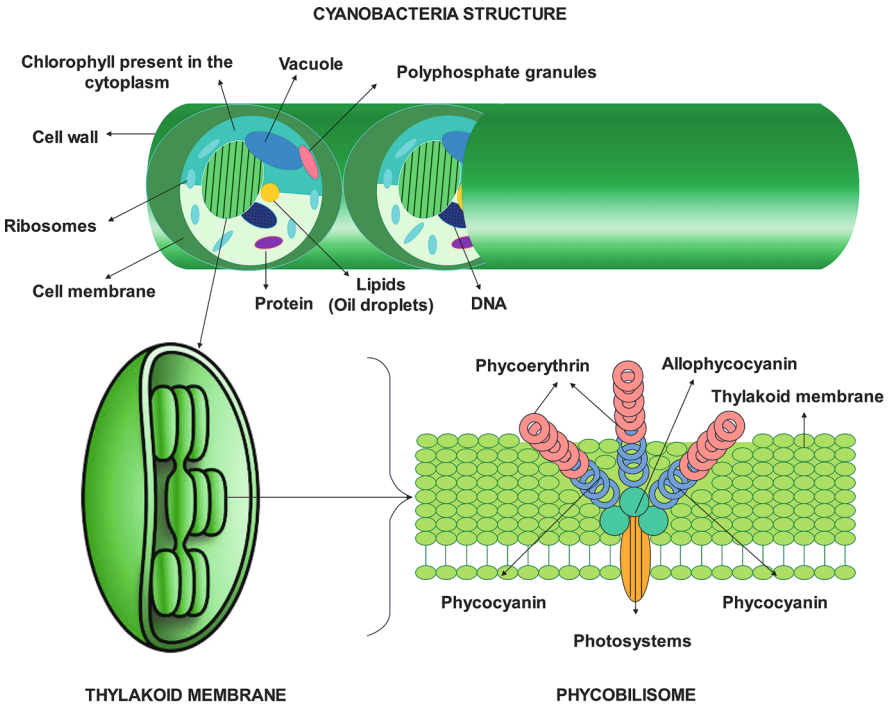


Fig. 5 Structure of cyanobacteria and phycobiliproteins (Adapted and modified from Morya et al. (2023); Pez-Jaeschke et al. (2021); Huokko et al. (2021))

approximately 14% of the total protein biomass of the cell (Wu et al., 2021; Morya et al., 2023).

Phycobiliproteins are colored, auto flowering proteins, classified according to their absorption spectrum into four large groups (see Fig. 5) which comprise the pathway through which the absorbed energy travels starting by phycoerythrin (pink color) until allophycocyanin (blue-green color) through by phycocyanin, the latter as a molecule with intense blue this coloration due to its structure that contains a linear tetrapyrrole called phycocyanobilin, which in its structure has alternating double and single bonds that allow it to delocalize electrons in response to a light excitation at a maximum wavelength of 620 nm; all the above phycobiliproteins are responsible of structural union of all the system and this tetrapyrrole is stabilized within the protein by binding to a cysteine residue by a thioester bond (Mysliwa-Kurdziel & Solymosi, 2016; Pez Jaeschke et al., 2021; Morya et al., 2023). The phycocyanin structure is added in the thylakoid membrane as Fig. 5 shows, and when the cell membrane is ruptured or broken during the extraction process, this compound comes off the system and easily dissolves in solution (Morya et al., 2023).

Proteins are produced as a result of active cyanobacteria cell division, and their accumulation is associated with exponential growth in the absence of nitrogen or

light limitation. In the same way, their maximum concentration of the phycobiliproteins is at the end of the exponential growth phase, under high light irradiance and low nutrient availability. Proteins have also relevance in the industry, because they play an important role in the production of human food, not only for the supply of essential amino acids and antioxidant activity but also for their techno functional properties as emulsifying, foaming, gelling in food formulation (Gifuni et al., 2019).

6 Conclusions and Perspectives

This chapter gives an overview of the current research in the field of photobioreactors for the production of proteins and phycocyanin from cyanobacterial biomass, starting for the importance of understanding the mechanism of nutrient transfer and the factors that affect the cyanobacterial growth in bubble column photobioreactors, as they provide a controlled and more efficient environmental conditions for the cultures to maximize the biomass production, and relevant products for the third generation of biorefineries. More studies of bubble column photobioreactors are necessary in terms of design, scaling-up strategies, optimization for microalgae and cyanobacteria biomass cultivation, and applications of alternative media cultures. Also from future perspectives the experimental data information could be used in the computational fluid dynamics (CFD) as tool to simulate and model hydrodynamics behaviors for large-scale cultivation and process for proteins and phycocyanin extraction to ensure its purity and stability for use as sustainable alternatives to conventional ingredients in the food industry.

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Configurations of Photobioreactors for Microalgae Cultivation in Third Generation of Biorefinery



Ana Mendoza, Rosa M. Rodríguez-Jasso, Luciane Maria Colla, and Héctor A. Ruiz

Abstract Nowadays, third-generation biorefineries have emerged as a viable method for capturing atmospheric CO_2 and are known as a promising platform for various applications, including biofuels, high-value compounds for nutraceuticals, cosmetics, pharmaceuticals, animal and aquaculture feed, and biofuels. For large-scale microalgae production, the cultivation stage is crucial. The success of microalgae cultivation depends on the configuration and design of systems. Key factors include the direction and rate of aeration, light/dark cycle duration, light-time ratio, average turbulent kinetic energy, and other variables critical to microalgal growth. This chapter aims to provide fundamental knowledge on cultivation techniques, growth modes, cultivation conditions, requirements of photosynthetic microorganisms, and the modeling and control of photobioreactors. Additionally, it discusses future trends to enhance performance in microalgal biomass production for high-value compound production that can focus on the main problems encountered in microalgae cultivation and propose a new configuration in photobioreactors to perform and achieve maximum performance.

Keywords High added-value compounds · Circular bioeconomy · Non-conventional photobioreactors configurations

1 Introduction

In natural source management, sustainability is a concept that encompasses socio-economic factors, environmental impact reduction, and operational effectiveness. The sustainability of the environment and the energy crisis caused by an over-

A. Mendoza · R. M. Rodríguez-Jasso · H. A. Ruiz (✉)
Biorefinery Group, Food Research Department, School of Chemistry, Universidad Autónoma de Coahuila, Saltillo, Coahuila, Mexico
e-mail: hector_ruiz_leza@uadec.edu.mx

L. M. Colla
Graduate Program in Civil and Environmental Engineering (PPGEng), University of Passo Fundo (UPF), Passo Fundo, Río Grande del Sur, Brazil

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reliance on fossil fuels are the two main problems facing humanity globally. Concerns about depleting fossil fuel supplies, volatile crude oil prices, greenhouses gas emissions from CO_2 emissions, and the acceleration of global warming all highlight how unsustainable it is to continue relying on fossil fuels for energy (Fu et al., 2019). To replace nonrenewable resources such as fossil fuels, some biomass feedstocks, including terrestrial plants and aquatic algae, have been discovered to generate renewable fuels and products. Aquatic microalgae offer an ideal solution for producing biofuels and high-value compounds due to their fast growth, high biomass yield, diverse product range, and ease of harvest. They hold promise as sustainable, environmentally friendly, carbon-neutral fuel sources. Microalgae are a single-cell, a large and polyphyletic group of O_2-evolving photosynthetic microorganisms, mostly aquatic, comprising bacteria such as cyanobacteria, diatoms, other protists, and unicellular plants (Chamilka et al., 2021; Nhat et al., 2019). High-value product manufacturing is one of the objectives of recent studies on microalgae culture. Global demand and the desire to minimize pollution are the main forces behind improving renewable energy and products. By converting CO_2 and nutrients into biomass and using photons to do so, microalgae help with CO_2 fixation. Numerous advantages come with this method, such as fast growth (0.7–3.2 day^{-1}) and a high photosynthetic rate (e.g., 6.9×10^4 cells/mL/h). Antioxidants, lipids, proteins, carbohydrates, polyunsaturated fatty acids, peptides, carotenoids, toxins, sterols, and natural colors are a few high-value products that can be produced from microalgae during photosynthesis (Zimermann et al., 2020). Commercially available applications stemming from these high-value products include human nutrition supplements, animal and aquatic feed additives, pigments, cosmetic ingredients, antiviral, stable isotope biochemicals, as well as the synthesis of antimicrobial, biofertilizers rich in valuable molecules, antibacterial, and anticancer medications (please see Fig. 1) (Abreu & Martins, 2023). Microalgae feedstock has great potential for biorefineries, as it represents the sustainable transformation of algae biomass into valuable chemical compounds and biofuels. Microalgae, acknowledged as a sustainable biomass source, serve as the cornerstone of an all-inclusive algae biorefinery, integrating upstream, bioreaction, and downstream processing. Oil, minerals, starch, and proteins may all be extracted and used with this method, which makes it easier to produce high-value compounds such as biofuels, biochemicals, feed, biogas, and more (Bhattacharya & Goswami, 2020; Khoo et al., 2019).

The biomass-based biorefinery sector aims to optimize resource utilization, maximize profitability, and minimize waste. It takes multiple procedures to extract microalgae biochemically because they grow in liquid media. Harvesting, extraction, and culturing are the three primary processes in the processing of microalgae biomass. First, there are two main systems for cultivation: open and closed. Since open systems are inexpensive, have straightforward architecture, and are easy to use, they are the most widely used for both large-scale commercial applications and environmental improvements. Nevertheless, open systems have drawbacks such as limited biomass output, contamination susceptibility, and unpredictable environmental conditions, even with their inexpensive equipment (Bhattacharya & Goswami, 2020). Closed methods to produce microalgae have been developed in

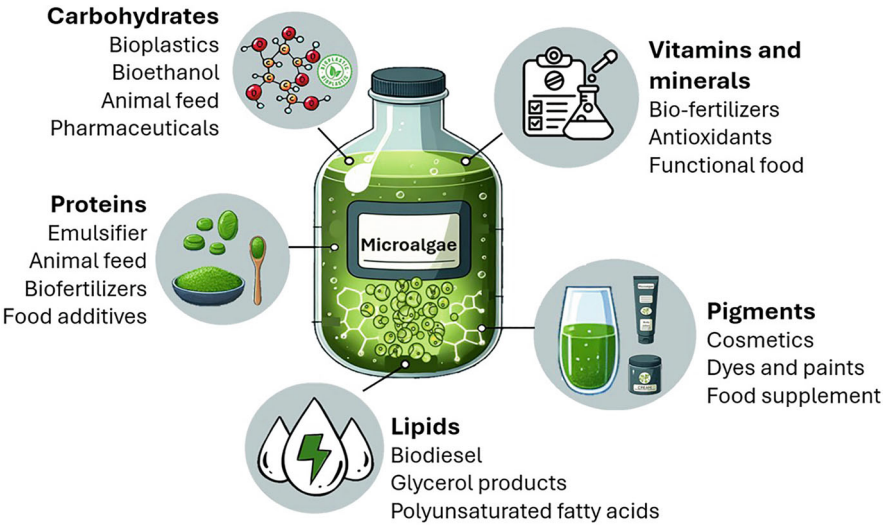


Fig. 1 Microalgae find diverse commercial applications across various sectors. (Adapted from Fu et al., 2019)

response to the problems with open systems, and many kinds of photobioreactors (PBRs) have been investigated. A PBR is an essential tool for the regulated production of microalgae, guaranteeing the attainment of intended results through growing and harvesting. In PBRs, critical variables including mass transfer, light impact, and pH are meticulously controlled to maximize cultivation efficiency and produce the desired outcomes (Razzak et al., 2023). Waste CO_2 and wastewater streams are recycled as inputs for algae cultivation in three different processing pathways for algal biomass recovery. This chapter provides a comprehensive overview of biomass processing and its possible future applications while highlighting the inadequacies of the present bioprocesses used to create microalgae biomass.

2 Microalgae Cultivation Techniques

Some techniques for microalgae cultivation have been reported. Microalgae techniques for cultivation depend on the investment, the desire products, cost, the CO_2 capture, and source of nutrients. The cultivation systems are divided into open and closed systems.

2.1 Open System

Open systems for microalgae cultivation are commonly known as open ponds, and the principal characteristic is that they are completely exposed to external environmental conditions. These systems encompass various designs, including tanks, circular ponds with rotating arms for mixing, raceway ponds with paddlewheels for circulation, and cascade systems with baffles. They are cost-effective and easy to build, maintain, and operate. However, they face potential issues such as contamination risk, inadequate mixing, shaded areas, water loss due to evaporation, poor mass transfer efficiency, and inefficient CO_2 utilization (Fu et al., 2019).

2.1.1 Unstirred Pond

Unstirred ponds are constructed in natural water bodies with depths of up to half a meter, allowing light to penetrate but lacking any stirring mechanism. This method offers an economical and convenient approach for cultivating microalgae. Commercially, unstirred ponds are used for certain species, such as *Dunaliella salina*, which thrives in these conditions. However, this type of pond has significant limitations, including poor microalgae growth due to suboptimal conditions and competition from contaminating bacteria, viruses, and protozoa. The lack of adequate mixing is a major drawback, but the method can be improved by incorporating agitation, either through circular ponds or through mechanical stirring devices (Alberto et al., 2019).

2.1.2 Raceway Ponds

Raceway ponds, also known as stirred paddle wheel open ponds, typically consist of single or multiple "raceway" channels where a mixture of microalgae, water, and nutrients is circulated. This circulation promotes agitation and suspension of the microalgae in the water, as well as the utilization of CO_2 from the external atmosphere. Some algae species commonly cultivated in this configuration include *Spirulina*, *Dunaliella*, *Chlorella*, and *Haematococcus* (Fu et al., 2019; Qin et al., 2019). Culture agitation, feeding, harvesting, carbon dioxide supply, drainage, possible overflow, and cleaning at a certain velocity are some of the variables that raceway ponds need to consider. The normal depth for large-scale raceway ponds is between 15 and 25 cm because to light penetration and scattering by microalgae cells. This could potentially achieve a production level of 50–60 g_{dw} (m^2 × day). Though, productivities are often in the range of 10–20 g_{dw} (m^2 × day). This arrangement of mixing allows for the coverage of a sizable region with microalgae. The eddies that are produced shorten the time that algae spend in dark areas, and turbulent mixing aids in maintaining a consistent microalgae density (Hong et al., 2023; Wayne et al., 2018).

2.1.3 Circular Ponds

The development of wastewater treatment ponds gave rise to this kind of system. In Asia, *Chlorella* sp. is mostly cultured in circular ponds. These ponds usually measure 40–50 meters in diameter and 20–30 centimeters in depth. A revolving arm that rotates axially mixes the culture medium and algae cells together. Circular ponds have a productivity of about 8.5 g/(m^2 × d). Compared to unstirred ponds, this structure offers improved mixing efficiency, but contamination risk still exists. The susceptibility of the microalgae and the absence of temperature control are the primary problems with open systems (Qin et al., 2019).

2.2 Closed Systems

To bring a solution which are generated from open systems and to achieve a better yield of microalgae biomass production, the closed systems has been proposed. In general, these closed systems involve microalgae cultures being cultivated in suspended form; also the cultivation in this configuration promotes an effective control of cultivation conditions and growth parameters such as temperature, pH, nutrients, light intensity, CO_2, and O_2 concentrations (Yin et al., 2020). The main purpose of closed systems is to promote a prolonged cultivation period of cultivation that will result in high productivity; some examples of these configurations designs are shown in Fig. 2. Some advantages and disadvantages of both systems are also presented in Table 1. Closed systems have significant input costs and a large initial capital investment, despite their potential for excellent performance. The need for research into creating a more functional and affordable photobioreactor is highlighted by the fact that maintenance frequently involves significant input and operating costs. Right now, tubular, flat panel, and column photobioreactors are the most often used closed photobioreactor designs (Amit Kumar et al., 2021b).

2.2.1 Tubular Photobioreactor

It is important to recognize the differences between bubble column and airlift vertical tube PBRs in this setup. Tubular photobioreactors use an airlift mechanism to move the culture around, strengthening the system. A sparger located at the reactor's bottom in all varieties creates tiny bubbles from gas to guarantee algal cell suspension, improve mass transfer, seize CO_2, and enable O_2 emission.

Table 1 Comparison between different types of closed photobioreactors, advantages and limitations

Types of photobioreactors	Advantages	Limitations
Flat panel	Large illumination area	Difficult in controlling culture temperature
	High rate of biomass productivity	Possibility of algae accumulation on the walls
	Low dissolved oxygen accumulation	Aeration induced high stress damage
	Cheap installation and operation	Scale-up process requires many support materials (instrumentation and control system)
Bubble column	Less physical stress in the cell	Precipitation of microalgae biomass
	Bubbles facilitate light penetration and dispersion	Oscillation flow in the culture
	High mass transfer	
Airlift column	High biomass concentration	Insufficient turbulence creation through airlift operation
	Cheap operation	Work volume limitation by oxygen removal
	High CO_2 utilization efficiency	Deficient scale-up
	Efficient light utilization and penetration	
Stirred tank bioreactor	Good light and mixing provision	Poor light irradiance
	Good aeration can achieve better gas solubility	Continuous and strong mixing required for optimum algae growth
		Difficulty in large-scale design

2.2.1.1 Bubble Column

Air bubbles created during fluid flow generation propel fluid flow in the most basic bubble column arrangement, which is a cylindrical tank devoid of any interior features. An external light source provides lighting, and a bubble column's height is usually at least twice its diameter (>0.1 m). As light intensity drops because of cell obstruction, the middle axial area stays darker. A "light flash effect" is produced in this arrangement, which increases photosynthetic efficiency and mixing by pushing algae cells from the lighted surface to the darker axial zone by means of bubbles. Because of their high surface area-to-volume ratio, minimal energy input, and compact, affordable, and simple operation, bubble columns provide adequate mass transfer. Predicting fluid dynamic parameters with accuracy is essential for the appropriate design and functioning of bubble column photobioreactors (Besagni et al., 2019; López-rosales et al., 2019).

2.2.1.2 Airlift Photobioreactor

An improvement on the bubble column, the airlift reactor is a pneumatic agitation device that uses energy supply to keep the tube homogeneous. A riser and a downcomer are the two connecting zones found in airlift reactors. Air is fed into the riser, which is usually a concentric tube, pushing the liquid upward and fostering circulation as well as the creation of dark zones. The mechanism of this type of photobioreactor consists of air bubbles dragging the liquid from the riser zone inside the tube up to the outside zone where algae cells can be illuminated "light flash effect" to enhance photosynthesis, while in this way causing the liquid to be recycled downward externally and finally return to the riser zone, also called loop. An isothermal expansion caused by the gas phase's velocity can result in three different regimes in the downcomer zone, depending on the gas phase's condition: (a) no air bubbles, (b) stationary bubbles, and (c) bubbles moving into the riser from below. The surface gas velocity immediately affects the regime and causes the hold-up that follows. Combining anaerobic, anoxic, and aerobic zones into a single reactor is possible, thanks to the standard design of an airlift photobioreactor. Multiple microbes can coexist in this configuration, allowing for the simultaneous removal of nutrients and carbon (Feng et al., 2024). Some advantages of airlift are the low operation costs and energy consumption. The fluidization of solids is not directly caused by gas bubbling but by the circulation of liquid within the reactor. This circulation helps maintain consistent shear forces throughout the reactor, preventing morphological and metabolic changes in the culture cells (Valdivia-Rivera et al., 2019).

2.2.2 Stirred Tank Photobioreactor

The stirred tank photobioreactor was modified for microalgae cultivation by adding an external light source, drawing inspiration from the fermentation tank. This concept uses an electric motor to drive an impeller, which moves mechanically, to provide agitation. In indoor growing, this arrangement is known for its superior mixing and transfer efficiency. A foam breaker is included to disperse and break up bubbles. However, they are connected in a cascade and arranged vertically to resemble a rectangular box. This arrangement is aerated and has two light sources. Air bubbling through a perforated tube or mechanical rotation powered by a motor are the two methods used to produce agitation (Hong et al., 2023; Suparmaniam et al., 2019). The benefits of a flat panel are in providing an appropriate design for scale-up process, opportunities for the accumulation of dissolved oxygen, and a high area to volume ratio for illumination, along with high photosynthetic efficiency in comparison with tubular type. Nevertheless, there are drawbacks to this configuration, including the surface biofouling and hydrodynamic stress caused by aeration. The possibility that algae may stick to the bioreactor walls and sustain harm from the intense aeration stress is another cause for concern. Temperature control, the distance between light sources and the panels, and whether all sides are lighted are

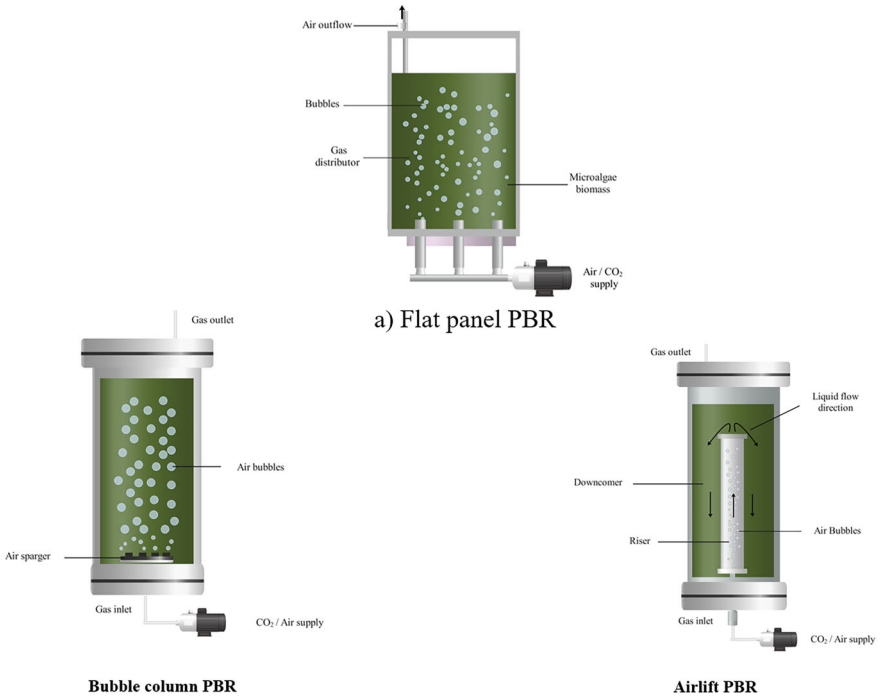


Fig. 2 Schematic representation of different photobioreactors. (**a**) Flat panel PBR, (**b**) Vertical tube PBR. Bubble column and airlift PBR. (Adapted and modified from Barboza-rodríguez et al., 2024)

important considerations when using flat panel systems, which are primarily intended for indoor use. Although controlling temperature might be difficult, devices like heat exchangers and sprinkler systems can be useful. Larger volumes result in greater hydrostatic pressure, which makes scale-up challenging for flat panel systems because they often cannot resist high pressures (Wayne et al., 2018; Yen et al., 2019) (Fig. 2). The advantages and drawback of the different photobioreactors are shown in Table 1.

2.3 Construction of Photobioreactor

The reactor material in a closed system for microalgae culture needs to fulfill specific ideal specifications. It ought to be simple to maintain, transparent, and non-corrosive or reactive. The most common materials used to build photobioreactors are glass and polyvinyl chloride (PVC), both of which can tolerate temperatures as high as 45 °C, which is higher than the ideal temperature for the growth of microalgae. They are

also resistant to UV deterioration. Furthermore, a broad pH range of 3–14 can be tolerated by materials like glass and PVC (Sathinathan et al., 2023).

Glass photobioreactors offer an ideal solution for maintaining sterility due to their ease of cleaning and sterilization using heat, providing excellent protection. However, they come with limitations such as high brittleness, which makes them riskier for fabrication compared to plastics. Glass can also develop structural imperfections due to broad grain boundaries, and it has lower tensile strength and Young's modulus. In contrast, cleaning photobioreactors can involve the use of chemicals, but this requires careful consideration as certain chemicals can compromise the material's integrity. For example, prolonged use of ethanol on acrylic materials can shorten their lifespan, as acrylic is soluble in this type of solvent (Maraveas, 2019).

3 Grow Modes and Cultivation Conditions

The characteristic growth of microalgae is significantly dependent on the cultivation condition. For microalgae growth, the culture depends on enough supply of carbon source and energy to carry out photosynthesis. Three types of metabolisms microalgae can assume, photoautotrophic, heterotrophic, and mixotrophic, and are capable to shifting the metabolism as a response to changes in the environment conditions.

3.1 Photoautotrophic Cultivation

Photoautotrophic conditions have long been recognized as the most usual and conventional culture conditions for microalgae. The pathway follows the use of inorganic carbon (carbon dioxide or bicarbonates) as the carbon source, microalgae use photosynthesis to produce chemical energy in this method of metabolism. Using processes like light absorption, electron transfer, photosynthetic phosphorylation, and carbon assimilation, microalgae fix CO_2 in photoautotrophic cultures by converting light energy into ATP and accessible reduced coenzyme II (NAD(P)H). First, the carbon supply (CO_2) that the microalgae utilize to power their own Calvin-Benson-Bassham (CBB) cycle causes biomass to accumulate. This is just one of the advantages that this microalgae culture offers (Dolganyuk et al., 2020; Shan et al., 2023).

Microalgae grown photoautotrophically have a higher capacity to absorb carbon dioxide at the same cell density as other types of culture, which helps to lower the amount of CO_2 in the atmosphere. Furthermore, since microalgae can use CO_2 from the environment for metabolism, they aid in the conversion and absorption of HCO_3^- to CO_3^{2-} in the culture water, helping to maintain a steady pH in the water (Shan et al., 2023). Microalgae could have high or low productivity depending

on the genus/species and that compounds accumulated intracellularly also depend on these conditions would be better with starch contents ranging from 18% to 25% and lipid productivity varying from 5% to 68% depending on the species under study. This phenomenon may be explained by microalgae's faster breakdown of organic nutrients that have accumulated in the CBB cycle in the absence of an organic carbon supply (Yun et al., 2021).

3.2 Heterotrophic Cultivation

Heterotrophic culture involves utilizing organic compounds like sugars and organic acids as the sole sources of carbon and energy in the absence of light. The efficacy of this microalgae cultivation method depends on several physiological characteristics: the ability of microalgae to adapt to environments with diverse organic carbon content, their reproductive capacity, the degree of biomass accumulation under dark conditions, the rate at which microalgae utilize organic matter when co-cultured with bacteria, and their ability to utilize various organic carbons such as lactate, pyruvate, acetate, glycerol, glucose, arabinose, and xylose. Not all microorganisms have the capacity to metabolize organic carbon without light, but some of them that can grow in heterotrophic conditions are *Thraustochytrium* sp., *Chlorella*, *Scenedesmus*, *Spirulina* sp., *Nannochloropsis* sp., *Haematococcus*, and *Neochloris oleoabundans* (Sui et al., 2021; Tian-yuan et al., 2019). In heterotrophic culture, microalgae no longer rely on external solar energy but instead utilize their tricarboxylic acid (TCA) cycle supplemented by organic carbon sources. This mode indirectly supplies the necessary organic carbon for the Calvin-Benson-Bassham (CBB) cycle and facilitates cellular biomass growth. Microalgae such as *Chlorella* sp., *Spirulina platensis*, or *Scenedesmus* sp. can grow in these conditions and accumulate up to 58% lipids and normally comprise 32–53% starch by dry weight (Shan et al., 2023). Theoretical biomass yields per ATP suggest that 1 mol of energy supports 19.3 g of biomass in heterotrophic cultures. This metabolic pathway generates ATP through mitochondrial oxidative phosphorylation, whereas illuminated cells derive additional energy from photophosphorylation in the chloroplasts. About 77% of the cell's overall need is met by the Calvin cycle's absorption of CO_2, considering the principal intermediate in heterotrophic metabolism is glyceraldehyde-3-phosphate (G3P), and is necessary 1.15 × 103 kJ of light energy to synthesize 2 mol of G3P, whereas the conversion of 1 mol glucose into 2 mol of G3P requires 2.1 kJ (Abreu et al., 2022). Some disadvantages in heterotrophic cultivation are the risk of contamination including different microalgae, fungi, and bacteria stains, reducing the final productivity of the technology. Moreover, without the external addition of inorganic carbon in this approach, there is a notable decrease in the rate of the Calvin-Benson-Bassham (CBB) cycle metabolism. As a result, there will be lower accumulation of products in the chloroplasts. Conversely, if the

efficiency of the CBB cycle is reduced, the energy generated by the TCA cycle remains unused, as excess energy that does not return to the mitochondria cannot be released (Shan et al., 2023).

3.3 Mixotrophic Cultivation

Mixotrophic cultivation consists of the performance of photosynthesis with the use of inorganic (CO_2) and organic compounds such as acetate, glycerol, and glucose. Mixotrophic growth does not occur in all species; some species that have shown increased growth rates and biomass production under this cultivation include *Chlorella* sp., *Asterarcys* sp., *Nannochloropsis salina*, *C. protothecoides, N. gaditana*, *C. vulgaris*, *N. oleoabundans*, *D. salina*, *Haematococcus* sp., *Botryococcus braunii*, *P. purpureum*, and *P. tricornutum* (Brito-bazan et al., 2021). Mixotrophic culture supplies lipid yields that are two to ten times greater, two to three times higher biomass, and a 40–60% reduction in ash content. A characteristic of heterotrophic culture, high cell density cultures, can be grown as well through superior bioprocessing ability in mixotrophic culture. It is crucial to keep a fast-stirring speed and the ideal concentration of CO_2 and O_2 because mass transfer at the gas-liquid interface is a limiting factor in this kind of culture with high cell density. A few advantages of mixotrophic respiration include using stored oxygen for respiration, avoiding damage from photooxidative stress brought on by high oxygen concentrations in closed reactors, and having the freedom to transition between dual trophic modes depending on the energy source available (please see Fig. 3) (Ghosh et al., 2024). Additionally, it compensates for growth losses during dark hours and lessens photoinhibition by activating alternate routes for photosynthetic processes. Mixotrophic cultures are more productive because they combine the advantages of light-dependent and organic carbon-based metabolic processes. Mixotrophic culture must be used in a biorefinery platform to produce important co-products, provide easy disinfection, and enable cost-effective bioprocessing (Anil Kumar et al., 2021a).

4 Operating Requirements for Microalgae Growth

According to some reports, the productivity of microalgae is determined mainly by intensity and type of light, nutrient supply, pH of the medium, agitation, temperature, and contamination by other organisms.

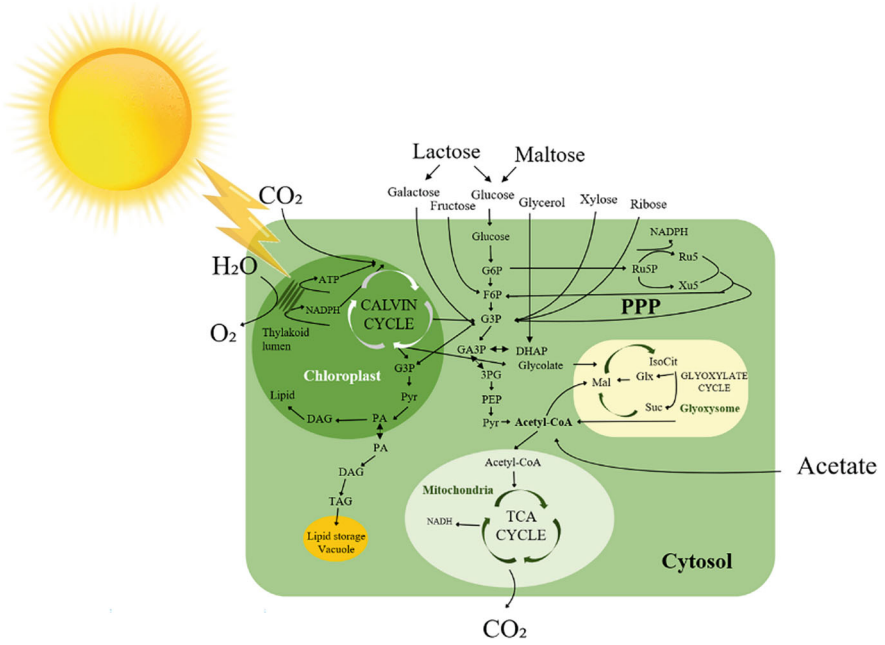


Fig. 3 Mixotrophic metabolism of microalgae cell with some carbon sources (Adapted and modified from Anil Kumar et al., 2021a)

4.1 Light

Microalgae are living things that use photosynthesis to transform light energy into chemical energy that is then stored in a variety of organic molecules. Light wavelength and intensity are important parameters that affect microalgal development and metabolism. The chemical makeup of the photosynthetic pigments in microalgae controls how much energy they absorb, mostly based on how different light wavelengths interact with the pigments (Abomohra et al., 2019). In a mixotrophic mode of cultivation, light intensity and photoperiod have a great effect on yield. Algae require light (at least 10–15 μmol/m^2/s) for glucose uptake; it did not grow in the completely dark condition. However, light/dark (L/D) cycles have an important impact and effect on the synthesis of organic compounds and nutrient metabolism. Adjusting the light-dark (L/D) cycle duration can influence various interactions in cell metabolism, including the rate of photon absorption and the management of photoinhibition effects, thereby impacting the overall energy dynamics of microalgae (Patel et al., 2019; Stev et al. 2019).

4.2 Nutrients Supply

Microalgae media is a vital source for good nutrition, but as mentioned before, depending on the nutrients used by the microalgae, it can be classified as autotrophic, heterotrophic, or mixotrophic. Nutrients that can sustain algae growth are micro and macronutrients. The principal macronutrients are carbon, nitrogen, and phosphorus, but they can also include magnesium, potassium, sodium, and calcium. Micronutrients or trace elements include selenium, boron, copper, manganese, molybdenum, vanadium, and zinc. Many of these trace elements are important for enzymatic reactions and synthesizing compounds necessary for metabolism.

4.2.1 Nitrogen Assimilation

As a macronutrient, nitrogen is important for the growth of microalgae and is also involved in the synthesis of lipids, carbohydrates, and proteins. Nitrogen from the medium is generally taken up by microalgae in a variety of forms, including urea, nitrate (NO_3^-), ammonium, nitrogen gas, and nitrogen oxides (NOx). In microalgae culture, nitrate is frequently chosen over ammonium salts because of its stability and decreased tendency for pH swings. Nitrogen limitation in the culture, however, can also lower the amount of biomass produced and the synthesis of proteins, lipids, and carbohydrates in microalgae. On the other hand, ammonia (NH_4^+) concentrations larger than 25 μm may be harmful to microalgae. Algal biomass normally contains between 1% and 10% nitrogen, depending on the kind and availability of the nitrogen source (Acid & An, 2021; Javad et al., 2019).

4.2.2 Phosphorus Assimilation

Phosphorus is also considered an important macronutrient for microalgal cell growth and compounds production such as phospholipids ATP, DNA, and RNA that are used for metabolic pathways, energy transfer, and nucleic acid synthesis are involved in the process. Phosphorus is taken from the medium as orthophosphate ($P\text{-}P0_4$). Factors such as an excessively high or low pH, or the absence of ions such as potassium, sodium, or magnesium, slow the phosphate uptake by microalgae. The necessary amount of phosphorus in the medium is less than that of nitrogen for the same amount of biomass generated. Several studies suggest that nutrient uptake in microalgae is influenced by the N ratio in the culture medium. Optimal growth and nutrient uptake tend to occur when this ratio closely matches the composition required by the microalgae. Low concentrations of phosphorus have been observed to increase lipid content and productivity, while carbohydrate content typically decreases under such conditions (Chakraborty et al., 2023). Phosphorus is slightly less than 1% of total algal biomass and is required at approximately 0.03–0.06% in the medium to sustain the growth (Acid & An, 2021).

4.3 pH

Some physiological and biochemical processes in microalgae are influenced by the pH of the medium; keeping the pH at an ideal level has an impact on nutrient uptake, enzyme activity, and cell development. A pH of 7.9–9.9 is neutral; a higher pH would result in less chlorophyll being present in the algae. The ideal pH varies depending on the type of microalgae; some can tolerate neutral and alkaline pH. Moreover, the pH regulation value can be changed by supplying carbon dioxide gas or by introducing acids and bases (Sathinathan et al., 2023).

4.4 Temperature

The temperature in the microalgae cultures affects biomass production and growth rate. This is caused by photosynthesis and the respiration process in microalgae. Microalgae survive in a temperature range of 15–40 °C at an optimum of 20–25 °C. An increment in the temperature would affect cell division and the rate of photosynthesis. Furthermore, microalgae have different temperature requirements, for example, *Chlorella* could grow between 5 °C and 42 °C (Sathinathan et al., 2023).

4.5 Agitation

A further crucial element influencing microalgae productivity is the photobioreactor's hydrodynamic environment. Aeration, mechanical agitation, or a combination of the two can be used to achieve agitation. To prevent harming the cells and upsetting the culture, though, great thought must be taken to choosing the right impeller speed and aeration rate. It is important to maintain a flow between 0.2 and 0.5 m/s to provide adequate agitation for large-scale cultivation, as reports suggest that shear pressures from velocities exceeding 1 m/s can injure cells (Sathinathan et al., 2023). Agitation plays a crucial role in several aspects: it ensures the uniformity of the medium, enhances the efficiency of stirring, prevents algae sedimentation and adhesion to reactor walls, homogenizes pH levels, and ensures proper distribution of gases and light. Aeration serves as a method for circulating the medium, improving mass transfer, regulating potentially harmful levels of dissolved oxygen and inhibitory levels of CO_2, and preventing CO_2 deficiency (Paula et al., 2022). However, not all species tolerate strong agitation that requires good mixing in the reactor, since they are sensitive to hydrodynamic stress and cell damage is caused by rupturing of the gas bubbles at the culture surface, and mechanical agitation of culture systems creates such gas bubbles (Ajala & Alexander, 2020; Orlando et al., 2018).

5 Innovations of Configurations and Operations of Photobioreactors

Effective large-scale culture techniques are essential for producing microalgae on a huge scale. The primary obstacles stem from design factors like hydrodynamics, light shading, and geometry, in addition to strict operational specifications like fertilizer supply and carbon dioxide levels. Innovative nutrition regimes can be explored by carefully adjusting photobioreactor setups to meet the unique kinetic and metabolic requirements of microalgae. This is important since it increases the productivity of microalgae biomass during culture. For the challenges mentioned above some innovations in photobioreactors are developed and are called hybrid photobioreactors or non-conventional PBRs. Non-conventional PBRs are defined as the combination and/or integration of the combination of the above with other technologies that result in PBR volume reduction, higher biomass yield, and pollutants removal efficacy; numerous configurations discussed have not yet reached the pilot or industrial stages; instead, they are still in the laboratory or research phase. This is a crucial point to recognize. Still, their use as case studies is beneficial in advancing the creation of novel and more appropriate designs for the large-scale production of microalgae biomass and chemicals (Assunçao, 2020; Nhat et al., 2019).

5.1 Geometry and Fluid Motion Issues in Microalgal Systems

5.1.1 Tubular PBR

Tubular photobioreactors (PBRs) have been enhanced with non-conventional designs to achieve high surface-to-volume (S/V) ratios. Examples include conical, helical, and inclined tube configurations (such as α-shaped or near-horizontal PBRs). Furthermore, the addition of static mixers has resulted in notable hydrodynamic enhancements.

5.1.1.1 α-Shaped PBR

The α-shaped PBR is a non-conventional version of the tubular photobioreactor and is made of two sets of parallel, translucent polyvinyl chloride tubes with a combined volume of 300 liters. Each set of tubes is 25 meters long and has an internal diameter of 2.5 cm. These tubes face sunlight at a 45-degree angle. A receiver tank is connected to the top of each set of tubes, and vertical air-riser tubes are connected to the base. The microalgae culture is propelled up to the reception tank by the design, and then it descends via the opposite set of tubes to form a loop-recirculation system (Assunçao, 2020).

5.1.1.2 Vertically Stacked Horizontal PBR

The tubular manifold rows are placed vertically in a horizontal PBR, mimicking the design of fence systems. S/V ratios of high values can be obtained with this design. However, only so many vertical rows can be employed, as the shadowing from the higher tubes would not allow the tubes at the bottom to get enough light. There needs to be at least 0.5 meters between vertical loops to guarantee high microalgae productivity. To further avoid shading between consecutive rows, it is imperative to position the bioreactor in a North-South direction. To accomplish degassing, an airlift system that releases air from the bottom is employed. High liquid velocities are necessary to prevent the accumulation of oxygen produced by photosynthetic processes, which would reduce the duration of the process.

5.1.1.3 Helical or Conical Tubular PBR

The helical shape of a helical tubular photobioreactor (PBR) sets it apart from other types of tubular PBRs because it maximizes light capture and improves the surface-to-volume (S/V) ratio. This arrangement consists of flexible plastic tubes that are looped or coiled to form a conical or helical framework. Although it can be used outside in direct sunshine, it is more frequently used indoors in artificial light, which raises operating expenses. A centrifugal pump raises the fluid, and the system usually consists of a heat exchanger and a separate gas exchange unit. Its strong S/V ratio makes effective light transmission possible throughout (Casazza et al., 2022; Díaz et al., 2019).

5.1.2 Column PBR

Since they were first used, column photobioreactors, or PBRs, have undergone several changes. The creation of annular column PBRs, intended to eliminate low-irradiation zones and shorten the light path inside the reactor, is one notable adaption. Further technological developments have been made to improve mass transfer rates, optimize hydrodynamics, and enhance light flashing effects (i.e., light/dark cycles).

5.1.2.1 Airlift Column with Static Mixers

Specific flow patterns are inherently linked to column PBR categories, leading to creative changes that make use of well-established engineering techniques to enhance hydrodynamics and gas-liquid mass transfer and give cells in the column more regular cycles of light and dark. Within-loop and external-loop PBRs are the two primary varieties of airlift columns. Constant draft-tube design characterizes internal-loop airlift PBRs; the downcomer is the annular space surrounding the

transparent inner column, which acts as the riser zone. An annular-loop airlift system is often achieved by sparging gas in the riser or annular area and forming the downcomer region as the core of the column. Concentric draft tubes can be modified to create an airlift baffled draft-tube by adding static mixers or baffles to improve mixing efficiency (Assunçao, 2020). Other unconventional designs include mechanical stirrers inside the draft tube with direct gas sparging through the inner cylinder or annular space, and split-tube systems where a flat plate or baffle divides the column into separate riser and downcomer sections. Furthermore, creative designs have been produced by incorporating consecutive baffles into bubble column (Putra, 2020).

5.1.2.2 Annular Bubble Column

The annular bubble column is another creative design. It consists of two Plexiglass cylinders that are nestled inside one another to create an annular chamber. This chamber contains a perforated plastic tube that is used as a gas diffuser for pH control and CO_2 delivery, as well as to aid in mixing and gas exchange. Artificial or natural illumination is usually placed inside the inner cylinder to minimize the light path and maximize the microalgae cells' utilization of artificial photon flux (Besagni, 2020).

5.1.3 Flat-Plate PBR

The term flat-plate or panel PBR refers to designs that emulate the light-harvesting approach of plant leaves. These configurations are specifically designed to maximize surface-to-volume ratios or to enhance mixing.

5.1.3.1 Curved-Chamber PBR

The curved-chamber PBR is a modification of a conventional flat-plate design. It has a vertical chamber with a top hole for gas disengagement made of Plexiglas that is 0.6 cm thick. The front and back glass walls are curved to create a nearly hemispherical dome. This arrangement was created to test the effects of spatial light dilution on growth and photosynthetic efficiency with the traditional cuboidal flat-plate method under artificial lighting. Reduced volumetric output was observed in the results, although photosynthetic efficiency increased (Assunçao, 2020).

5.1.3.2 V-Shaped PBR

Unconventional FP-PBRs include V-shaped PBRs, which have an enclosed glass tank fashioned like a fluidized bed reactor. At the bottom of the bioreactor, there is bubbling. Its shape removes escape routes, increasing mixing speeds without putting

a lot of strain on the shear bond. Large exposed areas are provided for effective light gathering by cells, and the arrangement also inhibits cell development on the reactor walls (Penloglou et al., 2024).

5.1.3.3 Flat Panel Airlift with Static Mixers

The most recent FP-PBR is the Flat Panel Airlift (FPA), which was created lately by Subitec GmbH Co. With this setup, the advantages of an internal airlift loop reactor and a flat-plate reactor are combined. The internal static mixers are formed by welding two half-shells made of deep-drawn plastic together. The culture broth is moved upward and then downward in a loop via vertical downcomers in a fluid motion that is based on the airlift-loop reactor principle, in which injected air or gas generates a pressure differential. While avoiding the generation of considerable shear forces close to the microalgae cells, this airlift operation ensures the turbulence required for effective gas exchange between the cells and dissolved gases. Cells can receive ideal light because of the system's thin layers and the fluid recirculation created by the stationary mixers (Al-nazwani et al., 2021; Penloglou et al., 2024).

5.1.4 Other PBR Designs

5.1.4.1 Torus-Type PBR

Torus-type PBRs are specially made for researching the effects of mixing on phototrophic microorganisms and the growing of microalgae to produce hydrogen. This type of system usually has a transparent material circular design with a liquid flowing in a continuous loop. Light is provided by external tubes that run parallel to the lit surface. Among its main benefits is that it can efficiently distribute liquids thanks to a marine propeller that stirs things up and causes Dean's vortices to form in the reactor's bends. Good mixing efficiency can be attained even at low impeller speeds, but the flow around the mechanical stirrer is still heterogeneous and may result in some shear stress (please see Fig. 4) (Ashok et al., 2019).

5.2 Strategies for Optimizing Light Utilization Efficiency in Photobioreactors

For the commercial production of diverse algal biochemicals, efficiently turning solar energy into useful bioproducts continues to be a major problem. Chlorophyll has a high light-harvesting efficiency, which allows microalgal cells to collect all incident photons from the visible range. All absorbed photons, however, are not used for photosynthesis; the maximum growth output is reduced the wider the light

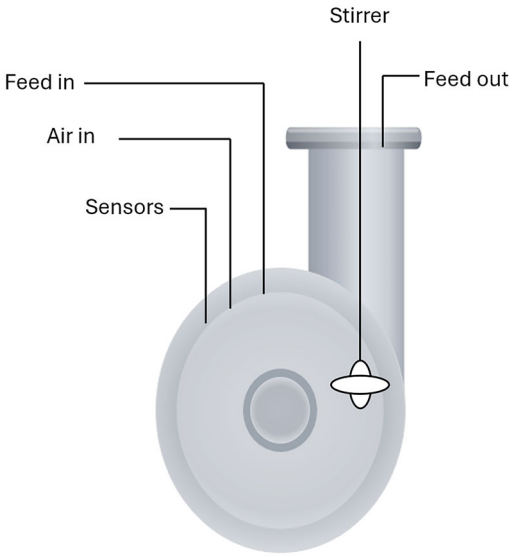


Fig. 4 Schematic representation of torus reactor (Adapted and modified from Ashok et al., 2019)

spectrum is, the lower the utilization efficiency. For that carbon fixation reactions can be improved by increasing light use efficiency (Nwoba et al., 2019).

5.2.1 Spectral Selection and Filtration

Microalgae's action spectra, which show which wavelengths they respond to best for photosynthesis, are used to gauge how well-absorbed photons are used. Many pigments used by microalgae to gather light are capable of absorbing photon energy in the 400–700 nm range of photosynthetically active radiation (PAR). Nevertheless, the wavelengths at which photosynthesis occurs are mostly determined by the unique characteristics of these pigments. Of all the sunlight that reaches Earth, 43% is made up of PAR wavelengths, which make up 28% of solar photons. Red (600–700 nm) and blue (400–525 nm) wavelengths are the most effective in promoting photosynthesis, and these are the wavelengths that chlorophylls a and b mainly absorb. With the use of wavelength filtration technologies, algal photosynthesis can be provided with a specified light spectrum (either the entire range or selected regions of PAR). Two crucial factors affect spectral selection (Nwoba et al., 2019). Customizing the precise wavelengths supplied to the culture media can not only provide a more efficient amount of PAR to the culture but also greatly minimize heat and UV radiation absorption. This decrease should lessen concerns about UV-induced cell damage and lessen the strain that warmth puts on the cultures, which will lessen the need for additional cooling systems (Yuan et al., 2020).

5.2.2 Solar Tracking Devices

Latitude, air scattering, and weather all affect solar resource availability. Because PBRs are the only source of photons that microalgal cultures can use, PBR placement plays a critical role in maximizing biomass productivity through optimal light management. PBR orientation (horizontal, vertical, or slanted) plays a crucial role in cultivation systems that have large surfaces that receive light to the length of the light path. In outdoor cultivation systems, horizontal reactors oriented north-south gather less solar energy than vertically positioned PBRs directed east-west, by an average of over 5% (Shang & Shen, 2023). Theoretically, these devices might be used to improve photon capture and boost photosynthetic efficiency in systems that grow algae. Solar trackers are movable gadgets that track the sun's location according to the season and time of day, increasing the efficiency of solar energy collecting. To optimize the amount of solar energy that PBRs can capture and gather, these devices move both horizontally and vertically. Compared to an open reactor, a solar-tracked reactor collects 69% more solar radiation. To achieve maximum production, the reactor must intercept as much light as possible. Microalgae cultures may suffer from photoinhibition and photooxidation as a result of extended exposure to high irradiance (Nwoba et al., 2019).

6 Conclusions and Perspectives

The classic or common configurations of different devices that are used for microalgae cultivation not always reach to the best performance. Main bottlenecks have been identifying among the use, such as light irradiance, mixing, nutrients supply, and gas transfer. For that, recent studies have been focused on the main problems and have proposed a new configuration in photobioreactors. Different and numerous photobioreactors have been designed for experimental, but very few have been used in a scale-up process. It is important to mention that before starting any process is required to verify the final product, because it provides a general idea what are the specifications and configurations to perform and reach to the maximum yield.

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Microalgae-Derived Biohydrogen



Walter José Martínez-Burgos, Andrea Monroy-Licht, Guilherme Anacleto dos Reis, Josilene Lima Serra, Roberta Pozzan, Hissashi Iwamoto, Adriane Bianchi Pedroni Medeiros, Carlos Ricardo Soccol, Ihana Aguiar Severo, Juan Carlos Ordóñez, José Viriato Coelho Vargas, and André Bellin Mariano

Abstract The increasing demand for clean and renewable energy sources has led to the exploration of hydrogen as a sustainable energy carrier due to its high energy content and eco-friendly by-products. However, traditional hydrogen production methods face environmental and energy efficiency challenges due to their reliance on fossil fuels. Biomass, recognized as a carbon-neutral and versatile energy source, offers a sustainable alternative to hydrogen production. Microalgae emerge as a promising candidate among biomass options due to their high growth rates, minimal land requirements, and adaptability to different environments. Microalgae can

W. J. Martínez-Burgos (✉) · G. A. dos Reis · H. Iwamoto · A. B. P. Medeiros · C. R. Soccol
Department of Bioprocess Engineering and Biotechnology, Federal University of Paraná, Centro Politécnico, Curitiba, PR, Brazil

A. Monroy-Licht
Chemistry and Biology Group, Chemistry and Biology Department, Universidad del Norte, Barranquilla, Colombia

J. L. Serra
Federal Institute of Maranhão, São Luís, MA, Brazil

R. Pozzan
Laboratory of Cell Toxicology, Department of Cell Biology, Federal University of Paraná, Centro Politécnico, Curitiba, PR, Brazil

I. A. Severo
Department of Mechanical Engineering, FAMU-FSU College of Engineering, Energy and Sustainability Center, Center for Advanced Power Systems (CAPS), Florida A&M University, Florida State University, Tallahassee, FL, USA

Sustainable Energy Research & Development Center (NPDEAS), Federal University of Paraná (UFPR), Curitiba, PR, Brazil

J. C. Ordóñez
Department of Mechanical Engineering, FAMU-FSU College of Engineering, Energy and Sustainability Center, Center for Advanced Power Systems (CAPS), Florida A&M University, Florida State University, Tallahassee, FL, USA

J. V. C. Vargas · A. B. Mariano
Sustainable Energy Research & Development Center (NPDEAS), Federal University of Paraná (UFPR), Curitiba, PR, Brazil

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produce biohydrogen under specific physiological conditions, making them a key focus for renewable hydrogen production. Therefore, this chapter provides an overview of biohydrogen production from microalgae, examining their characteristics, cultivation systems, metabolic pathways, and recent advances in improving hydrogen yields. Additionally, it explores biomass harvesting techniques, processing methods, and future perspectives for the large-scale application of microalgae in sustainable biohydrogen production.

Keywords Microalgae · Biophotolysis · Photobioreactors · Genetic engineering · Biohydrogen production · Biofuel

1 Introduction

The search for clean and renewable energy sources has gained significant momentum in recent decades due to concerns about climate change, fossil fuel depletion, and the environmental impact of conventional energy production. Hydrogen, as a clean energy carrier, offers considerable potential as part of a sustainable energy system due to its high energy content and the fact that it yields exclusively water as a by-product. In addition, hydrogen is non-toxic, odorless and colorless, and it has a very high heating value, of approximately 120 MJ/kg. It also has characteristics such as controllability, safety, and low pollution (Da Rosa & Ordonez, 2021).

Traditional hydrogen production methods, such as steam methane reforming (SMR), autothermal reforming (ATR), coal gasification (CG), and even water electrolysis, have several limitations and environmental impacts, such as high energy consumption and dependence on fossil fuels (Jiao et al., 2024). Biomass is considered a carbon-neutral energy source and a sustainable alternative to traditional fuels such as coal, oil, and natural gas, which are the three main global energy sources and account for more than 14% of global energy consumption (Raugei & Leccisi, 2016). Unlike other renewable energy sources, biomass is unique in its versatility; it serves as the only renewable carbon resource that can be efficiently converted into solid, liquid, or gaseous fuels through various conversion processes (Jiménez-Llanos et al., 2020). Biohydrogen, biodiesel, biogas, and bioethanol are examples of biomass-based energy produced through biochemical conversion (Rok Lee et al., 2024).

Among biomass-based energy strategies, microalgae are particularly promising due to their high-value biomass and potential for biofuel production. They are photosynthetic microorganisms capable of converting sunlight, CO_2, and nutrients into products such as biohydrogen. Microalgae are characterized by high growth rates, minimal land requirements, and the ability to grow in diverse environments such as fresh and salt water, making them suitable for sustainable hydrogen production (Deprá et al., 2018; Severo et al., 2019; Li et al., 2022). Additionally, microalgae can be grown in a variety of systems, including open ponds and closed photobioreactors, which provide different levels of control over growth conditions and hydrogen productivity (Corrêa et al., 2017; Dias et al., 2023). Biohydrogen production from microalgae is possible under certain physiological conditions, such

as an oxygen-deficient environment and high light exposure. The potential of microalgae to produce biohydrogen through different biochemical pathways can be exploited to increase their production (Nagarajan et al., 2021; Wang et al., 2021).

In this sense, this book chapter presents a comprehensive overview of biohydrogen production from microalgae, highlighting their characteristics, growth conditions, cultivation systems, metabolic pathways, recent strategies to enhance hydrogen production, harvesting and processing of microalgal biomass, and finally applications and future perspectives.

2 Characteristics and Growth Conditions of Microalgae

Microalgae are photosynthetic eukaryotic microorganisms that can reach various sizes; they can be unicellular or form multicellular colonies. Within this group, microalgae are the type of algae that has aroused the most interest in exploring clean energy. In general, about 50,000 different species of microalgae are recognized (S. Li et al., 2013).

These microorganisms are classified into six divisions based on their cellular structure, which is the most commonly used categorization scheme. These groups are *Pyrrophyta, Chlorophyta, Chrysophyta, Euglenophyta, Chlorophyta*, and *Rhodophyta*. Microalgae share similarities with terrestrial plants in terms of photosynthetic processes (Ahmad et al., 2022). However, their simpler cell form allows them to have more direct contact with water, CO_2, and other nutrients. Associated with these characteristics, they can use solar energy more efficiently than higher plants and produce a greater amount of biomass (Callegari et al., 2020).

Microalgae can be cultivated in photoautotrophic, heterotrophic, and mixotrophic modes, with photoautotrophic cultivation being the most prevalent (Arun et al., 2022). In this mode, microalgae utilize carbon dioxide or bicarbonates as carbon sources and synthesize organic matter through photosynthesis under light conditions (Sharma et al., 2021). Under controlled environmental conditions, a large number of microalgae produce mainly hydrocarbons, triacylglycerol, and neutral lipids, which are the raw materials for different biofuels (Sacristán de Alva et al., 2018).

The most significant factors influencing microalgae growth (biomass accumulation) to produce biohydrogen, bioethanol, and other biofuels of interest include temperature, pH, light intensity, and the type and amount of nutrients. The ideal ranges for these parameters vary depending on the species and are mutually dependent (Rathinavelu et al., 2024). Table 1 shows examples of microalgae growth conditions in hydrogen production studies conducted at laboratory scales, using different substrates. In some cases, these studies combine microalgae with bacterial activity. Below are some systems that have been used to cultivate microalgae at different scales to stimulate greater efficiency in biomass generation.

Table 1 Examples of microalgae growth conditions in laboratory-scale hydrogen production studies using various substrates and bacterial activity

Algae (organism strains)	pH	Temperature	Light intensity	Photoperiod	Shaking	Initial biomass concentration	Growth medium	H_2 production rate or accumulation	Type of cultivation	Reference
Chlorella sp, supplemented with *Bacillus amyloliquefaciens* (Bacteria as promoters of algal biomass)	7.5	25 °C	50 µmol photons $m^{-2}{\cdot}s^{-1}$	16 h/8 h	180 rpm	10×10^7 cell/mL (algae) 10×10^5 cell/mL (Bacteria)	SWW[a]	40 $mLH_2/L.\ d^{-1}$	PBR (Lab-scale)	Hupp et al. (2023)
Chlorella pyrenoidosa	7.2	25 °C	2000 Lux	12 h/12 h	–	$OD_{750} \geq 0.3$ (10 mg/mL)[b]	TAP medium supplemented with 50 mM glucose[c]	14.97 mmol H_2/L	PBR(Lab-scale using flocculation method)	Chen et al. (2024)
Chlamydomonas reinhardtii with *Mesorhizobium sangaii* (facultative aerobic and nitrogen fixing bacteria)	7.0	25 °C	120 µmol photons $m^{-2}{\cdot}s^{-1}$	14 h/10 h	180 rpm	$OD_{600} \geq 1.0$	TAP medium supplemented with 3 g/L $NaNO_2$	67.46 µmol/mg chl a	PBR(Lab-scale)	Yu et al. (2021)
Dunaliella primolecta and *D. tertiolecta* (used as substrate for dark fermentation by *Thermococcus eurythermalis)*	7.0	25 ± 2 °C (algae cultivation for increased biomass yield); 85 °C for dark fermentation	4000 Lux (algae cultivation for increased biomass yield)	–	–	10×10^6 cell/mL (bacteria)	TRM supplemented with lipid-extracted *Dunaliella* residue[d]	192.35 mLH_2/g VS[e] (*D. primolecta*) 183.02 mLH_2/g VS[e] (*D. tertiolecta*)	Dark fermentation in anaerobic conditions	Chen et al. (2020)

Chlorella vulgaris and *Scenedesmus obliquus*	7.5	25 °C	140 μE $m^{-2}·s^{-1}$	Continuous irradiance	100 rpm	10×10^7 cell/mL (algae)	SWW[a]	128 mLH_2/L d (*S. obliquus*);60.4 mLH_2/L. d (*C. vulgaris*)	first stage: growth photosynthetically under normal cultivation conditions; the second stage: anaerobic conditions and sulfur is limited.	Ruiz-Marin et al. (2020)
Spirullina platensis hydrolysate (1% w/v); *Rhodobacter sphaeroides* and *Bacillus firmus*	I Stage: 6.5 II Stage: 7.0	32 ± 2 °C	I Stage: no light II Stage: 2.5 klux	II Stage: continuous irradiance	100 rpm	Inoculum volume-20.0% (v/v) in both stages	I Stage: nutrient medium supplemented with 1% w/v cyanobacterial biomass hydrolysate of *S. platensis* as carbon source supplement	23.06 ± 3.63 mmol H_2/L (first stage);1510 ± 35 mL H_2/L (second stage)	Sequential dark fermentative (I-stage) and photofermentative (II-stage)	Pandey et al. (2021)
Chlorococcum minutum	7.0	25 °C	2000 Lux	Continuous irradiance	140 rpm	Log phase	TAP medium supplemented with 0.8 mM Na_2SO_3	310 ± 3.0 μmol H_2/L	PBR (Lab-scale)	Paramesh and Chandrasekhar (2020)
Chlamydomonas reinhardtii	7.0	24 ± 2 °C	180 μE $m^{-2}·s^{-1}$	Continuous irradiance	–	3×10^7 cell/mL (algae)	TAP medium supplemented with 0.4 mM of Triton × 100; 0.05 mM MV+21 and 0 mg/L Chloramphenicol	216 μmol H_2/L	Photoelectrocatalytic-enzymatic hybrid system	Chatzitakis et al. (2013)

[a]*SWW* Synthetic Wastewater with the following concentrations (mg/L): NaCl, 7; $CaCl_2$, 4; $MgSO_4.7H_2O$, 2; KH_2PO_4, 15 and NH_4Cl, 115 (Ruiz-Marin et al., 2010)

[b]The growth of the *Chlorella pyrenoidosa* was monitored by measuring the absorbance at 750 nm wavelength (OD750)

[c]*TAP* medium Tris/acetate/phosphate

[d]*TRM* Thermococcales Rich Medium

[e]*VS* volatile solid

2.1 *Types of Microalgae Culture Systems*

Open ponds are good systems for growing microalgae, which are used for treating wastewater. They are easy to operate and inexpensive. However, they can be easily contaminated and have low productivity. Additional nutrients, such as phosphorus, iron, nitrogen, and rarely silicon, must be added in the case of open tanks (Dubini & Ghirardi, 2015). On the other hand, photobioreactors, closed systems, are typically employed in producing pharmaceuticals and valuable products (Bennett et al., 2014). Due to their increased control over operational variables and nutrients and higher biomass productivities, photobioreactors outperform open pond reactors (Deprá et al., 2021). Figure 1 illustrates the different photobioreactor systems.

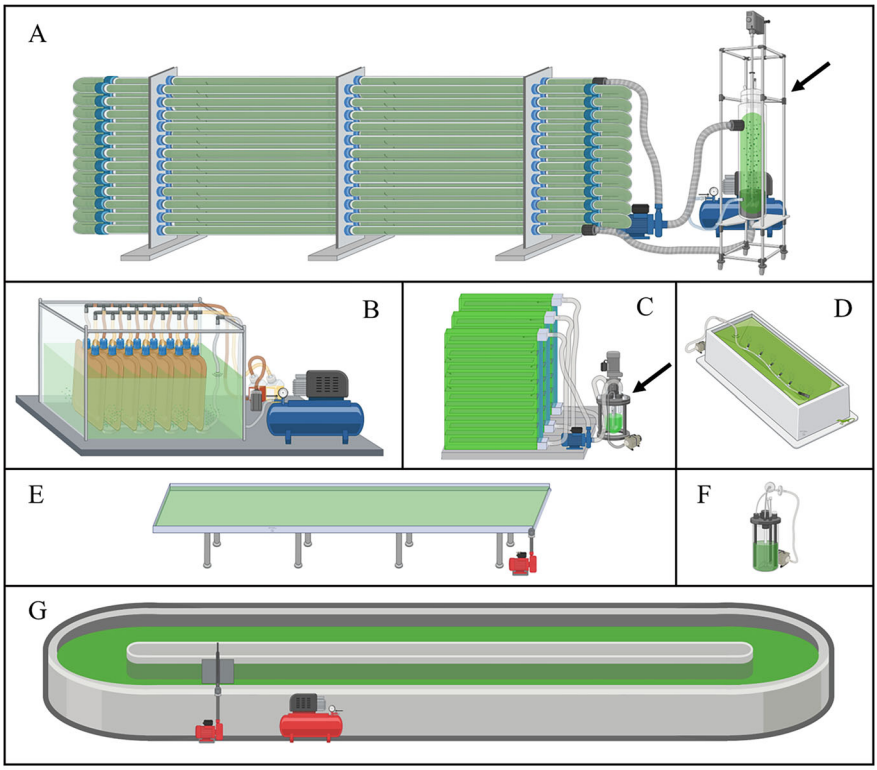


Fig. 1 Illustration of different types of photobioreactors. (**a**) Tubular photobioreactor. (**b**) Membrane photobioreactor. (**c**) Flat plate photobioreactor. (**d**) Pond photobioreactor. (**e**) Thin layer photobioreactor. (**f**) Conventional bench-scale photobioreactor. (**g**) Raceway photobioreactor. The black arrows are the gas exchange systems commonly used in closed and large systems

2.1.1 Unstirred Ponds and Circular Ponds

Non-stirred ponds and circular ponds represent simple culture systems (Fig. 1f). They include open systems and lakes. They are economical culture processes that use evaporative cooling, eliminating the need for refrigeration systems (Ranga Rao et al., 2012). However, their main disadvantage is that they are only useful for organisms with good adaptability that can grow well by dominating other microorganisms. They have wide growth ranges in terms of temperature, pH, oxygen, and CO_2, since these parameters are not easily controllable under these operating conditions. *Dunaliella salina* and *Dunaliella tertiolecta* are organisms that show a high capacity for acclimatization. It can grow under conditions of 10% NaCl, maintaining high photosynthetic rates (Kumar et al., 2018).

2.1.2 Raceway Ponds

Raceway ponds are surface structures open to the environment, in which water and nutrients are kept in continuous movement by paddles (Fig. 1g). This is the most popular open pond reactor setup for large-scale microalgae production (Borowitzka, 1999). The advantage of these systems is their low construction and operating cost, suitable for wastewater treatment and large-scale biomass production. The system allows the growth of *Chlorella, Spirulina, Dunaliella,* and *Haematococcus* very efficiently (Shekh et al., 2021), depending on the nutrients required for each species. This technique can be used to grow algae from various wastewater sources (Shekh et al., 2021). However, light intensity is a challenge, and the growth rate can be affected by seasonal and geographic variations in temperature, and evaporation and infiltration of CO_2 from the atmosphere can cause losses. Generally, a considerable amount of land is needed. Low biomass production as a result of insufficient agitation presents another difficulty for mass transfer (Ahmad et al., 2022).

2.1.3 Tubular Photobioreactors (TPBRs)

TPBRs are bioreactors made of transparent tubes (glass or polyethylene) in the form of a serpentine loop (Fig. 1a), used for microalgae cultivation (Molina et al., 2001). These reactors are closed devices that have a high surface-to-volume ratio, which improves the light exposure of the microalgae (Fernández del Olmo et al., 2022). Their design includes a gas exchange system (Fig. 1a, pointed with a black arrow) that supplies CO_2 and cools the water, and their efficient radial mixing helps distribute light evenly, reducing the shading effect and increasing radiation intensity (Arbib et al., 2013). As its design improves light exposure and photosynthesis, it minimizes photoinhibition (Assunção & Malcata, 2020). The radiated mixture generates an efficient distribution of nutrients and gases. *Arthospira sp*. grows very well in such systems (Ravishankar & Ambati, 2019).

However, inadequate mass transfer can occur, which can produce large amounts of dissolved oxygen (DO), adversely affecting the algae. Photo-inhibition can occur, especially in outdoor TPBRs due to high light exposure. It is a challenge to control the temperature in the system. It has also been observed that cells tend to adhere to the walls of the tubes. Gas transfer gradients can occur in long TPBRs involving oxygen and CO_2 (Huang et al., 2017).

2.1.4 Flat Panel Photobioreactor (FPBR)

The FPBR is a rectangular version of conventional bioreactors used for indoor and outdoor algae cultivation (Fig. 1c). It may require a certain amount of artificial light. It is designed to optimize the use of solar energy and is built with transparent materials such as glass, plexiglass, polycarbonate, and plastic bags (Suparmaniam et al., 2019). FPBRs are relatively affordable, although they accumulate dissolved oxygen more slowly than horizontal tubular photobioreactors. This is due to its high-lighting surface, which facilitates efficient light distribution and penetration (Behera et al., 2019). Among the species that grow best in this type of system are *Arthospira platensis, Chlorella sorokiniana, Spirulina sp*., and *Arthospira platensis* (Aslam et al., 2019).

Despite their photosynthetic efficiency, FPBRs face photoinhibition risks, especially in outdoor or early-stage cultures. They are suitable for large-scale algae production and are used to treat wastewater with low organic load and high light transparency, such as in urban and residential waters. They have difficulties in temperature control. A critical parameter in their design is the hydraulic retention time (HRT), due to the self-shading effect in dense cultures that reduces biomass production and nutrient uptake (Sarker, 2022).

2.1.5 Membrane Photobioreactor (MPBR)

MPBRs (Fig. 1b) improve the mass transfer of gases and liquids by avoiding turbulence and taking advantage of the large surface area provided by the membranes (Kumar et al., 2018). They stabilize the production process by continuously removing extracellular metabolites. This process is very efficient in nitrogen and phosphate removals and promotes high biomass proliferation (Bilad et al., 2014).

2.1.6 Thin Film Photobioreactor

The goal of AlgoFilm© PBR technology is to maximize light conversion to achieve very high volumetric efficiency. It increases the specific surface area that is lit and the photon flux density by applying the thin film technique to form a layer that is between 1.5 and 2 mm thick (Chuka-Ogwude et al., 2021). For a surface area of 2 m^2, these reactors (Fig. 1e) have a highlighted particular area of 500 m^2 (Yan et al.,

2016). This enables 5.7 kg m^{-3} day^{-1} of biomass yield, which is 3–6 times greater than photoautotrophic production and nearly equal to the heterotrophic process. For example, *Chlorella sp*. improved its biomass and productivity when grown under this method (Pruvost et al., 2017).

2.1.7 Vertical Photobioreactors (VPBRs)

VPBRs are vertical bioreactors designed for microalgae cultivation, with a structure that facilitates mixing and light exposure, even at high cells. The design includes an upper section for gas/liquid separation and an air sparger at the bottom that supplies air or CO_2, generating bubbles that create turbulence and mix the reactor contents (Cuaresma et al., 2011). These bubbles help to mix the contents and improve the exposure of the cells to light. The top of the reactor allows separation of the gas from the liquid. The gaseous liquid rises through the inner column, while the degassed liquid descends from the outside. Among its advantages are efficient mixing, simple design, and easy cleaning (Vargas et al., 2019; Catrawedarma et al., 2021). This system has the disadvantage that although it improves mixing, the design can be more complex and costly to implement. On the other hand, maintaining optimal light and gas conditions can be a challenge. Variations in gas concentration along the column can affect culture performance (Ahmad et al., 2022).

Typically, the goal of these systems is to promote a photoautotrophic metabolism. However, in the case of heterotrophic culture, a high surface-to-volume ratio is not necessary, and the design of the bioreactor is greatly simplified (Hu et al., 2012). For instance, when grown with various carbon and nitrogen sources, *Nannochloropsis salina* produced more biomass (Marudhupandi et al., 2016). Heterotrophic microalgae obtain their carbon from organic sources, whereas autotrophic organisms obtain theirs from CO_2. The cost of the procedure is raised by these organic ingredients. Systems have concentrated on producing microalgae in wastewater to offset increased process costs (Yu et al., 2020).

2.1.8 Mixotrophic Cultivation Systems

Mixotrophic culture is the method to increase the productivity of microalgae culture by combining photoautotrophic and heterotrophic metabolisms. These organisms can thrive in both photoautotrophic and heterotrophic environments due to their ability to digest organic and inorganic carbon in this type of culture (Hu et al., 2012). Mixotrophic culture has been observed to result in faster growth rates and higher CO_2 fixation (Baldisserotto et al., 2014). In general, anaerobically digested wastewater and sludge have a low C/N ratio. However, by adding acetic and n-butyric acid to raise this ratio to 10, it is possible to control the amount of nutrients from the nitrogen and phosphorus families, while increasing biomass and lipid production. This high metabolic capacity is what distinguishes microalgae as highly promising organisms (Rajendran et al., 2020).

3 Metabolic Pathway for Hydrogen Production

Microalgae are capable of fixing CO_2 and producing H_2 naturally through biophotolysis. They use different biological mechanisms to produce hydrogen gas as part of their metabolic processes. These mechanisms can be categorized into direct and indirect photolysis, dark fermentation, and photo-fermentation. Each of these routes has its own unique pathways and conditions, and therefore, understanding them is critical to optimizing hydrogen production in microalgae-based systems (Nagarajan et al., 2021).

Table 2 summarizes the main advantages and disadvantages of the different biological pathways to produce hydrogen from microalgae. The ability of these microorganisms to produce H_2 via biophotolysis is influenced by various factors, including the characteristics of the microorganisms, environmental conditions, enzyme activity, metabolic pathways, and the biorefinery process (Show et al., 2018). Microalgae release H_2 and O_2 gases via biophotolysis process. Equation 1 represents the process by which H_2 is generated via direct photolysis of water in anaerobic conditions (Bergene, 1996).

$$2H_2O + \text{Light Energy} \rightarrow 2H_2 + O_2 \quad (1)$$

Chlamydomonas sp., *Chlorella* sp., and *Scenedesmus* sp. are species known to produce hydrogen either through direct photolysis (DP) or indirect photolysis

Table 2 Advantages and disadvantages of hydrogen production mechanisms

Mechanism	H_2 production yield	Duration cycle	Advantages	Disadvantages
Direct biophotolysis	1.2–73.5 mL/L	10 days (depending on microalgal species)	Sunlight and water are used directly to produce H_2	Low productivity and inhibitory effect of H_2
Indirect photolysis	6.5–243 mL/L	148–168 h	Cost-effective, simple growing medium and overcomes O_2 sensitivity	Inhibitory effect of H_2 on nitrogenase activity; low yield
Dark fermentation	16–135 mL/H_2/g VS	48–72 h	Light and O_2 requirement are not mandatory, and a variety of substrates can be used as carbon sources, generating many by-products	Low efficiency of substrate conversion and production of CO_2 and H_2S
Photo-fermentation	125 mL/g VS	96–120	High substrate transformation efficiency and use of different organic biomass	Low H_2 yield, light requirements and need for large areas and specific reactor

Adapted from: Jiao et al. (2024) and Khetkorn et al. (2017)

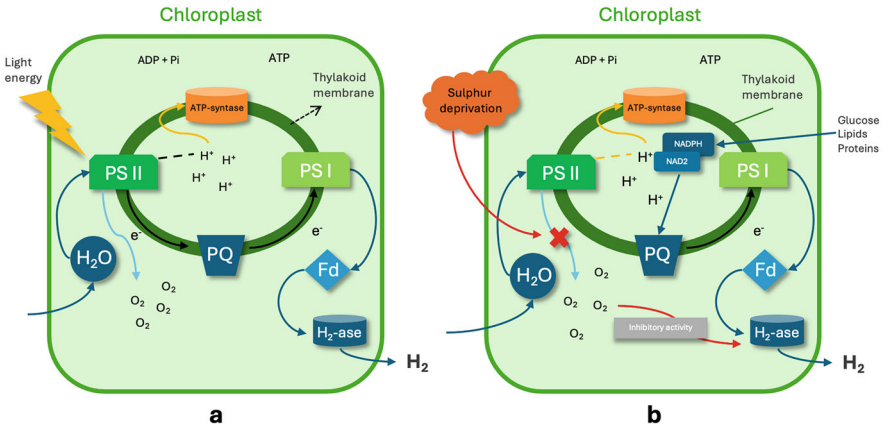


Fig. 2 A summarized representation of the H_2 pathway in microalgae through photolysis. (**a**) represents the DP of H_2 using light energy to drive electron movement; (**b**) represents the IP of H_2 by organic carbon storage materials to drive electron movement; sulphur deprivation halts the PSII activity and reduces the level of O_2. Fd ferrodoxin, H_2-ase hydrogenase, PQ plastoquinone, PS photosystem, NAD2 nicotinamide adenine dinucleotide, e^- electron, H^+ protons, O_2 oxygen

(IP) mechanisms (Nagarajan et al., 2021), as illustrated in Fig. 2. The DP, also known as the light-dependent pathway (Fig. 2a), is a natural process in microalgae comparable to photosynthesis that involves two photochemical oxidation reactions as described by Show et al. (2018). In the first step, sunlight activates Photosystem II (PSII), leading to the splitting of water (H_2O) into protons (H^+), electrons (e^-), and oxygen (O_2) with the help of the enzyme H_2O-plastoquinone oxidoreductase, which is located in the thylakoid membrane. The electrons then pass through Photosystem I (PSI) and ferredoxin (Fd). In the second step, the electrons are transferred from ferredoxin to hydrogenase, which produces molecular hydrogen (H_2). The movement of these electrons is driven by light energy. Additionally, the protons generated from water oxidation create a proton gradient that ATP synthase uses to produce ATP. This process is advantageous because it does not require additional nutrient sources (such as carbon) or external ATP for stability and can be regulated by solar energy. However, it is enzyme-dependent, and the hydrogenase enzyme is sensitive to oxygen, functioning optimally in low-oxygen or anaerobic conditions (Goswami et al., 2021; Show et al., 2018). The reactions involved in the DP are described below, according to Eqs. 2 and 3 (Fay, 1983; Bergene, 1996):

$$2H_2O + \text{Sunlight} \rightarrow 4H^+ + 4e^- + O_2 \quad (\text{Photosynthesis reaction}) \tag{2}$$

$$4H^+ + 4e^- \rightarrow 2H_2 \quad (\text{Hydrogen production reaction}) \tag{3}$$

The IP (Fig. 2b) overcomes the limitations of DP through a strategic approach. The CO_2 fixation for internal carbon storage reduces in-situ O_2 and sets a better condition for the H_2 synthesis. The O_2 levels halt the e^- transport chain. The energy for e^-

movement is obtained from the catabolism of biomolecules. The e^- are transferred to plastoquinone and then transferred towards the hydrogenase enzyme for IP production (Jiménez-Llanos et al., 2020). In addition, sulphur deprivation conditions inhibit the oxygenic photosynthesis in PSII and reduce the O_2 level. As a result, it reduces photosynthesis activity and switches to the respiration path. The complete anaerobic condition is created as the in-situ O_2 and mitochondrial activity decreases. This condition is favorable for IP for H_2 production (Goswami et al., 2021; Show et al., 2018). The reactions involved in the IP are described below, according to Eqs. 4 and 5:

$$6H_2 + 6CO_2 + \text{Solar energy} \rightarrow C_6H_{12}O_6 + 6O_2 \quad \text{(Aerobic reaction)} \tag{4}$$

$$C_6H_{12}O_6 + 6H_2O + \text{Solar Energy} \rightarrow 6CO_2 + 12H_2 \quad \text{(Anaerobic reaction)} \tag{5}$$

Dark fermentation refers to the anaerobic breakdown of organic compounds in the absence of light to produce hydrogen and represents a promising method for biohydrogen generation due to its simplicity and the ability to utilize a wide range of organic feedstocks. In this process, microalgae use a series of metabolic pathways and enzymes to ferment organic substrates such as carbohydrates, lipids, and proteins, resulting in the production of hydrogen gas and organic acids (Ahmed et al., 2021). Typically, the breakdown of glucose serves as the primary pathway, represented by the following simplified reaction (Eq. 6):

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \tag{6}$$

Hydrogenases play a crucial role in dark fermentation by mediating the reversible conversion of protons and electrons into hydrogen. The two main types of hydrogenases found in microalgae are [FeFe]-hydrogenases and [NiFe]-hydrogenases, which mediate the transfer of electrons from reduced ferredoxin or NADPH to protons, resulting in hydrogen production (Li et al., 2022). The efficiency of these enzymes in generating H_2 depends on their stability, electron transfer rates, and the conditions within the microalgal cells. In addition, the efficiency of dark fermentation for hydrogen production is influenced by several factors, such as enzymatic stability, substrate availability, pH, temperature, and microbial community composition. However, low hydrogen yields and competition for substrates with other metabolic pathways pose significant challenges for hydrogen production via dark fermentation in microalgae. Optimization of these parameters can significantly increase hydrogen yields. For instance, maintaining an optimal pH range (typically between 5.5 and 6.5) and temperature (mesophilic or thermophilic conditions) can favor hydrogenase activity and maximize hydrogen production. Additionally, substrate selection and pre-treatment could improve the availability of fermentable sugars, thereby enhancing the efficiency of the fermentation process (Show et al., 2019b; Jiao et al., 2024).

Finally, unlike dark fermentation, which occurs in the absence of light, photofermentation is a type of fermentation that combines the light-dependent reactions of photosynthesis with the anaerobic metabolism of organic matter (such as

carbohydrates, organic acids, or waste biomass) to produce biohydrogen and CO_2. In general, photo-fermentation exploits the synergistic effects of anaerobic and light-dependent pathways to increase hydrogen production rates compared to dark fermentation alone, using nitrogenase enzymes to catalyze the reactions (Li et al., 2022). Equation 7 represents the photo-fermentation reaction:

$$CH_3COOH\ (\text{acetic acid}) + H_2O + \text{Sunlight} \rightarrow 2CO_2 + 4H_2 \quad (7)$$

The photo-fermentation process for biohydrogen production by microalgae is gaining more attention due to the wide range of renewable organic compounds that can be used as substrate sources, offering potential applications in bioenergy and bioremediation. However, process optimization strategies such as modulation of light conditions, nutrient availability, and microbial community composition should be improved to achieve better biohydrogen production yields (Putatunda et al., 2023).

4 Strategies for Enhancing H_2 Production

In the biotechnology field, microalgae represent a promising source for producing valuable inputs such as proteins, enzymes, nutraceutical foods, and biofuels such as biohydrogen. Genetic engineering has been an important tool to achieve cheap, efficient, and large-scale production. Among the most diverse applications are the overexpression, knockdown, and knockout of specific genes (Khatodia et al., 2016). Thus, genetic engineering offers a way to optimize the genome and the biosynthetic pathways of microalgae to increase the rate and yield of metabolites of interest (Ng et al., 2017).

There are currently many studies and papers in the scientific literature on editing the genome of microalgae to produce bioproducts. However, compared to editing the genetic material of plants and animals, studies are still at an early stage and are also restricted to laboratories. The following is an analysis of the main gene editing tools that are revolutionizing the world and driving the development of research in biohydrogen-producing microalgae (Fayyaz et al., 2020).

4.1 The Main Genetic Engineering Technologies

4.1.1 RNAi

Ribonucleic acid interference, better known as RNAi, is an effective tool for silencing genes. It is considered a complementary RNA to messenger RNA since it can inhibit gene expression during translation or hinder gene transcription.

However, this tool has some disadvantages such as inducing off-target gene alterations, silencing desired phenotypes, and incomplete repression of target genes (Boettcher & McManus, 2015).

4.1.2 Zinc Finger Nucleases (ZFNs)

To develop new approaches to manipulating and optimizing genetic material, researchers have developed programmable sequence-specific nucleases (SSNs). These enzymes can cut the genetic material at specific locations so that specific deletions, substitutions, or insertions can be made in the genome. One type of these enzymes is zinc finger nucleases (ZFNs). They are based on proteins derived from an endonuclease from the *Flavobacterium okeanokoites* strain, called *Fok I* (Kim et al., 1996). It works by having a DNA-binding domain and a non-specific domain at the N-terminus and C-terminus, respectively. These endonucleases are obtained by fusing zinc finger proteins to the C-terminus of *Fok I* (Durai et al., 2005).

4.1.3 Transcription Activator-like Effector Nuclease (TALENs)

TALEN enzymes were first discovered in phytopathogenic bacteria of the genus *Xanthomonas* (Nemudryi et al., 2014). Structurally, these proteins have three domains, where the first is DNA binding, the second transcriptional activation, and the third is nuclear localization signal (NLS). TALENs can be made up of 12–27 matrices (Boch & Bonas, 2010). Like ZFNs, TALENs are also designed by fusing the DNA-binding domain with the *Fok I* cleavage domain (Joung & Sander, 2013). Because of this, TALENs together with ZFNs are widely used in gene editing, including microalgae such as *Chlamydomonas reinhardtii* (Gao et al., 2014).

4.1.4 CRISPR/Cas9

CRISPR/Cas9 consists of two molecules, a nuclease (Cas9) capable of cutting DNA in a specific region and a small piece of RNA sequence (CRISPR RNA or crRNA) located inside a longer RNA structure. This RNA molecule is what directs Cas9 to the part of the genome to be cut (Gasiunas et al., 2012; Jinek et al., 2012). CRISPR/Cas9 has received a lot of appreciation in scientific literature because of its efficient results, easy handling, and accurate results (Jeon et al., 2017). It works in a similar way to RNA, where the activity of the target gene can be suppressed or increased by fusing the effector molecule to dCas9/sgRNA (Piatek et al., 2015). In addition to gene editing, CRISPR/Cas9 can also be used to characterize new genes. In the scientific literature, you can find many studies where it has been used to increase the production of lipids, pigments, and biohydrogen in microalgae (Naduthodi et al., 2019).

4.2 Applications of Genetic Engineering

4.2.1 Increased Carbohydrate Content

In biohydrogen production, optimizing the ability to fix carbohydrates to increase the amount of biomass produced is very important. A great way to achieve this is through the overexpression of enzymes involved in carbohydrate synthesis, the knockout of carbohydrate suppressor genes, and the knock-in of genes related to carbohydrate synthesis (Barati et al., 2021).

A well-known enzyme related to this property in photosynthetic cells is ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco). Increasing the activity of this specific enzyme improves carbon fixation. In a study aimed at increasing Rubisco activity, the Rubisco Activase (RCA) enzyme gene was overexpressed, which is responsible for hydrolyzing adenosine triphosphate (ATP) molecules to remove inhibitors from the Rubisco catalytic site. This modification was made to the microalgae strain *Nannochloropsis oceanica*, culminating in a 32% increase in biomass generation and a 28% increase in photosynthetic activity (Wei et al., 2017).

4.2.2 Increased Photosynthetic Efficiency

Another way that genetic engineering has been found to improve biomass accumulation, and therefore increase biohydrogen production, is by increasing the photosynthetic efficiency of microalgae. Through techniques aimed at knocking out, replacing, or inserting genes into the genetic material of microalgae, it is possible to improve the conversion of sunlight into organic molecules and biomass (Kumar et al., 2021b).

An interesting thing about microalgae is that they have a light-harvesting complex (LHC) (Grama et al., 2022). This complex captures solar energy before transferring it to the cell's photosystem. What limits this photon harvesting system is the cell's ability to transmit light (Razu et al., 2019). In one study, where large-scale cultivation of *C. reinhardtii* was carried out, it was noticed that the cells in the upper layer of the culture obtained a greater absorption of light than the rate of photosynthesis, while the cells in the lower layer were unable to carry out the photosynthetic process. According to the study, this represented a waste of 95% of the light energy received (Fayyaz et al., 2020). In this way, genetic engineering sought to end the limitation of the LHC by improving carbon fixation and the efficiency of light conversion in photosynthesis. The NAB1 protein binds to the mRNA of the LHC gene to prevent its translation. The absence of this binding protein leads to an increase in the LHC antenna (Zhang et al., 2023).

In one study, a plasmid containing a mutated NAB1 coding sequence was inserted, which resulted in the expression of a reduced LHC antenna in *C. reinhardtii*. This smaller antenna reduced the excessive absorption of light and allowed the cells in the lower layers to acquire some of the sun's energy. The mutant

strains then had an 82% increase in the photosynthetic activity of the cells (Polle et al., 2003). In another study, using the same procedure to reduce the LHC antenna, it was possible to obtain a 50% higher biohydrogen production yield (Beckmann et al., 2009).

In addition to the LHC antenna, reducing the size of chloroplasts proved to be another strategy for improving photosynthetic efficiency. A mutant strain of *C. reinhardtii* had a lower chlorophyll content per unit cell volume of a chloroplast. This resulted in better light transmittance and solar energy conversion efficiency, leading the mutant strain to produce up to 5.2 times as much as the wild strain (Ban et al., 2019).

4.2.3 Altering Hydrogenase and Nitrogenase Activity

When the entire biochemistry behind the formation of biohydrogen in microalgae is studied, it is discovered that two enzymes play a central role in this bioproduction. These enzymes are hydrogenase and nitrogenase. To help optimize and improve the activity of these enzymes, genetic engineering has sought to alter their structures and oxygen uptake and reduce hydrogen consumption (Li et al., 2022, 2019).

Hydrogenases are divided into three types: Fe-Fe, Ni-Fe, and Fe. This classification is based on the metals (iron or nickel) attached to their active sites (Pandey et al., 2021). Of all these enzymes, Fe-Fe hydrogenase has been shown to have an activity 100 times greater than all the other hydrogenases (Razu et al., 2019; Von Abendroth et al., 2008). It is worth noting that the transcription, translation, and activity of the hydrogenase enzyme are controlled by the presence of oxygen. Because of this, the gene responsible for the hydrogenase enzyme has been overexpressed, as well as using hydrogenase genes from other microorganisms, which have more favorable conditions for the activity of this enzyme (Sivaramakrishnan et al., 2021). An example would be a study carried out with the bacterial strain *Clostridium acetobutylicum*, which has the ability to overexpress hydrogenases, but in a smaller quantity. To solve this, the expression vectors of the microalgae strain *C. reinhardtii* were inserted, which led to a high hydrogenase content (Von Abendroth et al., 2008).

Oxygen functions as a hydrogenase inhibitor, as it can cause the degradation of 2Fe and 4Fe-4S clusters when it binds to the enzyme's active sites (Lu & Koo, 2019). To solve this problem, the structure of the oxygen gas inlet channels in microalgae is usually reduced, along with an increase in oxygen tolerance. The aim is to increase the yield of hydrogen production. One study that carried out this procedure managed to achieve a 30-fold increase in hydrogen production compared to the wild *C. reinhardtii* strain (Yang et al., 2019).

In microalgae cells, under normal conditions, intracellular oxidases are not able to consume all the oxygen produced by photosynthetic activity. Reducing the amounts of intracellular oxygen has therefore become an area of great interest in the scientific literature. Researchers isolated the genes for pyruvate oxidase from *Escherichia coli* (POX) and catalase from the *Synechococcus elongatus* strain (CAT) and cloned them into vectors to insert into the *C. reinhardtii* genome. This led to a reduction in

oxygen content and a 100-fold increase in hydrogen production compared to the wild culture. The great advantage of all this is being able to reduce the oxygen concentration without interfering with the cell's photosynthetic rate (Xu et al., 2011).

Another mechanism that genetic engineering has found to increase hydrogen production is to decrease the amount of sulfur in the cell's culture medium. The lack of sulfur limits the synthesis of methionine and cysteine, which inactivates photosystem II (PSII), causing the photosynthetic release of oxygen at a slower rate than cellular respiration. The secret behind this lies in microRNAs (miRNAs), which are small non-protein-coding RNAs linked to the regulation of gene expression in animals and plants (Xu et al., 2019). Thus, through the expression of miRNAs, induced by blue light, a positive regulation system was built, under conditions of lack of sulfur, which manages to target a protein of the PSII reaction center in *C. reinhardtii* (Wang et al., 2017). A major advantage of applying sulfur-deficient crops in partnership with genetic engineering is that the culture media does not need to be altered or refined to the point of increasing production costs on a large scale (Li et al., 2015).

As previously mentioned, in addition to hydrogenases, other key enzymes for good biohydrogen production are nitrogenases. These enzymes are responsible for helping microalgae fix nitrogen (Giri et al., 2022). Nitrogenase is capable of catalyzing the unidirectional production of hydrogen (Masukawa et al., 2012). To be able to produce large quantities of biohydrogen is necessary a large number of electrons, a reducing agent, and ATP (Razu et al., 2019).

An example of an applied study was when they placed the minimal cluster of the *Bacteroides nif* gene into the genome of *Synechococcus elongatus PCC* 7942, a strain of non-nitrogen-fixing bacteria. The aim was to get the microorganism to start expressing the nitrogenase enzyme. This minimal cluster of *nif* genes is made up of six segments, each with their own independent promoters and terminators. In this way, a bioelectrochemical nitrogen fixation (e-BNF) system was developed, where methyl viologen (MV) provided the nitrogenase reducing power, and the PSII inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea (DCMU) was used to inhibit photosynthetic oxygen production. The recombinant strain of *S. elongatus PCC* 7942 was able to perform nitrogen fixation and hydrogen production simultaneously. After 48 h of culture, the bacterium was able to acquire a level of nitrogen fixation efficiency 21 times greater compared to its wild variant (Dong et al., 2021).

4.2.4 Optimizing Electronic Delivery

Microalgae are capable of producing biohydrogen from light in two different ways. The first is through the decomposition of water by photosystem II (PSII) and the transfer of electrons to hydrogenase enzymes, or the oxidation of organic substrates present in the culture medium (Manoyan et al., 2019). The first pathway obtains electrons from the decomposition of water which are transferred from PSII to ferredoxin (FDX), resulting in the production of ATP and NAD(P)H. In the end,

FDX supplies electrons to the hydrogenase enzyme, which forms biohydrogen by combining protons and electrons from the decomposition of water by PSII (Khetkorn et al., 2017).

Through this biochemical mechanism, it is clear that the lack of electrons can become a barrier to biohydrogen synthesis (Esper et al., 2006). The iron-sulfur hydrogenase (FeS) cluster in the FDX center plays a role in electron transfer in microalgae cells (Oey et al., 2016). FDX ends up acting as a regulator between carbon fixation and hydrogen production in the microalgae cell (Esper et al., 2006). The hydrogenase enzyme ends up having to compete with other metabolic processes for electrons (Kumar et al., 2016). To overcome this, genetic manipulation can increase the flow of electrons to hydrogenase (Giri et al., 2022).

One way they found to reduce electron competition was to improve the affinity of the hydrogenase for FDX (Dubini & Ghirardi, 2015). In one study, FDX was fused with Fe-Fe hydrogenase and expressed in the microalga *C. reinhardtii*. The mutation improved the electronic competitiveness of the hydrogenase, and the rate of photosynthetic hydrogen production was 4.5 times higher than that of the wild-type strain (Eilenberg et al., 2016). Other genes can also be adjusted to improve the flow of electrons to the hydrogenase. Ferredoxin NADP reductase (FNR) is an enzyme found in microalgae that converts FDX into NADP (nicotinamide adenine dinucleotide phosphate). Under anaerobic conditions, the reaction catalyzed by this enzyme competes with that of hydrogenase. Using RNAi technology, double-stranded RNA targeting the FNR gene was introduced into *C. reinhardtii* to inhibit its expression. As a result, hydrogen production in the mutant increased by 250% compared to the wild type (Sun et al., 2013).

For the hydrogenase enzyme, the main competing bioprocess for electrons is carbon fixation, carried out by the Rubisco enzyme, as discussed above (Pinto et al., 2013). As shown, although the overexpression of Rubisco significantly helped to improve carbon fixation, and therefore increase biomass and biohydrogen production, it also ended up leading to an increase in oxygen production, which negatively affects the activity of the hydrogenase enzyme (Liang & Lindblad, 2016). However, inhibiting the activity of Rubisco can increase the activity of hydrogenase (Eroglu & Melis, 2016).

In a study carried out with a mutant strain of *C. reinhardtii*, in the gene coding for the Rubisco enzyme, at positions 67, 68, and 72, the tyrosine residues were replaced by alanine, weakening the enzyme's stability. The strain acquired the ability to produce hydrogen under normal sunlight conditions, without modifications to the culture medium, obtaining a biohydrogen production 10–15 times higher than that of the wild type. The hydrogenase was then widely expressed to relieve the metabolic pressure in the cells caused by the degradation of Rubisco enzymes and the excessive reduction in photosynthetic electron transport (Pinto et al., 2013).

Another important electron transport system for biohydrogen production is photosystems I and II (PSI and PSII). This photosynthetic electron transport chain is located in the thylakoid membrane and the stromal layer (Tolleter et al., 2011). In an anaerobic culture of microalgae, the PSII protein binds to PSI, sacrificing PSII to increase light absorption capacity. When there is an increase in PSI cyclic electron

flow (CEF) compared to PSII linear electron flow (LEF), the proton gradient in CEF limits the supply of hydrogenase electrons, leading to an increase in ATP synthesis and a decrease in hydrogen production (Dubini & Ghirardi, 2015; Kruse et al., 2005). Therefore, inhibiting CEF can improve the efficiency of photosynthetic hydrogen production (Kruse et al., 2005). The random insertion of resistance genes to produce knockout mutants of *C. reinhardtii* resulted in a lack of photosynthetic CEF in the mutant. This led to the flow of electrons to the hydrogenase. Under higher light intensity, the mutant's hydrogen production rate remained high, while that of its wild-type counterparts decreased rapidly (Tolleter et al., 2011). As in other studies, the mutant strains were able to prolong their hydrogen production for significant periods under mixed nutritional conditions (Elman et al., 2022).

4.2.5 Regulating Metabolic Engineering

One area that ended up developing within genetic engineering was metabolic engineering. This area consists of editing the microorganism's genome to the point of changing its metabolism in order to produce efficient "factories" of biological products (Aamer Mehmood et al., 2021).

The production of biohydrogen by microalgae, under natural conditions, is transient and controlled by the cell's metabolic network (Nagarajan et al., 2021). In this way, metabolic engineering has great potential for increasing microbial hydrogen production (Mathews & Wang, 2009). The metabolic networks of microalgae are extremely complex and non-linear. The cells carry out various metabolic processes according to the demands imposed by the environment. Initially, cell metabolism focuses on growth rather than hydrogen production, but with the help of metabolic engineering, the hydrogen production pathway can be improved systematically and quantitatively (Mathews & Wang, 2009; Oh et al., 2013).

With this, the microalgae were transformed to increase substrate utilization, and alternative metabolic pathways were also introduced (Singh & Das, 2019). Through advances in metabolic engineering, it is hoped to solve the problem of low metabolic efficiency. In the scientific literature, it is possible to find approaches where energy has been supplied to break down thermodynamic barriers, express heterologous proteins, achieve more complete substrate conversions, and increase the flow of proton-reduction electrons (Hallenbeck et al., 2012).

The natural metabolic pathways of microalgae are diverse and can be applied to both dark and light-phase production. Dark fermentation, in particular, has the advantage of requiring a low energy demand and being simple to carry out (Mahidhara et al., 2019; Margareta et al., 2020). In large-scale industrial production, dark fermentation proved to be more viable, easy to control and stable (Boshagh, 2021). The big problem lies in the fact that only one-third of the substrates are used for hydrogen production, as the remaining two-thirds are used to produce additional fermentation products of low commercial interest (Jain et al., 2024). In order to overcome this production bottleneck, studies have focused on the nitrate assimilation

pathway in unicellular cyanobacteria (*Synechocystis* sp.), as it was discovered that this pathway competes with the hydrogenase enzyme for electrons in dark fermentation. In one study, this nitrate assimilation pathway was excluded, and as a result, they managed to create a strain capable of producing hydrogen in an amount of 140 times greater than that of the wild type (Baebprasert et al., 2011).

Another metabolic point that received attention was the hexose transporters in the plasma membrane, which transport exogenous glucose to the intracellular cytoplasm. The plasma membrane of *C. reinhardtii* microalgae lacks these hexose transporters, so an attempt was made to overexpress these proteins (Huang et al., 2022). By modifying the metabolism and placing the hexose transporter from *Chlorella HUP1* in *C. reinhardtii*, it was possible for carbohydrates from external sources to supply protons and electrons to the hydrogenase, improving hydrogen production by 150% (Doebbe et al., 2007).

Based on all this information, a microalgae metabolism model (AlgaGEM) based on *C. reinhardtii* was developed. This made it easier to predict the effect of known mutations on hydrogen production. Using this metabolic model, it was predicted that, under dark phase conditions, blocking the flow of circulating electrons would increase hydrogen production. This effect on the increase in hydrogen production is in line with the findings of studies described in the literature (Gomes et al., 2010).

5 Harvesting and Processing of Biomass Microalgae

Biohydrogen production from microalgae is a promising pathway for sustainable energy generation, utilizing renewable resources such as solar radiation and carbon dioxide. This process is underpinned by a sophisticated sequence of upstream and downstream operations, each critical to optimizing the overall yield and process efficiency (Fig. 3). The upstream phase involves the meticulous selection of microalgal strains, alongside the implementation of advanced cultivation techniques and modes, which are tailored to enhance biomass productivity and biohydrogen output (Ayub et al., 2024; Rady et al., 2024; Sharma et al., 2021).

Microalgal cultivation necessitates precise environmental control, including parameters such as light intensity, nutrient availability, pH stability, and temperature regulation. These factors are crucial for maximizing biomass accumulation and biohydrogen production potential. Furthermore, microalgae are cultured using various carbon sources, both organic (e.g., glucose, glycerol) and inorganic (e.g., CO_2, bicarbonates), which significantly influence growth rates and overall productivity (Kumar et al., 2021a).

The cultivation environment plays a critical role in determining the overall biofuel yield. Microalgae can be cultured in open pond reactors or closed systems, such as photobioreactors, with the latter being more commonly used due to their ability to operate in batch, fed-batch, continuous, and semi-continuous modes under controlled conditions. Parameters such as light, temperature, nutrients, pH, salinity, and dissolved oxygen are all integral in optimizing microalgal productivity (Ayub et al., 2024).

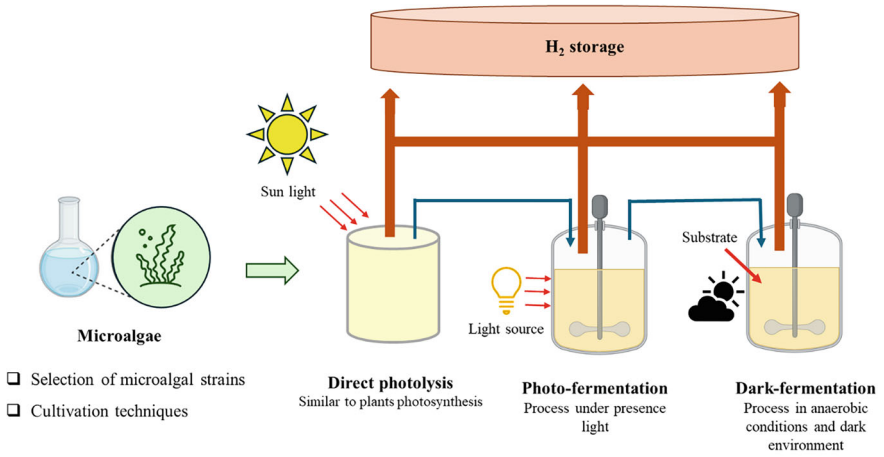


Fig. 3 Biological systems for biohydrogen production

The downstream processing phase begins with harvesting, which involves the concentration of dilute microalgal cultures into a denser slurry or paste. This step is pivotal within the downstream process to produce biohydrogen. Due to the colloidal nature of microalgal cells, achieving high efficiency during harvesting is challenging yet essential, as it directly affects subsequent processing stages (Singh et al., 2013). The careful selection of microalgal species, therefore, is paramount in optimizing this phase and ensuring maximal process efficiency (Ahmed et al., 2022).

The disruption of microalgal cells is also essential for the extraction of biohydrogen. The biofuels derived from microalgal biomass are advantageous due to their non-toxic nature compared to conventional fossil fuels (Onumaegbu et al., 2018). The biohydrogen production phase capitalizes on the high carbohydrate content of microalgal biomass, where up to 50% of the dry weight is composed of carbon (Rady et al., 2024). Pretreatment processes are crucial for disrupting the rigid cellulose structures of microalgal cell walls, thereby facilitating the release of fermentable carbohydrates. Techniques such as thermal, mechanical, enzymatic, and chemical pretreatments are employed to enhance the efficiency of subsequent microbial fermentation steps, which are essential for the generation of biohydrogen and other valuable products (Ahmed et al., 2022; Elshobary et al., 2024).

Following cell disruption, biohydrogen can be produced through various pathways, including photo-fermentation, dark fermentation, and biophotolysis. Photo-fermentation, a process that occurs under light exposure, converts microalgal biomass into biohydrogen and other products (Fig. 3). However, a significant limitation is the inhibition of hydrogenase by oxygen, which reduces the efficiency of hydrogen production (Ayub et al., 2024; Mona et al., 2020).

Dark fermentation, an anaerobic process that does not require light, is highly effective for hydrogen production from organic substrates such as glucose, starch, and organic waste. The integration of pretreatment techniques with dark

fermentation has been shown to significantly enhance hydrogen yields (Iqbal et al., 2022). For instance, the use of residual cassava water as a substrate in a microbial consortium resulted in a 40% increase in scaled-up biohydrogen production while also reducing medium costs (Martinez-Burgos et al., 2021).

Biophotolysis can be categorized into light-dependent (direct biophotolysis) and light-independent (indirect biophotolysis) pathways (Ayub et al., 2024). In indirect biophotolysis, microalgae and cyanobacteria produce biohydrogen from water, leveraging their ability to fix atmospheric nitrogen through enzymatic systems involving hydrogenase and nitrogenase. These enzymes facilitate biohydrogen production through direct light absorption and electron transfer, with nitrogenase catalyzing hydrogen synthesis and nitrogen reduction to ammonia (Iqbal et al., 2022). Direct biophotolysis, on the other hand, directly converts water into biohydrogen using sunlight, offering a conversion potential significantly higher than terrestrial plants. However, the sensitivity of hydrogenase to oxygen and the limited light conversion efficiency are notable challenges in this method (Mona et al., 2020).

In summary, biohydrogen production from microalgae presents a viable alternative to traditional energy sources, with significant potential for sustainability. The process involves intricate upstream and downstream operations, each requiring optimization to maximize yield and efficiency. Ongoing research and technological advances are crucial to overcome current challenges and realize the full potential of microalgae-derived biohydrogen as a clean, renewable energy source.

6 Applications and Future Perspectives

Although biohydrogen production has shown significant potential in laboratory and pilot settings, the transition to industrial-scale production is hampered by a lack of studies focused on optimizing process parameters and assessing scalability. Unlike chemical hydrogen synthesis, biohydrogen production presents numerous technical challenges. Addressing these issues could cost around $1.42 million. To compete with gasoline, which costs $2.50 GJ^{-1}, the target cost for hydrogen production should be $0.30 per kilogram, as 1 kg of hydrogen provides energy equivalent to 3.79 kg of gasoline (Ahmed et al., 2021; Show et al., 2018).

The design of the microalgae cultivation system and reactor is a major factor influencing the cost of biohydrogen production. For instance, biohydrogen produced through biophotolysis in a system involving a 140-hectare open pond and a 14-hectare photobioreactor could cost $6 per square meter and $100 per square meter, respectively. The capital investment for such a setup would be about $43 million, with annual operational costs of $10 million, leading to a production cost of $10 GJ^{-1}. Capital costs account for nearly 90% of the total expenses, with an annual capital charge of 25%. Operating at 90% capacity, the system is expected to generate 1200 TJ of biohydrogen annually (Mona et al., 2020). However, the costs associated with hydrogen storage and handling, which could add approximately $0.58 million, were not included in this calculation (Ahmed et al., 2021; El-Dalatony et al., 2020).

A study by Gholkar et al. (2021) explored the techno-economic feasibility of a biohydrogen production plant in India. The plant cultivated microalgae in an open pond at a rate of 12,790 kg per hour, producing 1239 kg of biohydrogen per hour. The total capital investment was estimated at $144.6 million, with 11% allocated for purchasing the gasifier and its accessories. Microalgae cultivation accounted for 76% of the annual operating costs, which totaled $7692, with the remainder spent on utilities. Assuming a market price of $10 per kilogram, the study concluded that biohydrogen production was economically viable, with a payback period of 3.78 years and an internal rate of return of 22%. The study emphasized that profitability depends heavily on the composition of biohydrogen and the cost of microalgae production, recommending the development of more selective catalysts (Ahmed et al., 2021).

In another study, James et al. (2009) estimated the cost of producing biohydrogen using oxygen-tolerant microalgae at $1.38 per kilogram, excluding storage costs. In this scenario, 42% of the costs were attributed to operating the photobioreactor, 34% to biohydrogen collection, and 13% to recycling and pumping systems. Additionally, 4% of the costs were associated with the microalgae feed assembly, with the rest allocated to other control systems and consumables. Storage remains a significant cost factor, posing a major challenge. To make direct biophotolysis economically viable, the study recommended increasing conversion efficiency and biomass production to 13.4% and 9.4%, respectively. Assuming that biohydrogen is entirely produced through photosynthesis, estimated that with a plant operating at half capacity and producing 80 kg of biohydrogen per acre per day, the cost would be about $2.80 per kilogram, which could compete with gasoline (Jiménez-Llanos et al., 2020).

Most economic assessments of biohydrogen production were conducted 10–20 years ago, making it challenging to evaluate the feasibility of modern biohydrogen production technologies. Furthermore, many existing studies present an overly optimistic view of biohydrogen economics. Current estimates suggest that biohydrogen production costs range from $10 to $20 GJ^{-1} (Show et al., 2019b). However, these costs are still too high for biohydrogen to be a competitive alternative to gasoline, which costs $0.33 GJ^{-1}. Future research should focus on comprehensive evaluations of facility feasibility, operational costs, and the impact of factors such as light, weather, and land availability. Additionally, recycling residual metabolites and biomass could improve economic feasibility (Ahmed et al., 2021).

7 Conclusions

Biohydrogen production holds significant potential as a renewable energy source, but it faces considerable challenges in terms of cost and scalability. Current economic assessments highlight that while the process shows promise, particularly in laboratory and pilot settings, the transition to industrial-scale production requires overcoming significant technical and financial hurdles. The high capital investment,

operational costs, and the need for advanced technologies like highly selective catalysts contribute to the overall expense of biohydrogen production. Despite the potential for biohydrogen to be competitive with traditional fuels, such as gasoline, further research and innovation are essential to reduce costs, enhance efficiency, and improve economic feasibility. Future efforts should focus on optimizing the entire production process, from cultivation systems to storage solutions, to make biohydrogen a viable and sustainable energy alternative.

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Part II
Unlocking Market Potential—Economic Insights and Innovation in Microalgae Biotechnology

Bio-green Evolution: The Journey of Microalgae Products from Lab to Market—An Overview of Microalgae-Based Processes and Products—Part I



Ambika H. D. and Hema Vijayan P. U

Abstract Microalgae, a diverse group of photosynthetic microorganisms, play a pivotal role in sustainability and innovation. Renowned for their high photosynthetic efficiency, they convert sunlight and carbon dioxide into oxygen and biomass at rates far surpassing terrestrial plants. Key species such as *Spirulina* and *Chlorella* are valued for their adaptability, rapid growth, and rich nutritional profiles, including proteins, essential fatty acids, vitamins, and pigments. These attributes enable wide-ranging applications across industries, including food, animal feed, pharmaceuticals, cosmetics, and biofuels. This exploration highlights advancements in cultivation techniques, such as open ponds, photobioreactors, and hybrid systems, which enhance productivity while maintaining eco-friendliness. Furthermore, the integration of microalgae into circular economy models and their use in carbon capture and wastewater treatment exemplify their role in environmental sustainability. The growing global market for microalgae products is fueled by consumer demand for natural and health-promoting solutions. With ongoing innovations in biotechnology and process optimization, microalgae continue to emerge as a transformative resource, addressing critical global challenges while unlocking economic opportunities.

Keywords Microalgae · *Spirulina* · *Chlorella* · Biofuels · Functional foods · Aquaculture · Cosmetics · Sustainability · Photobioreactors · Circular economy · Carbon capture

A. H. D. (✉) · H. Vijayan P. U
ProAlgaeTech, Research and Development, Stuttgart, Baden-Württemberg, Germany
e-mail: hema.vijayan@proalgaetech.com

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1 Introduction

Microalgae, including cyanobacteria, are photosynthetic organisms that are crucial for ecosystems as primary producers. They are diverse and can be found in areas of the ocean where sunlight can reach. Microalgae use sunlight to convert it into chemical energy, producing oxygen as a byproduct. They have adapted to extreme habitats and have a wide range of biodiversity. They play a vital role in aquatic food webs and nutrient cycles. Additionally, microalgae have potential applications in biotechnology and environmental sustainability (Pulz & Gross, 2004; Metting, 1996; Cadoret et al., 2012; Chisti, 2014).

Microalgae, which primarily inhabit the euphotic zone in aquatic ecosystems, play a crucial role in primary production by absorbing external energy and nutrients. They use photosynthetic processes to absorb carbon dioxide and release oxygen. While most microalgae exist as independent cells or colonies, some engage in symbiotic relationships. The utilization of microalgae by humans dates back to ancient times, but commercial exploitation has only recently gained attention due to concerns over protein scarcity (Pulz & Gross, 2004). Microalgae are known for their high protein and nutritional composition, but research now focuses on their physiologically active substances such as polyunsaturated fatty acids and pigments. Microalgae are classified based on their taxonomy and phylogeny, with common types including blue-green algae, green algae, diatoms, and golden algae (Fig. 1).

1.1 Classification of Microalgae

Algae are basic, autotrophic organisms that can be either unicellular or multicellular. They can produce oxygen and lack tissue differentiation. Primitive algae were already thriving 1.5 billion years ago (Gerrienne et al., 2016). Cyanobacteria and microalgae present today are descendants of early photosynthesizing microorganisms (Wikipedia contributors, 2024a). Eukaryotic microalgae emerged around 1 to 2 billion years ago, while seaweed or macroalgae emerged later, around 1 billion years ago. Some microalgae transitioned from water to land around 465 million years ago, with some originating from freshwater habitats and others from saltwater. Among the algae that established themselves on land, only the freshwater charophytes evolved into more advanced plants (Lewin & Andersen, 2024; Khan et al., 2020; Andersen & Lewin, 2024). Multicellular land plants share a single common ancestry according to genetic data. The descendants of the freshwater algae that first emerged on land are primarily found as subaerial algae, which thrive in contact with air. However, the classification of algae remains uncertain and differs significantly.

Microalgae can be classified as prokaryotic or eukaryotic and show high evolutionary diversity (Khan et al., 2020). Eukaryotic microalgae have a phylogenetic history of 1.9 billion years, younger than cyanobacteria, which have a history of 2.7 billion years (Khan et al., 2020; Andersen & Lewin, 2024). The ancestor of algae



Fig. 1 A variety of unicellular and colonial freshwater microalgae (Figure 1 Microalgae – Wikipedia, n.d.)

formed an endosymbiotic association with cyanobacterium, giving rise to two distinct evolutionary lineages: green and red algae. Secondary endosymbiosis led to the emergence of heterokont algae, Dinoflagellates, Cryptophytes, and Euglenida (Andersen & Lewin, 2024). Green microalgae are classified into two main families: Chlorophyta and Conjugaphyta. The Conjugaphyta group is larger, but none of its species have been used for biotechnological engineering yet. Another classification of microalgae includes prokaryotic blue-green (Cyanobacteria), eukaryotic green (Chlorophyceae), eukaryotic brown (Phaeophyceae), eukaryotic red (Rhodophyceae), and eukaryotic diatoms (Bacillariophyceae) (Leliaert, 2015).

1.2 Cyanophyta/Blue Green Algae

The classification of microalgae is constantly changing due to new genetic data, but they can generally be categorized into prokaryotic and eukaryotic algae (Demoulin et al., 2019). Prokaryotic microalgae, also known as cyanobacteria or blue-green algae, belong to the taxon Cyanobacteria and have five orders: Pleurocapsales, Oscillatoriales, Nostocales, Stigonematales, and Chroococcales. Another category of Cyanobacteria, called Prochlorophyta, is distinguished by their distinct color and the absence of phycobiliproteins. Cyanobacteria obtain energy through photosynthesis and played a significant role in changing the Earth's atmosphere from a reducing to an oxidizing one. They are found in various terrestrial and aquatic environments, including freshwater, marine habitats, soil, and even wet desert rocks. They can exist as planktonic cells, phototrophic biofilms, or as endosymbionts in other organisms. Cyanobacteria are known for forming visible blooms in freshwater and marine environments.

One of the primary traits of cyanobacteria is their ability to quickly absorb and store substances like phosphate, cyanophycin, and branching α-1,4 polyglucan. Some cyanobacteria can produce hepatotoxins and neurotoxins, while others can produce medicinal substances such as antioxidants, immunomodulators, and antivirals. *Nostoc commune*, *Aphanizomenon flos-aquae*, and S*pirulina (Arthrospira) platensis* are three significant cyanobacteria used in biotechnology (Wikipedia contributors, 2025; Sánchez-Baracaldo & Cardona, 2019).

2 Characteristics of Different Eukaryotic Microalgae

Eukaryotic algae can exist as either unicellular or multicellular organisms and are classified into several divisions. The largest group is Chlorophyta, commonly known as green algae, which can be found in aquatic habitats and are either unicellular or filamentous. Euglenophyta are unicellular organisms with flagella that originated from a secondary endosymbiotic interaction between algae and a protozoan-like host. Red algae, or Rhodophyta, are characterized by their high concentration of phycoerythrin. Cryptophyta consists of unicellular, mobile, photosynthetically active organisms. Dinophyta, or Dinoflagellata, is a broad category of organisms, but many of its species are not used due to the production of harmful compounds. Freshwater algae belong to the Prymnesiophyta and Glaucophyta taxa, also known as Haptophyta, which have a photosynthetic organelle called the cyanelle. Brown algae (Phaeophyta), yellow-green algae (Eustimatophyta or Xanthophyta), golden algae (Chrysophyta), and diatoms (Bacillariophyta) are part of Heterokontophyta (Sánchez-Baracaldo & Raven, 2024; Fawley & Fawley, 2020; Leliaert et al., 2012; Zhen et al., 2016; Gimpel et al., 2015).

2.1 Chlorophyta/Green Algae

Green algae, a diverse group originating from higher plants or embryophytes, are part of the paraphyletic group, with green algae and embryophytes forming a monophyletic Kingdom Plantae. This group includes microalgae, unicellular and colonial flagellates, and macroscopic seaweeds, all characterized by chlorophyll a and b in a double-membraned chloroplast. Notable commercially important species include *Chlorella vulgaris*, a freshwater alga with a complex cell wall and a pyrenoid for CO_2 fixation (Biology Online, 2022; Wikipedia contributors, 2024b); *Dunaliella salina*, a photoautotrophic biflagellate with a cup-shaped chloroplast that reproduces both vegetatively and sexually (Ayad, 2021; Pierangelini et al., 2017; Aigner et al., 2020); *Haematococcus pluvialis,* which can assume palmelloid or biflagellate forms, featuring a gelatinous layer and a cup-shaped chloroplast with dispersed pyrenoids (McCourt et al., 2023); and *Botryococcus braunii,* a colonial alga embedded in a hydrocarbon matrix (Weis et al., 2012).

2.2 Rhodophyta/Red Algae

The Phylum Rhodophyta, also known as red algae, is an ancient group of eukaryotic algae, comprising around 5000–6000 marine algae species. Most are multicellular and include seaweed. Freshwater or unicellular species are rare in this group. The commercialization of the unicellular red alga, *Porphyridium cruentum,* is still in the research and development stage in France and Israel. This spherical microalga has a single double-membraned chloroplast with a central pyrenoid, and thylakoids with phycobilisomes composed of pigments phycocyanin and phycoerythrin (Gantt & Conti, 1965). *Rhodella reticulata* has a mucilaginous sheath of brown to olive-colored unicells and a single pyrenoid-containing chloroplast (Deason et al., 1983).

2.3 Haptophyta/Pavlovophyceae

Haptophyta, a microalgal phylum, primarily inhabits marine habitats and can be single-celled or colonial. However, some species have been found in freshwater environments. *Isochrysis galbana* and *Pavlova salina* are well-known members used in aquaculture. These algal cells have one or two chloroplasts containing pyrenoids connected to the nuclear envelope and endoplasmic reticulum (Edvardsen et al., 2000).

2.4 Ochrophyta/Eustigmatophyceae

Eustigmatophyceae, a class of microalgae with chlorophyll a, is found in marine, freshwater, and terrestrial habitats. *Nannochloropsis oculata*, a microalgae used in aquaculture, is one of five species in the genus *Nannochloropsis*, with only *N. limnetica* found in freshwater. These cells are non-motile, oval, or spherical, with a distinct papilla (Suda et al., 2002).

2.5 Ochrophyta/Bacillariophyceae

Bacillariophyceae, a class of diatoms found in freshwater and marine environments, are mainly unicellular, with some being colonia. *Skeletonema costatum, Chaetoceros muelleri,* and *Thalassiosira pseudonana* are commercially cultivated and have distinctive diagnostic features. These organisms are used in aquaculture due to their diagnostic features. *Thalassiosira pseudonana,* the first marine phytoplankton with a genome sequenced, is a marine small, centric, unicellular diatom belonging to the Coscinodiscophyceae (Sarno & Kooistra, 2005; Armbrust et al., 2004).

2.6 Dinophyta/Dinophyceae

Dinophyta, a phylum of unicellular organisms, is primarily found in marine environments, with only a few species inhabiting freshwater habitats. About 50% of Dinophyta can photosynthesize, while the remaining 50% are heterotrophs lacking chloroplasts. *Crypthecodinium cohnii*, a commercially valuable marine dinoflagellate, is known for its ability to produce docosahexaenoic acid (DHA) through heterotrophic means (Prabowo et al., 2013).

3 Historical Context and Discovery of Microalgae

3.1 Early Evolution and Ancient Significance

Microalgae, originating 3.5 billion years ago, played a crucial role in oxygenating Earth's atmosphere through photosynthesis and oxygen generation. The Great Oxidation Event led to the emergence of advanced aerobic organisms (Schirrmeister et al., 2015). These ancient microorganisms also developed stromatolites, sedimentary structures with distinct layers, providing evidence of life on Earth and highlighting the persistence of microalgae throughout the planet's history (Falkowski & Knoll, 2007).

3.2 Discovery and Early Studies

The scientific exploration of microalgae began in the seventeenth century with the introduction of microscopy, with Antonie van Leeuwenhoek being a pioneer. Leeuwenhoek used homemade microscopes to record and analyze microorganisms found in pond water, including microalgae. Carl Linnaeus used the term "algae" in the eighteenth century, but it wasn't until the nineteenth century that more intricate categorization and examination began (Lane, 2015). German biologist Christian Gottfried Ehrenberg made significant contributions to the field of phycology by finding and characterizing many microalgae species (Chapman, 2013).

In the twentieth century, microalgae gained significant understanding and use. In the early 1900s, scientists investigated their biochemical characteristics and potential as a chemical reservoir. Hans Molisch's 1920s studies highlighted their protein content, leading to further research on their food and fuel sources (Spolaore et al., 2006). World War II intensified interest in microalgae, with the US and Germany studying its growth for specific purposes. The commercialization of microalgae also improved with large-scale outdoor algal growth systems (Technology Networks, 2021).

3.3 Modern Developments and Industrial Applications

Microalgae have gained renewed interest due to concerns about sustainability, climate change, and resource depletion. Advancements in biotechnological and genetic engineering have enabled the cultivation of microalgae for various purposes, including biofuels, medicines, and nutraceuticals (García et al., 2017). They are also being grown for producing valuable compounds like omega-3 fatty acids, pigments, and antioxidants. Microalgae's industrial applications have increased due to their carbon capture and wastewater treatment capabilities (Rumin et al., 2020). Algae have a long history and have been extensively studied and explored by scientists. Recent developments in algal research offer hope for environmentally friendly practices and the creation of sustainable ecosystems. Algae are photosynthetic organisms that can monitor water contamination, eliminate harmful substances through bioremediation, and generate renewable energy. Industries like food and pharmaceuticals are using algae to create valuable products and enhance crop development. Microalgae have great potential to contribute to global sustainability and strengthen corporate sustainability initiatives. They can capture carbon, generate renewable energy, cleanse water, and support sustainable agriculture, enhancing resource efficiency, fostering new product creation, and promoting corporate social responsibility.

3.4 Contribution of Microalgae to Global Sustainability

Due to their high photosynthetic efficiency, CO_2 is converted into oxygen and biomass more rapidly than land plants, making them effective for climate change mitigation by reducing greenhouse gas concentrations. They are utilized in renewable energy (biofuels), bioremediation (wastewater treatment), and as biofertilizers, benefiting agriculture and sustainable food production. Microalgae farming is cost-effective, requiring minimal land and utilizing non-potable water sources. Products derived from microalgae, such as natural colorants, antioxidants, and bioactive chemicals, have potential applications in cosmetics, pharmaceuticals, and food industries. They are also being explored for bioplastics to reduce petrochemical dependency. Water quality can be assessed through monitoring phytoplankton and pollutants, while eco-toxicological studies use diatoms and algae to measure toxicity, promoting environmental sustainability (Yu et al., 2024). Industrial effluents, often containing hazardous chemicals and metals, pose risks to aquatic life and health, and traditional treatment methods are expensive and inefficient. Microalgae offer a cost-effective, scalable solution for wastewater treatment, potentially generating biomass and blooms for remediation. Types of algae commonly found in wastewater include Chlorophytes, Euglinophytes, Chrysophytes, and Cyanophytes. Integrating microalgae into industrial processes can enhance Corporate Social Responsibility, support environmental sustainability, and create economic and ecological benefits.

4 Microalgae Species in Focus

Microalgae are increasingly recognized for their rich nutritional content, offering a natural, sustainable source of "superfood biomass." Despite their potential, further efforts are needed to integrate these substances into mainstream human diets. Among the estimated 200,000 to 800,000 species, only a few are economically viable. Chlorella and Spirulina, which have been cultivated as nutritional supplements, are particularly valued for their high levels of chlorophyll, proteins, minerals, vitamins, and antioxidants, boosting immunity and overall vitality. These microalgae have been vital in aquatic ecosystems for millions of years.

4.1 Spirulina

4.1.1 Biological Characteristics

Arthrospira (Spirulina), particularly species like *Arthrospira platensis* and *Arthrospira maxima*, is a cyanobacterium characterized by spiral-shaped trichomes within a mucilaginous sheath, thriving in alkaline, brackish, and saline waters, often

forming large blooms (Belay, 1997). Historically, *Arthrospira platensis* was the first cyanobacterium commercially farmed using advanced biotechnology, initially for human and animal consumption and now also for medical diagnostics, research, and cosmetics (Habib et al., 2008). The species remains a focus for the cyanobacterial biotechnology industry due to its medicinal potential.

Spirulina is known for its helical filaments, ranging from 0.5 to 8 micrometers in diameter and extending several hundred micrometers in length, optimized for photosynthesis (Fig. 2). Its septa, or cross-walls, are a primary taxonomic feature, with structure varying by species and environmental conditions. Culinary use of Spirulina dates back to the Aztecs and has continued in African and Mexican diets since the seventeenth century. The three most cultivated species globally are *Spirulina maxima, Spirulina platensis,* and ***S****pirulina fusiformis*, used in food, health products, and as sources of valuable chemicals (Markou et al., 2021; Jung et al., 2021). *Spirulina* efficiently converts sunlight, CO_2, and water into organic molecules and oxygen, with a unique ability to fix nitrogen through heterocysts, enhancing its growth in nutrient-limited environments (Rastogi et al., 2020). It reproduces via binary fission, facilitating rapid population growth under favorable conditions (Dillon et al., 1995). Spirulina's resilience allows it to thrive in extreme environments unsuitable for other organisms, making it ideal for bioremediation projects (Aiello et al., 2019). Its rich pigmentation, including chlorophyll a, phycocyanin, and carotenoids, contributes to its metabolic functions and health benefits, such as anti-inflammatory and immune-enhancing properties (Lee, 1997). *Spirulina* is a highly nutritious food, rich in protein (up to 70%), minerals, vitamins, tocopherols, γ-linolenic acid, and phenolic acids. It is easily digested due to the absence of cellulose cell walls (Costa et al., 2019a). Spirulina's protein content and amino acid profile make it a valuable human food source, particularly for addressing protein malnutrition (Zhan et al., 2017). Its biological traits, including photosynthetic efficiency and adaptability, underscore its value as a dietary supplement and contributor to ecological sustainability.

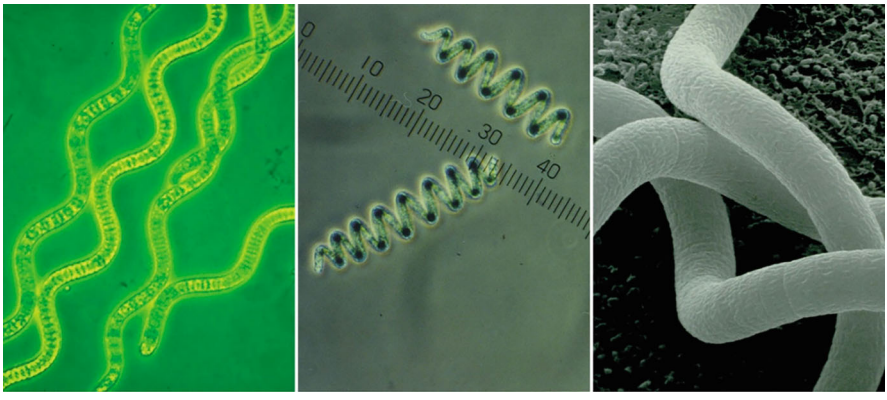


Fig. 2 View of Spirulina under microscope: (1) long filaments; (2) perfect spiral coils; (3) electron microscope (Habib et al., 2008)

4.1.2 Cultivation Methods

Spirulina, a microalga thriving in freshwater environments like brackish lakes and soil marshes, is produced using both open pond technologies and closed photobioreactors. Species selection, notably *Arthrospira platensis* and *A. maxima*, is vital due to their beneficial properties (Lee, 2001). Since the late 1970s, commercial production has predominantly occurred in the Asia-Pacific region. Cultivation technology—whether open ponds, closed bioreactors, or hybrid systems—affects growth by influencing light exposure and nutrient availability. Open systems, such as raceway and circular ponds, are cost-effective but face challenges like low light efficiency and contamination risks. Optimal Spirulina growth requires careful management of temperature (ideal between 29 °C and 35 °C), light (especially blue and red wavelengths), pH, and nutrients like sodium bicarbonate and potassium. Alkaline brackish water enhances biomass productivity, while proper salinity and light intensity are crucial for maintaining growth and metabolic processes.

4.1.2.1 Laboratory-Scale Cultivation

Cultivating Spirulina involves preparing a nutrient-rich culture medium, introducing a starter culture, and maintaining optimal conditions such as temperature, pH, light intensity, and aeration. The process begins at the lab scale, progresses to pilot scale, and culminates in commercial production. Continuous monitoring and fine-tuning are crucial for achieving optimal growth. After harvesting, the biomass is processed to remove contaminants and extract specific proteins, maximizing both quantity and quality. At the lab scale, cultivation occurs in small settings like shake flasks, open ponds, or closed photobioreactors (PBRs). Shake flasks, which are illuminated and placed on shakers, allow for controlled growth conditions. Laboratory-scale PBRs are enclosed systems used to study Spirulina growth patterns and refine culture parameters. Open ponds, often used for large-scale microalgae culture, offer scalability and cost-efficiency but carry a higher risk of contamination and variability due to environmental factors. As cultivation scales up to the pilot stage, the choice of growing method becomes critical for increasing output, productivity, and efficiency.

4.1.2.2 Large-Scale Cultivation

To achieve economic viability in large-scale microalgae cultivation, developing cost-effective operational methods to enhance biomass and target molecule production is essential. Current cultivation technologies, including photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic methods, each have their benefits and limitations (Oswald & Goleuke, 1967). Photoautotrophic methods use solar energy for CO_2 conversion, while mixotrophic and photoheterotrophic methods offer flexibility in varying conditions (Abu-Ghosh et al., 2021). Open pond systems

are simple and cost-effective but struggle with environmental fluctuations. Enclosed photobioreactors provide better control but are costly. Heterotrophic cultivation, using organic substrates like glycerol or wastewater, can reduce costs and boost growth. Integrating microalgae cultivation with wastewater treatment enhances lipid and biomass production, while mixotrophic cultivation combines autotrophic and heterotrophic approaches for improved yield and efficiency (Abu-Ghosh et al., 2021).

4.1.2.3 Open Pond Cultivation

The raceway pond is a type of open pond design used for cultivating Spirulina at a pilot scale. It was first introduced in the 1960s and has remained relatively unchanged in structure and appearance since then (Velarde et al., 2014; Nadal, 1971). Other open pond designs include rectangular ponds, unstirred ponds, natural ponds, and covered ponds. In the raceway pond system, paddle wheels are used to create a continuous flow of water, ensuring even distribution of nutrients and preventing the formation of oxygen-deprived areas. Open ponds are cost-effective and easy to implement, making them suitable for large-scale production, especially in areas with ample sunlight and favorable temperatures. However, open pond cultivation is susceptible to contamination from other microorganisms and can be affected by environmental fluctuations, which may result in inconsistencies and compromised quality of the Spirulina biomass (Becker, 2007a).

4.1.2.4 Photobioreactor

Closed photobioreactors provide a controlled environment for cultivating Spirulina, allowing for precise regulation of factors such as light intensity, temperature, and carbon dioxide levels. This leads to increased production and purity of the Spirulina culture. The enclosed design of these systems also reduces the risk of contamination and water loss, making them suitable for high-value output. However, the complexity and cost of building and maintaining photobioreactors limit their use to smaller-scale or specialized applications. Regardless of the growing technique used, effective nutrient control is crucial for successful Spirulina development. The growth medium must contain essential nutrients such as nitrogen, phosphorus, potassium, and trace elements. Nutrients can be provided through inorganic fertilizers or organic waste products, depending on the farming arrangement and sustainability goals. Continuous monitoring and adjustment of nutrient concentrations are necessary to maintain optimal growth conditions and prevent imbalances that could hinder Spirulina productivity (Nadal, 1971).

4.1.2.5 Hybrid System

Hybrid systems combine the advantages of open ponds and photobioreactors, reducing contamination, evaporative losses, and CO_2 desorption. They offer better control over culture variables, higher productivity, and reduced power consumption. By combining closed systems' regulated environment with open ponds' scalability and cost-effectiveness, hybrid systems can optimize Spirulina production with high protein content (Nuhu, 2013; Braga et al., 2018).

Algal biomass **harvesting** involves several steps, including centrifugation, filtration, and flotation. Centrifugation is a fast and effective technique for collecting concentrated algal cells, while filtration is efficient for separating large quantities of biomass. Flotation is a clustering process that can be enhanced by centrifugation or filtration. Combining various harvesting methods can improve efficiency and protein yield. Concentrated Spirulina biomass is processed to extract protein, including cell disruption, protein isolation, filtration, and desiccation. This protein-concentrated powder is suitable for various uses, including dietary supplements, livestock feed, and biotechnological purposes. The collected biomass is then thoroughly cleansed and dehydrated using techniques like spray drying, freeze drying, or sun drying, to maintain nutritional value and prolong storage time.

4.1.3 Nutritional and Commercial Value Analysis of Spirulina

Spirulina is considered the "food of the future" due to its ability to produce high-quality, concentrated food faster than any other algae. It contains all necessary amino acids, making up around 60% of complete protein, and abundant nutrients in a bioavailable form. Spirulina is rich in beta-carotene, with a concentration 10 times higher than carrots. It also contains vitamin B12, a nutrient primarily found in animal tissues, with a teaspoon of spirulina providing 2 1/2 times the Recommended Daily Allowance.

Protein Spirulina, a blue-green algae, contains ribosomes, essential for protein synthesis, despite its simple shape and lack of starch-storing cells. This arrangement allows for protein synthesis and photosynthesis processes. Spirulina protein content varies between 65% and 71%, making it physiologically complete and more easily digestible than soy or beef proteins. The eight essential amino acids in Spirulina are isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine. It also contains 10 out of 12 non-essential amino acids, including alanine, arginine, aspartic acid, cysteine, glutamic acid, glycine, histidine, proline, serine, and tyrosine (Sajilata et al., 2008a; Rangel-Yagui et al., 2004).

Fatty acids Spirulina, a microalgae, has been used in biotechnological industries as a nutraceutical and regenerative medicine due to its high content of very-long-chain polyunsaturated fatty acids (VLCPUFAs). These fatty acids are essential for human physiology and are found in *Spirulina platensis*, which contains stearic acid, linoleic acid, and gamma linolenic acid. These fatty acids are essential for regulating

cholesterol levels and are found in the lipid levels of *Spirulina platensis* and *Spirulina maxima*, which range from 5.6% to 7% (Rastogi et al., 2020; Madhyastha & Vatsala, 2007). However, experts suggest that the lipid content could potentially reach 11% with improved extraction technologies (Ogbonda et al., 2007). Spirulina has a significant proportion of fatty acids, accounting for about half of its total lipids. Polyunsaturated fatty acids (PUFAs) make up 30% of the total lipid content, while γ-linolenic acid makes up 36% (Rastogi et al., 2020). Other fatty acids include linoleic acid, stearidonic acid, eicosapentaenoic acid, docosahexaenoic acid, and arachidonic acid. These fatty acids are crucial for respiration, skin, hair, and nails, and the decomposition of blood cholesterol. They also help the body synthesize Prostaglandins, hormones that regulate blood pressure and capillary strength.

Carbohydrates Spirulina is a plant with a high carbohydrate content, primarily composed of polysaccharides, which make up 10–15% of its total carbohydrate content. These polysaccharides are easily absorbed by human cells, providing rapid energy without causing hypoglycemia. Spirulina provides about 50–70% of the body's energy needs, with 1 gram of carbohydrates providing 4 kilocalories of energy. Its absence of cellulose makes it easily assimilated, making it an ideal dietary supplement for conditions like diabetes and obesity. Carbohydrates are essential for the liver, central nervous system, heart, and muscles (Wikipedia contributors, 2024c).

Minerals and Vitamins Spirulina, a plant rich in trace minerals, is essential for metabolism and overall health. It thrives in alkaline waters and converts minerals into their biological forms, enhancing their absorption by the body when chelated with amino acids. Inorganic minerals, such as iron, are often consumed without physiological benefits, leading to illnesses caused by mineral shortages. Spirulina is rich in essential minerals, including potassium, calcium, phosphorus, magnesium, manganese, copper, iron, selenium, sodium, and zinc (Rastogi et al., 2020; Grosshagauer et al., 2020). It is beneficial for bone and teeth health, blood regeneration, and blood regeneration due to its high levels of calcium and iron. Spirulina contains a variety of vitamins, including A, B1, B2, B6, B12, E, and H. It contains 21% of the Recommended Dietary Allowance for riboflavin and thiamine, and is a highly abundant source of beta-carotene, which is converted into vitamin A for optimal vision, immune support, and healthy skin. Vitamin E, a powerful antioxidant, is also found in spirulina, safeguarding cardiovascular and vascular well-being, enhancing cellular oxygenation, and slowing down the aging process (Chentir et al., 2018). Spirulina is also beneficial for treating anemia due to its high levels of folic acid and vitamin B12.

Pigments Spirulina is a rich source of protein, minerals, and vitamins, as well as essential pigments like Phycocyanin, Carotenoids, and Chlorophyll. These pigments have antioxidant effects, enhance immune system performance, stimulate red blood cell generation, and promote muscular functionality. Spirulina's beta-carotene content is 15 times higher than carrots. Chlorophyll, a green pigment found in plants, regulates hemoglobin levels and aids in digestion. Phycocyanin, the primary

pigment in Spirulina, is a rare light blue dye with anti-inflammatory, antioxidant, and anticarcinogenic properties. It is commonly used in food products and is similar to bilirubin, a pigment found in humans. Carotenoids, a spectrum of yellow to orange, are fat-soluble compounds that aid in plant and microorganism recovery from light-induced damage. Spirulina contains a high concentration of natural carotenoids, which serve as precursors for Vitamin A. These antioxidants neutralize free radicals, cellular aging, and cholesterol, preventing heart disease. They also elicit an "ocular protective response" and enhance the immune system. Overall, Spirulina is a valuable source of essential nutrients.

Spirulina, a high-value protein source, is gaining popularity as a sustainable alternative to traditional animal feed due to its high crude protein content and availability of essential amino and fatty acids. It can be cultivated in bioreactors or open ponds, utilizing resources not included in the food production system. Spirulina's nutritional richness and diverse health advantages make it a popular ingredient in animal feed, enhancing growth, health, and productivity in cattle, poultry, aquaculture, and pets. Spirulina farming requires less land, water, and energy, making it an eco-friendly alternative (Fernández-Rojas et al., 2014).

4.2 Chlorella

4.2.1 Biological Characteristics

Chlorella, a green algal species, has been on Earth since the pre-Cambrian epoch, 2.5 billion years ago. It is a unique green algal member with bioactive compounds like protein, vitamins, pigments, and sterols. Chlorella microalgae have a wide distribution across various habitats, including freshwater, sea water, soil, and symbiotic relationships with lichens and protozoa (Colla et al., 2007; Lyer et al., 2010; Wikipedia contributors, 2024d). They have a spherical morphology and lack flagella, with chloroplasts that can be parietal, cup-shaped, or plate-like. Pyrenoids, a prominent feature in most Chlorella species, are positioned near the center of the chloroplast and enclosed by the starch sheath (Wikipedia contributors, 2024d; Wawrik & Harriman, 2010; The Editors of Encyclopaedia Britannica, 1998).

Chlorella has a sturdy cell wall that is resistant to degradation by microorganisms in the absence of oxygen (The Editors of Encyclopaedia Britannica, 1998). Their chloroplasts house green photosynthetic pigments chlorophyll-a and -b. Chlorella cells undergo fast multiplication, needing only carbon dioxide, water, sunlight, and a minimal amount of minerals for reproduction (Admin_Echlorial, 2023; Rani et al., 2018). Chlorella is considered a viable source of food and energy due to its photosynthetic efficiency, which can reach up to 8%. The cells contain a high concentration of proteins, vitamins, and minerals, particularly B-complex vitamins, making them a promising source of nutrition (Fig. 3). Chlorella is being extensively manufactured as a health food in Germany, China, Japan, and other Asian nations.

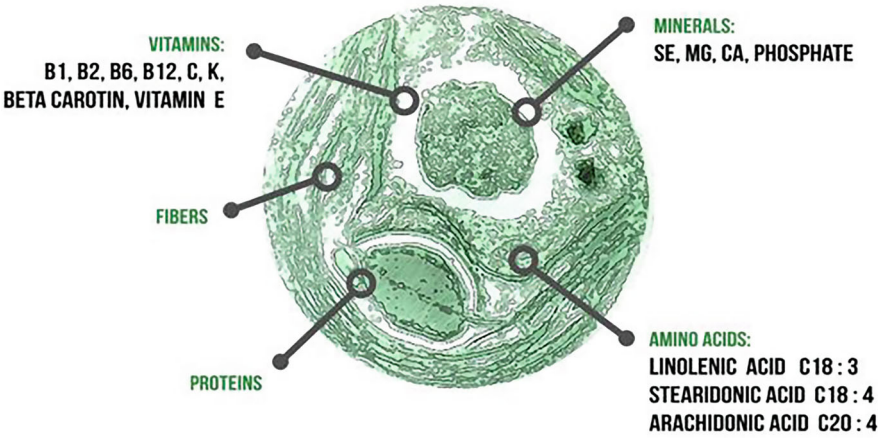


Fig. 3 Composition of Chlorella (The Editors of Encyclopaedia Britannica, 1998)

4.2.2 Cultivation

Chlorella cultivation began in the late 1940s in the US, Germany, and Japan. This led to a surge in algae cultivation, influencing algal biotechnology. Algal cultures can be grown photoautotrophically, heterotrophically, or mixotrophically (Sakarika & Kornaros, 2018; Li et al., 2021). Chlorella, with high photosynthetic efficiency, is commonly grown outdoors using sunlight in open ponds or closed photobioreactors. This led to the development of various culture methods for large-scale cultivation (Lin, 2005; Masojidek et al., 2011; Moheimani, 2013).

4.2.2.1 Open Pond Cultivation

Open ponds, such as circular and raceway ponds, are popular for their simplicity, durability, and low construction and operational costs. Circular ponds, originally from Japan and later spread to China, are widely used for large-scale production of Chlorella in Asia. However, they have drawbacks such as heavy reinforced concrete structures, excessive energy consumption, and inefficiency in land utilization. Raceway ponds, originally used for Spirulina production, are also widely used for Chlorella cultivation. They are constructed using poured concrete or excavated dirt and lined with plastic liner to avoid liquid absorption. These ponds are typically maintained at a shallow depth of no more than 30 cm to allow sunlight to penetrate. Maintaining microalgae in motion and in contact with the medium is crucial for their growth. However, these systems can decrease photosynthesis efficiency, risk evaporation, and require large construction dimensions (Masojidek et al., 2011; Moheimani, 2013). Additionally, they may be susceptible to contamination from other organisms, making them only recommended for species resistant to these

challenges. Cultivation of Chlorella in open ponds is vulnerable to undesirable algae forms, such as diatoms, cyanobacteria, and protozoans, which consume the algae, reducing the output of Chlorella biomass (Wikipedia contributors, 2024d). A comprehensive management plan containing best practices preventing and remediate contamination could enhance the economic efficiency of Chlorella production using open systems.

4.2.2.2 Closed System

Photobioreactors (PBRs) are photobioreactors designed using transparent materials with a high surface area to volume ratio. The tubular PBR design is widely used and allows for precise control over cultivation conditions, higher biomass production, and preventing pollution release. It can include bubble columns, airlift columns, agitated, conical, and tubular helical tanks (Kunjapur & Eldridge, 2010; Lee et al., 1995; Schenk et al., 2008; Hu et al., 1998). Advantages include greater control over experimental conditions, ease of sterilization, simple acclimatization, and reduced photo-oxidation. However, they are more expensive than open systems (Godos et al., 2009). PBRs can be arranged in three ways: straight tubes, cross tubes, or coiled tubes. A large tubular PBR system in Germany, with a 700 cm^3 volume and the capacity to produce up to 100 metric tons of Chlorella biomass annually, was installed (Liu et al., 2013). Panel PBRs, on the other hand, require less capital for building and operation, have a smaller dark volume, and collect less dissolved oxygen (Zemke et al., 2013; Zhang & Richmond, 2003; Ursu et al., 2014; Sui et al., 2012). Both PBRs offer advantages in terms of control over experimental conditions, ease of sterilization, and reduced photo-oxidation.

4.2.2.3 Hybrid System

Chlorella cells are cultivated in PBR-pond systems for fast development and used as seeds in raceway ponds for biomass production. In a fermenter-photobioreactor (PBR) system, cells are cultivated heterotrophically in fermenters for high-density biomass accumulation. The cells are then moved to thin PBRs for desired products like oils or astaxanthin. The optimization of production systems depends on achieving a cost balance between output and input.

4.2.3 Nutritional and Commercial Value of Chlorella

Chlorella, a genus of 13 single-celled green algae, is the most researched species, rich in fatty acids, dietary fibers, carotenoids, vitamins, minerals, and other bioactive components, making it a nutritious meal.

Protein Chlorella is a protein-rich microalgae with a protein content of up to 68%, three times higher than beef. Its low molecular weight makes it easily digestible in damaged cells. *Chlorella vulgaris* peptides have significant prophylactic effects against cellular damage. Its amino acid composition includes aspartic acid, threonine, serine, glutamic acid, glycine, alanine, cysteine, valine, methionine, isoleucine, leucine, tyrosine, phenylalanine, histidine, lysine, arginine, tryptophan, ornithine, and proline (El-Naggar et al., 2020).

Carbohydrates *Chlorella* sp. is a rich source of polysaccharides, including starch, cellulose, and reducing sugars. Its yield varies between 13% and 19%, with neutral sugars like glucose, galactose, mannose, arabinose, xylose, ribose, fucose, and rhamnose (Raposo et al., 2013a; Shi et al., 2007; Montero-Sánchez et al., 2012; Ferreira et al., 2017). β-1,3-glucan, a prominent Chlorella constituent, can act as an antioxidant and immunostimulator. Cellulose, a β1-3 glycosidic bond, can be used in functional goods promoting human health.

Lipids Chlorella produces long-chain polyunsaturated fatty acids (PUFAs) through synthesis, which are influenced by the strain and cultivation conditions (Rismani, 2017; Gutiérrez-Pliego et al., 2018; Shanmugam et al., 2020). *Chlorella* sp. PUFAs are mostly composed of oleic fatty acid, while *Chlorella homosphaera* contains linoleic acid, gamma-linoleic acid, and α-linoleic. EPA and DHA accumulate in *C. homosphaera* when cultured on BG-11 medium, and their accumulation increases with glucose addition. DHA and EPA have anti-inflammatory properties, and Chlorella has a high amount of ALA, which is the precursor of DHA and EPA (Kim & Chojnacka, 2015; Bewicke & Potter, 1984). Both EPA and DHA have immunomodulatory properties, hindering the generation of proinflammatory proteins. Administering EPA and DHA from Chlorophyceae to healthy and diabetic mice resulted in a decrease in the release of cytokines, including IFN-γ, TNF-α, and IL-4. The fatty acid composition of *C. vulgaris* consists of 70.18% saturated fatty acids, 16.85% monounsaturated fatty acids, and 8.72% polyunsaturated fatty acids, resulting in a diverse lipid profile (Bewicke & Potter, 1984).

Pigment Chlorella sp. produce significant pigments, including chlorophyll and carotenoids, with a higher concentration of chlorophyll compared to processed supplements (Geetha et al., 2010). Chlorophyll has health benefits, including wound healing, regulating menstruation, and beneficial for individuals with hemophilia, diabetes, and asthma. Chlorophyll is an antioxidant compound with medicinal properties, including wound healing and anti-inflammatory effects. Carotenoids in *Chlorella* species vary, with β-carotene, α-carotene, lutein, zeaxanthin, astaxanthin, and neoxanthin found in *C. pyreniodesa*; violaxanthin, anteraxanthin, and zeaxanthin in *C. ellipsoidea*; lutein in *C. vulgaris*; and keto-carotenoids in *C. zofingiensis*. Carotenoids are widely used in food, feed, nutraceutical, and pharmaceutical sectors due to their vibrant coloring properties, potent antioxidant activity, and positive impact on human health (Kumar et al., 2011; De Fretes et al., 2012; Liu & Chen, 2014).

Table 1 Nutritional information and health benefits of Chlorella and Spirulina (CBI Ministry of Foreign Affairs, n.d.)

Chlorella	Spirulina
Chlorella is a good source of: Protein (50–60%, including all 9 essential amino acids) Vitamin A Zinc Iron Phosphorus Magnesium	Spirulina is a good source of: Protein (60–70%) Thiamine (vitamin B1) Riboflavin (vitamin B2) Niacin (vitamin B3) Copper Iron
It is also a source of B-vitamins, including riboflavin (B2), thiamine (B1), and folate (B9), and contains a wide range of antioxidants such as omega-3s, vitamin C, and carotenoids.	Spirulina also contains vitamins E, C, and B6, as well as magnesium, plant-based protein phycocyanin, and antioxidants.
Potential health benefits of Chlorella include: Detoxifying the body of heavy metals Reducing blood pressure and cholesterol levels Boosting the immune system Anti-inflammatory effects	Potential health benefits of chlorella include: Reducing blood pressure and cholesterol levels Boosting the immune system Anti-inflammatory effects

Phenolic Compound Phenolic compounds play a crucial role in microalgae's chemical defense systems, protecting them from metal pollution, bacterial colonization, and UV radiation (Muszyńska et al., 2018; Bito et al., 2020; Nutritionist, 2024). They shield vital biological components like cell membranes, nucleic acids, structural proteins, and enzymes from free radicals. Phenolic compounds found in *C. vulgaris* and *Chlorella sorokiniana* have therapeutic attributes like antioxidant, anti-tumor, and antibacterial effects (Nutritionist, 2024). Chlorella's phenolic compounds can inhibit tumor formation in liver cells by preventing lipid oxidation, counteracting free radicals, and safeguarding DNA.

Functional food, containing essential nutrients and functional elements, is on the rise due to the growing demand for these meals. Microalgae like Chlorella offer promising compounds for functional food components, including beneficial nutrients like amino acids, antioxidants, and vitamins. Chlorella may also boost probiotic survival in fermented dairy products (Table 1).

5 Microalgae in Food Industry

Microalgae are nutrient-rich, containing abundant carbohydrates, proteins, enzymes, fiber, vitamins, and minerals like vitamins A, C, B1, B2, B6, niacin, iodine, potassium, iron, magnesium, and calcium. Traditionally used in Asian countries like China, Japan, and Korea as a food source, microalgae are now consumed globally for their nutritional value. Key species like *Chlorella vulgaris,*

Haematococcus pluvialis, Dunaliella salina, and cyanobacteria such as *Spirulina platensis* and *Spirulina maxima* are commercially significant, primarily used as nutritional supplements and animal feed additives. *Spirulina platensis* has gained global popularity as a dietary supplement due to its high nutritional content, particularly as a source of proteins, polyunsaturated fatty acids (Hills & Nakamura, 1978), pigments (Wells et al., 2017; Raposo et al., 2016), vitamins, and phenolics (Becker, 2004a; Moreno et al., 2017). The primary commercial use of Spirulina is in the isolation of phycocyanin, a blue pigment involved in photosynthesis. Microalgae and their biocompounds are also utilized in various culinary products, such as isotonic drinks, cereal bars, instant soups, and biscuits, enhancing their nutritional value, particularly in terms of protein and mineral content (Garcia et al., 2017; Becker, 2004b). Chlorella is economically significant for producing by products used in food preservation (WHO, 2003; Granato et al., 2010; Roberfroid, 2000), while *Dunaliella salina* is a major source of beta-carotene, an orange pigment and vitamin A precursor. *Arthrospira, Chlorella,* and *Nannochloropsis* are rich in proteins and are also significant sources of polysaccharides or oligosaccharides, making them potential candidates for prebiotics (Becker, 2004a; Hills & Nakamura, 1978).

The microalgal market is primarily dominated by Chlorella and *Spirulina* due to their high protein content, nutritional value, and ease of cultivation, with their biomass commercially available in pills, capsules, and liquids as dietary supplements (Wells et al., 2017; Raposo et al., 2016). The World Health Organization (WHO) emphasizes that dietary patterns and lifestyle behaviors are key modifiable risk factors for chronic illnesses like obesity, type 2 diabetes, and hypertension, making functional foods, which include microalgae, increasingly important for enhancing health and managing disease (Moreno et al., 2017; Garcia et al., 2017; Becker, 2004b; WHO, 2003).

The growing interest in natural resources with high nutritional value has positioned microalgae as a promising and sustainable option for developing innovative functional foods due to their diverse bioactive compounds and extensive taxonomic variety (WHO, 2003; Granato et al., 2010; Roberfroid, 2000; Dewapriya & Kim, 2014). Microalgae offer a rich nutritional profile, including proteins, carbohydrates, lipids, vitamins, and minerals, along with bioactive substances like carotenoids, polyphenols, and polyunsaturated fatty acids, which possess antioxidant, anti-inflammatory, anticancer, and antibacterial properties (Plaza et al., 2008; Vieira et al., 2020; Rumin et al., 2020; De Morais et al., 2015).

While *Arthrospira platensis* and *Chlorella vulgaris*, recognized as Generally Recognized as Safe (GRAS), have been widely studied and utilized as functional foods, species like *Nannochloropsis oculata* and *Porphyridium purpureum* hold potential for creating healthier food items but require further research (Koyande et al., 2019a; de Jesus Raposo et al., 2013; Caporgno & Mathys, 2018; Bernaerts et al., 2018) (Fig. 4). Notably, *Haematococcus pluvialis* is valued for producing astaxanthin, a potent antioxidant with significant health benefits, currently used in dietary supplements and as a feed component to enhance salmon color (Li et al., 2019). Microalgae-derived bioactive substances, such as polyunsaturated fatty acids and phycobiliproteins, are increasingly explored by the food industry. Studies have

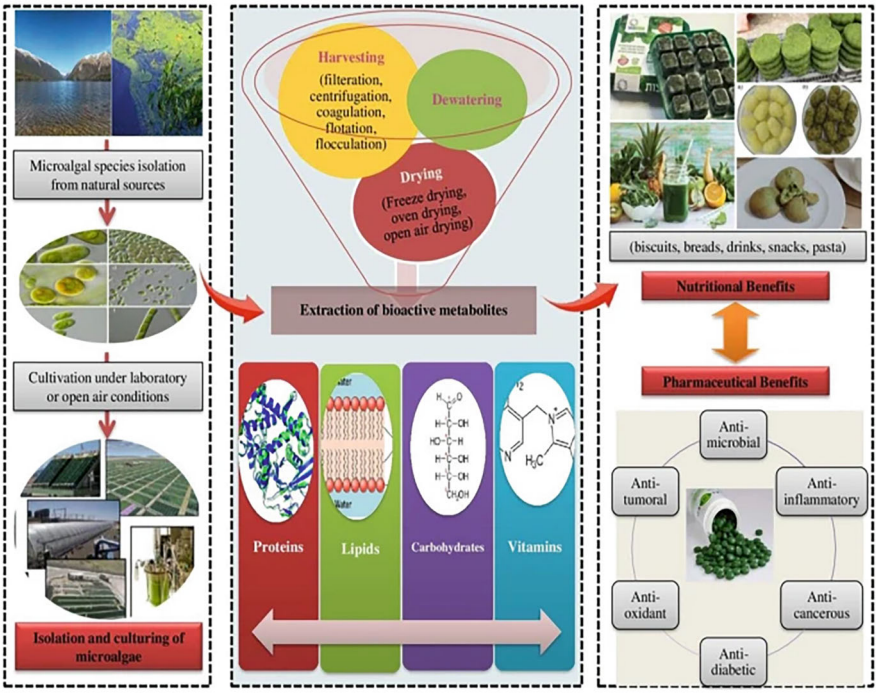


Fig. 4 Commercial applications of microalgal products (Bernaerts et al., 2018; Li et al., 2019; Matos et al., 2016; Villaró et al., 2021; Qazi et al., 2022)

shown that incorporating microalgae like *Tetraselmis chuii, Nannochloropsis gaditana,* and *Chlorella vulgaris* into gluten-free bread improves its nutritional content but may affect sensory qualities, highlighting the need for further research to optimize their use in food products (Table 2).

6 Microalgae in Feed Industry

Microalgae, a diverse aquatic resource, display significant genetic and physiological diversity, with both autotrophic and heterotrophic species. Phototrophic species like *Arthrospira* (formerly *Spirulina*), *Chlorella*, *Dunaliella*, and *Haematococcus* are well-known for their efficient photosynthesis, making them valuable for generating proteins, carbohydrates, lipids, and other bioactive compounds. Additionally, heterotrophic species such as *Crypthecodinium*, *Schizochytrium*, and *Ulkenia* are utilized for producing n-3 fatty acids. This versatility has led to extensive research into microalgae's potential in food, feed, and biofuel production, offering a sustainable alternative to traditional resources (Gouveia et al., 2008; Adarme-Vega et al., 2012).

Table 2 High-value bioproducts extracted from microalgae (Li et al., 2019)

Product group	Application	Source
Phycobiliproteins, carotenoids	Pigments, cosmetics, pro vitamins	Phycocyanin (*Spirulina platensis*), β carotene (*Dunaliella salina*), astaxanthin and lutein (*Haematococcus pluvialis*), Eicosapentaenoic acid - EPA (*Chlorella minutissima*), docosahexaenoic acid (DHA) (*Schizochytrium sp.*), Arachidonic acid (AA) (*Parietochlorisincise*), Biotin (*Euglena gracilis*)
Polyunsaturated fatty acids (PUFAs)	Food additive, nutraceuticals	
Vitamins	Nutrition	α-tocopherol (Vitamin E) (*Euglena gracilis*) ascorbic acid (Vitamin C) (*Prototheca moriformis*, a Chlorella spp.

In the context of protein sources, microalgae offer a promising solution to the rising demand for sustainable nutrition. Traditional protein sources like chicken, beef, and soybeans face competition from human consumption, driving up costs and impacting industries like cattle farming. To address this, innovative protein alternatives, including Single Cell Protein (SCP) derived from microalgae, are being explored. With over 100,000 species identified, microalgae are not only effective in CO_2 fixation and biomass production but also enhance the nutritional value of animal products. Their growing application in pet food, driven by the nutritional benefits of species like *Spirulina*, is contributing to the expansion of the global pet food market (Adarme-Vega et al., 2012; Gimpel et al., 2015; Priyadarshani & Rath, 2012; Pirwitz et al., 2016).

6.1 Microalgae in Animal Feed

Microalgae play a crucial role in the feed industry, accounting for nearly 30% of global microalgae production, with species like *Schizochytriu*m sp., *Chlorella* sp., and *Arthrospira* sp. being prominent. Incorporating microalgae into the diets of animals such as ruminants, pigs, poultry, and rabbits has shown to enhance growth, improve meat quality, and provide essential nutrients like DHA and EPA. For instance, *Schizochytrium* sp. in pork and chicken meat boosts fatty acid composition due to its high DHA content. Additionally, microalgae like *Phaeodactylum tricornutum* and *Nannochloropsis* sp. are valuable in animal feed for their anti-inflammatory properties and high EPA content, making them a sustainable alternative to traditional protein sources in pet food and livestock diet (Priyadarshani & Rath, 2012; Pirwitz et al., 2016; Souza et al., 2019; Zhang et al., 2001; Calder, 2012). The integration of microalgal biomass into animal feed not only provides essential nutrients such as vitamins, amino acids, and fatty acids but also supports sustainable agricultural practices by reducing competition with human food resources. Species like *Chlorella, Isochrysis*, and *Nannochloropsis* are especially vital in aquaculture, supporting the growth of fish larvae, while microalgae like *Chlorella* and

Arthrospira enhance poultry feed by improving the yellow hue of grill skin and egg yolks. Given their high protein content and diverse nutritional benefits, microalgae are increasingly recognized as a valuable alternative feed source, contributing to the growth and health of livestock and pets while promoting sustainability in the animal feed industry (Gimpel et al., 2015; Batista et al., 2013).

6.2 Microalgae in Aquafeed

Microalgae biomass is being explored as a valuable feed for fish and prawns in sustainable aquaculture, with studies highlighting its potential benefits (Camacho-Rodríguez et al., 2018; Ahmad et al., 2022). Despite its promise, several challenges must be addressed, including safety concerns, antinutritional factors (ANFs), and low digestibility due to high cellulose content in microalgal cells (Gominho-Rosa et al., 2015). Aquafeed represents a significant portion of aquaculture costs, and with traditional feed ingredients like fish meal and soybean meal becoming less viable, microalgae could offer an ecological and economically advantageous alternative. Properly optimized microalgae-based feed could reduce production costs, minimize waste, and lessen environmental impact. Microalgae species such as *Spirulina* sp., *Dunaliella* sp., and *Isochrysis* sp. are used in fish hatcheries, but their large-scale cultivation remains limited (Hodar, 2020). Research shows that microalgae offer the highest net biomass productivity compared to land-based plants or animals (Rizwan et al., 2018; Chu, 2012). Incorporating microalgae derivatives into feed can enhance weight gain, provide antioxidant benefits, and improve fish coloration. Unlike terrestrial plants, microalgae lack lignin and hemicellulose, making them easier to digest and potentially improving immunological health through prebiotic and probiotic properties.

7 Microalgae in Pharmaceutical and Cosmetic Industry

Microalgae are emerging as significant resources in the nutraceutical and medicinal sectors due to their ability to produce a wide range of biologically active metabolites. Key components include phycobilins, carotenoids, β-carotene, omega-3 fatty acids, polysaccharides, and vitamins, which have various health benefits (Sathasivam & Ki, 2018; Koyande et al., 2019a). Carotenoids, such as β-carotene and astaxanthin, are particularly valued for their antioxidant properties, which help combat oxidative stress linked to chronic diseases like cancer and cardiovascular conditions (Maoka & Akiomoto, 2011). For instance, β-carotene from *Dunaliella salina* has been shown to reduce cancer risk and prevent UV-induced damage (Ruperez et al., 2009), while astaxanthin from *Haematococcus pluvialis* is recognized for its potent antioxidant effects and broad protective benefits (Anila et al., 2016).

Microalgae also offer high-quality protein with superior digestibility, comparable to traditional sources like soy and egg (Amorim et al., 2020; Masten Rutar et al., 2022). They can be cultivated without competing for arable land or freshwater, making them a sustainable protein source. In addition, microalgae provide valuable polysaccharides with antimicrobial, antitumor, and immune-modulating activities (Arad & Levy-Ontman, 2010). They are also a primary source of essential omega-3 and omega-6 fatty acids, crucial for human health and commonly found in fish (Kothri et al., 2020; Barkia et al., 2019). As traditional sources of these fatty acids face challenges, microalgae present a promising alternative due to their nutritional benefits and sustainable production (Hamed, 2016).

7.1 Biological Activities of Microalgae

The search for new antibiotics is intensifying due to the rise of antibiotic-resistant bacteria, leading to a focus on microalgae as a potential source of antimicrobial agents. Studies have shown that microalgae produce various antibacterial compounds, including free fatty acids, polysaccharides, cyclic compounds, terpenes, and halogenated hydrocarbons (Mishra et al., 2021). Fatty acids and their derivatives, such as γ-linolenic acid, eicosapentaenoic acid, and docosahexaenoic acid, have demonstrated significant antibacterial properties, with their effectiveness influenced by factors like saturation level, carbon chain length, and double bond arrangement (Desbois et al., 2008; Desbois & Smith, 2010). Microalgae often secrete free fatty acids into their environment as a defense mechanism against pathogens and other competing organisms (del Pilar Sa'nchez-Saavedra et al., 2010; Kumar et al., 2018). These fatty acids can cause bacterial cell lysis, making them promising candidates for new antibiotic development. While the use of these antimicrobial compounds is still in the early stages, they hold potential for creating new antibiotics to address resistant bacterial strains (Mishra et al., 2021).

7.2 Microalgae in Cosmetic Industry

Microalgae are increasingly being utilized in the cosmetics and cosmeceuticals industry due to their bioactive compounds, which offer various benefits for skin and hair care. These compounds, including carotenoids, β-carotene, and phycobiliproteins, are incorporated into products such as anti-aging creams, sunscreens, and hair care items. For example, *Spirulina platensis* is used for skin rejuvenation, while *Chlorella vulgaris* enhances collagen production and tissue regeneration (Mishra et al., 2023; Wang et al., 2015; Ariede et al., 2017; Rizwan et al., 2018; Tiwari et al., 2018; Balić & Mokos, 2019). Additionally, microalgae-derived pigments and phenolic compounds provide antioxidant protection and support skin health (Besednova et al., 2020; Jimenez-Lopez et al., 2021;

Sahin, 2018). The microalgae sector in personal care is expected to grow significantly, with projections estimating a market value of US $107.0 million by 2033 and a compound annual growth rate (CAGR) of 6% (Ariede et al., 2017). This growth is driven by increasing demand for natural and vegan products and eco-friendly production methods of microalgae. The unique properties of microalgae, including their high nutrient content and sustainable production, align with consumer preferences for environmentally conscious and cruelty-free cosmetics, potentially boosting their market presence (Mishra et al., 2023; Ariede et al., 2017; Mobin et al., 2019).

8 Microalgae in Biofuel Industry

8.1 Biodiesel and Bioethanol Industry

Microalgae offer a promising alternative to fossil fuels due to their advantages over first- and second-generation biofuels. They can be cultivated in diverse environments, including seawater, freshwater, brackish water, and wastewater, using open raceway ponds or closed photobioreactors (Saratale et al., 2022; Das et al., 2019a; Al-Jabri et al., 2021). Microalgae produce a range of macromolecules such as proteins, lipids, and carbohydrates, which can be converted into various biofuels including bioethanol, biodiesel, biocrude oil, bio-jet fuel, pyrolysis oil, biomethane, and biohydrogen (Das et al., 2019b; Aljabri et al., 2022; Kumar & Sharma, 2014; Ohse et al., 2015; Brennan & Owende, 2010; Adesanya et al., 2014) (Fig. 5).

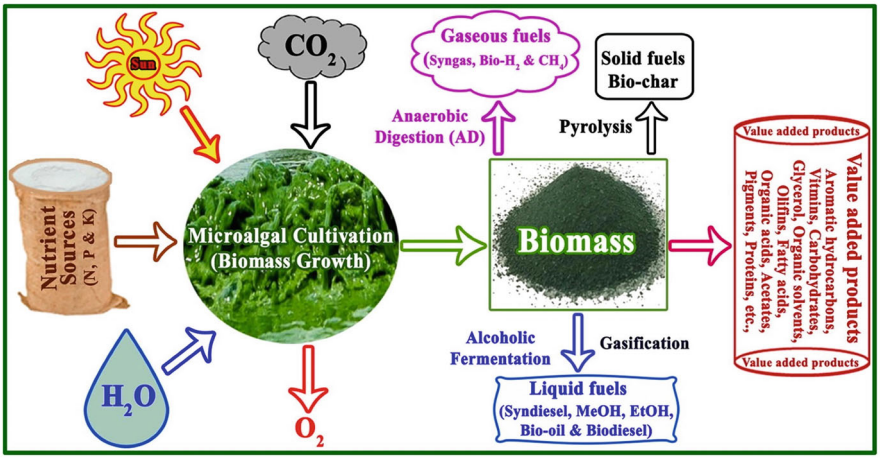


Fig. 5 Microalgae for biofuel production (Brennan & Owende, 2010)

8.2 Biodiesel

The use of algal biomass for biodiesel production has gained importance due to microalgae's rapid growth and high oil yield, which can be up to 30 times more than terrestrial crops (Ohse et al., 2015). Microalgae have lipid concentrations ranging from 5% to 75% of their dry biomass and produce fatty acids similar to those used in biodiesel, with a significantly smaller environmental footprint compared to petroleum diesel (Brennan & Owende, 2010; Adesanya et al., 2014; Sharma et al., 2012). Lipid accumulation in microalgae is influenced by nutrient depletion (Batista et al., 2018). Biodiesel from microalgae features high cetane numbers, which enhance fuel quality and reduce emissions (Ebhodaghe et al., 2022). The production process, involving the recycling of carbon and transesterification of lipids, contributes to carbon neutrality and has advantages such as high growth rates and resilience to environmental stress (Song et al., 2022). The fatty acid profile of microalgae directly affects biodiesel's combustion efficiency and heating power, making it a viable alternative to fossil fuels (Chhandama et al., 2021). Microalgae-derived biodiesel has excellent performance characteristics, including high mobility, renewable nature, and low pollutant levels (Bartley et al., 2014). Cultivation conditions significantly affect lipid concentration, with stress factors like nutrient shortages and environmental changes enhancing lipid accumulation (Suyono et al., 2015; Park et al., 2019). Biodiesel synthesis involves converting fatty acids into methyl esters through either direct transesterification or a two-step process involving lipid extraction (Vignesh et al., 2021; Qi et al., 2017; Menegazzo & Fonseca, 2019). Although there are no absolute obstacles to the development of microalgae biofuel, advances in biological and technical methods are needed to optimize production (Sudhakar et al., 2018).

8.3 Bioethanol

Bioethanol derived from microalgal biomass is a promising alternative to petroleum-based fuels and is widely used in transportation, with Brazil and the US leading in global production, accounting for 62% of the total output. Microalgae are valuable for biodiesel production due to their lipid content and efficient photosynthesis. They also offer potential for bioethanol production because they can contain carbohydrates that can be converted into ethanol through hydrolysis, unlike land plants which require complex structural biopolymers (Olabi et al., 2022; Khan et al., 2018a). Microalgae species with high starch content, such as *Chlorella*, *Dunaliella*, *Chlamydomonas*, and *Scenedesmus*, are particularly useful for bioethanol production. These algae do not require energy for molecule dispersion and transportation, simplifying the bioethanol production process. The efficiency of ethanol generation depends on factors such as algal development, biomass accumulation, sugar types in the feedstock, and fermentation conditions, all influenced by the specific algal

species and their growth conditions (Siddiki et al., 2022; Reyimu & Özçimen, 2017). Optimizing the growth conditions, including light, pH, temperature, and nutrients, is critical for enhancing ethanol output. Green algae, known for their efficient photosynthesis, are preferred for bioethanol production due to their high carbohydrate and sugar yields (Shen, 2020). Additionally, genetic factors play a significant role in carbohydrate production, making genetic modifications in algal cells essential for improving biomass and carbohydrate yields. The asexual reproduction of most algal species also prevents gene escape, which is advantageous for genetic engineering (Olabi et al., 2022; Khan et al., 2018a).

9 Technological Challenges and Advancements in the Biofuel Sector

Algae biofuels are emerging as a sustainable energy solution amid growing global energy demands and climate change concerns. Utilizing photosynthesis and the high lipid content of algae, these biofuels offer a more efficient alternative to traditional feedstocks and can be produced in various environments, including wastewater and non-arable land. This adaptability and potential for reducing greenhouse gas emissions make algae biofuels a promising option for mitigating climate change (Lee & Low, 2018; Chen, 2017; Roy & Dasgupta, 2018). Technological advancements have significantly enhanced the algae biofuel industry. Key improvements include genetic engineering for strain enhancement, such as using CRISPR-Cas9 to increase lipid production and stress tolerance (Lee & Low, 2018). Cultivation methods, including photobioreactors (PBRs) and open pond systems, have been optimized for better light exposure and CO_2 utilization (Chen, 2017; Roy & Dasgupta, 2018). Conversion technologies like hydrothermal liquefaction (HTL) and transesterification have seen progress in catalyst efficiency and reaction conditions, improving biodiesel production (Elliott, 2017; Zeng, 2018; Brennan & Owende, 2019). Economic feasibility has been boosted by integrated biorefineries that produce biofuels alongside valuable by products like proteins and pigments. Circular economy models focus on recycling nutrients and water, enhancing sustainability. Automation and data analytics have improved operational efficiency, while environmental and economic evaluations guide ongoing development. Future prospects for the industry include hybrid systems, advances in genome editing, sustainable practices, and robust policy support (Su, 2020; Wang, 2019).

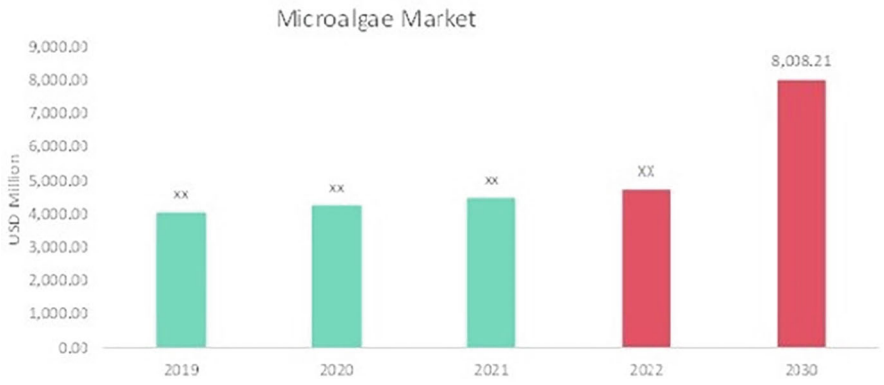
Despite their potential, algal biofuels face several challenges that hinder their widespread adoption. Key obstacles include high costs and scalability issues related to algae farming and extraction processes. Technological advancements are needed to improve biofuel production efficiency and reduce overall costs (Olguín et al., 2022). Integrating algal biofuels into existing infrastructure also presents significant hurdles. Microalgal biorefineries encounter multiple constraints in achieving zero-waste production. Variations in algal biomass composition, influenced by factors

such as pH, temperature, light intensity, and nutrient availability, complicate processing and increase costs (Ryan et al., 2016). The downstream processing, which accounts for about 40% of production costs, further impacts cost-effectiveness (Norsker, 2017). Additionally, there is a lack of comprehensive data comparing different microalgal culture processes, which hampers the development of more efficient methods.

Mixotrophic cultures have shown promise in improving productivity by enhancing stability and survival in varying environmental conditions. Utilizing environmental bioremediation techniques, such as wastewater and flue gas utilization, and optimizing resource use can enhance output and reduce costs. Strategies include using aquaculture water to reduce water usage, improving oil yield, and leveraging biogas for upgrading (Sari et al., 2021; Slade & Bauen, 2013). A thorough Techno-Economic Analysis (TEA) and Life Cycle Assessment (LCA) of microalgal cultivation and biomass conversion processes are crucial due to the high production costs and competitive market prices. Implementing a comprehensive approach that includes data collection, technology application, and addressing limitations can help overcome these challenges and improve overall efficiency (Sari et al., 2021; Slade & Bauen, 2013).

10 Global Microalgae Market Overview

Microalgae Market Size was valued at US $4735.28 million in 2022. The microalgae market industry is projected to grow from US $8008.21 million by 2030, exhibiting a compound annual growth rate (CAGR) of 6.93% during the forecast period (2023–2030).



10.1 Microalgae Market Segment Insights

10.1.1 Microalgae Type Insights

The microalgae market is categorized by type, including *Dunaliella Salina, Chlorella, Spirulina*, *Haematococcus,* and *Nannochloropsis,* among others. *Spirulina*, a cyanobacterium often called blue-green algae, is renowned for its high nutrient content, making it a superfood. It is rich in protein, vitamins, copper, fatty acids, and iron, with two main types: *Arthrospira platensis* and *Arthrospira maxima* (Fortune Business Insights, n.d.). The increasing consumer demand for natural food ingredients and cosmeceuticals has significantly driven the growth of Spirulina, which holds a major market share and is expanding at a CAGR of 6.32%.

10.1.2 Microalgae Source Insights

The microalgae market is categorized into two main segments based on form: dry/powder and liquid. Liquid microalgae, suspended in water, are used in industries like biofuels, cosmetics, and medicine. Cultivation techniques include open ponds, photobioreactors, and closed systems. Open ponds are cost-effective but prone to contamination and lower quality output. Photobioreactors, while more expensive, offer better control over growing conditions and produce higher-quality microalgae. The properties of liquid microalgae determine the types of products that can be derived, such as natural colorants from pigments or biofuels from lipids. However, challenges such as contamination, nutrient availability, and light intensity require significant research and development efforts to improve culture and processing efficiency.

10.1.3 Microalgae Application Insights

The microalgae market is segmented into various uses, including food & beverages, medicines, cosmetics, animal feed, and biofuels. Rising awareness of healthy living and organic foods has led to increased demand for high-quality, natural ingredients. Microalgae, particularly *Spirulina*, are used in bakery, dairy, and confectionery products to enhance taste, color, and nutritional value, attracting health-conscious consumers. This growing preference for natural ingredients is driving food manufacturers to invest in microalgae to improve their product offerings. The food and beverages sector holds the largest share of the microalgae market, representing about 25% of the total market (Fortune Business Insights, n.d.).

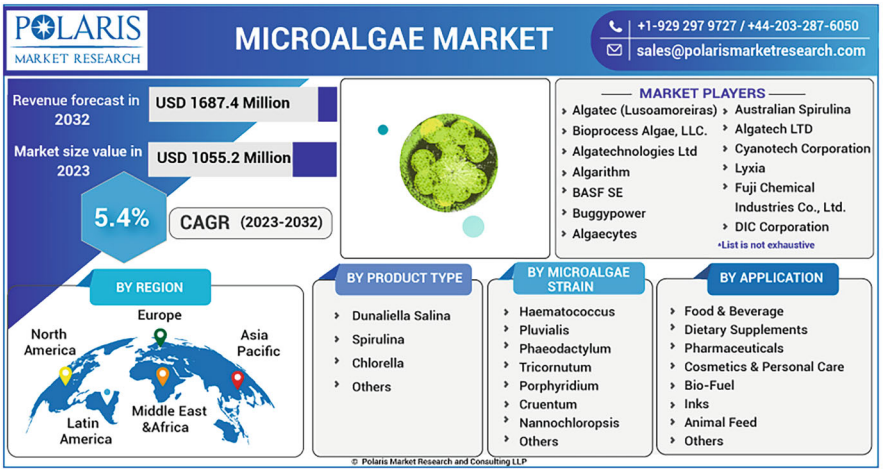
10.1.4 Regional Insights

The global microalgae market is segmented by region, including North America, Europe, Asia-Pacific, South America, and the Middle East & Africa. In North America, the market is growing due to increasing demand for sustainable and eco-friendly products. Microalgae are used across various sectors, and North America's growth is driven by the rise in natural goods and sustainable practices, with the US leading the market and Canada showing potential. In Europe, the market is expanding due to a preference for health-beneficial, organic, and chemical-free products. The rising demand for vegan and organic items is expected to boost market growth. The Asia-Pacific region, led by China, Korea, and Indonesia, is experiencing significant growth due to the prolific production of Spirulina for nutraceuticals and the cosmeceutical industry's rising demand. China is the dominant player, with Japan focusing on advanced technology for microalgae products, and India and Australia emerging as key markets due to interests in sustainable agriculture and biofuels. Overall, the global microalgae market is expected to grow substantially, driven by their potential as biofuels, food, feed, and nutraceuticals, supported by increased R&D and consumer preference for natural ingredients.

10.1.5 Distribution Channels

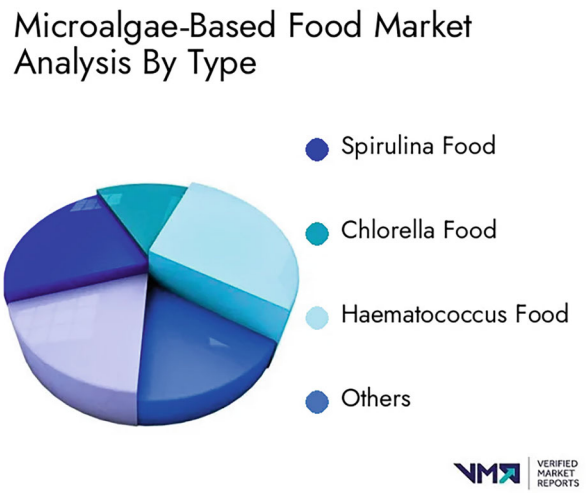
The microalgae market is segmented into B2B and B2C, with various distribution channels including direct sales, distributors, e-commerce platforms, biotechnology firms, and research organizations. The market is expected to grow due to significant investment in research and development by key industry players. The global market is populated by many global, regional, and local participants, with tier-1 and tier-2 corporations dominating approximately 64% of the market share. Microalgae are abundant and diverse organisms with numerous health and nutritional benefits. They have been used in food, feed, cosmetics, and agricultural inputs, offering a sustainable means of enhancing nutrients and eliminating toxins from the environment. By 2050, the increasing population will result in a significant increase in the need for food, as agriculture plays a vital role in food production. Government initiatives aiming at standardizing the farming process of microalgae and rapid technical improvements aimed at developing novel strains that improve energy efficiency and can withstand high salinity are expected to drive the expansion (Fortune Business Insights, n.d.).

Prominent companies in the microalgae business include Algenol Biotech, Cellana Inc., Cyanotech Corporation, DIC Corporation, E.I.D Parry ltd, Fuqing Spirulina Co. Ltd., Kent BioEnergy Corporation, Taau Australia, and Astareal AB (Polaris Market Research, n.d.).



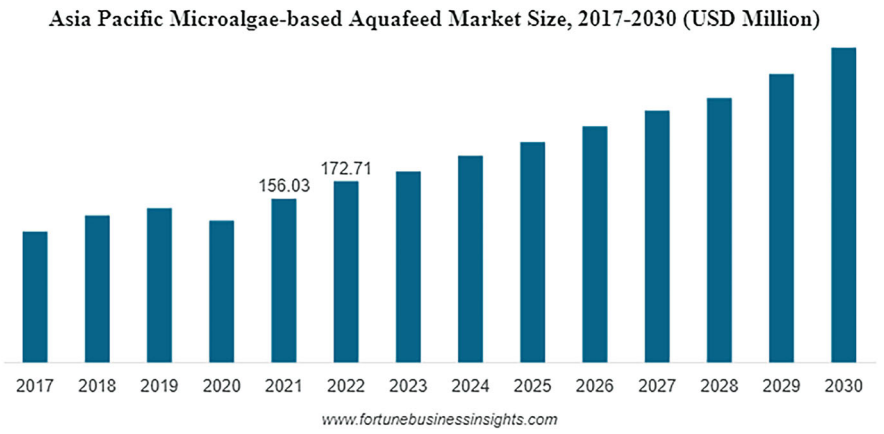
The microalgae market is expanding due to the increasing demand for bioactive molecules in pharmaceutical applications. Microalgae have the capacity to create bioactive molecules like antibiotics, monoclonal antibodies, neurotoxic compounds, enzymes, subunit vaccines, hormones, and more. They are also promising for vaccine delivery systems due to their non-toxic properties and high protein content in a single chloroplast. Microalgae also hold potential as a renewable energy source for medicines and biofuels. The market's growth is driven by limited substrate supplies, increased productivity, and the Generally Recognised as Safe (GRAS) status of compounds obtained from microalgae (Fortune Business Insights, n.d.; Polaris Market Research, n.d.). The global microalgae-based products market is segmented into North America, Europe, Asia-Pacific, Middle East & Africa, and South America. The market's growth is driven by the wide range of uses and useful characteristics of microalgae, making them highly desirable in various industries. The rise in food allergies and the growing demand for vegan options are driving interest in algae-derived protein. The Centers for Disease Control and Prevention (CDC) predict that food allergies will increase, boosting the demand for natural, allergen-free protein sources. Algae, both macroalgae and microalgae, offer a rich nutrient profile, including vitamins, minerals, omega-3 fatty acids, and up to 70% protein content. This makes algae a valuable ingredient for functional foods aimed at vegetarians and vegans. Companies like Solazyme are leveraging microalgae to create innovative vegan food products. Solazyme's Chlorella-based AlgaVia Whole Algae Flour and AlgaVia Whole Algae Protein are examples of allergen-free, gluten-free, and sustainable ingredients. This positions microalgae as a promising solution in the vegan food sector, potentially capturing a significant share of the alternative protein market by 2054. Key players in the microalgae market include Algae Health Sciences, Bluebiotech International GmbH, Cargill, Cellana Inc., and others. These companies are investing in mergers, acquisitions, and R&D to expand their global presence and market reach. The microalgae food market is growing due to increased demand for nutrient-dense, eco-friendly food options and advancements

in biotechnology. Technological improvements in cultivation and processing have made microalgae products more affordable and accessible. Additionally, microalgae are gaining traction in aquaculture as a sustainable feed alternative, enhancing the nutritional quality of aquatic diets and supporting eco-friendly practices. Innovations in photobioreactors and processing techniques are further driving the industry's expansion.



Aquaculture has been growing rapidly over the past 30 years, significantly contributing to global food security. "The State of World Fisheries and Aquaculture 2022" report forecasts that aquaculture production will reach 109 million tons by 2030 and over 140 million tons by 2050. This growth is driven by technological advancements, innovative production practices, and high-quality feed. Currently, feed accounts for 70–80% of aquaculture costs, with a historical reliance on marine resources. As demand increases, the industry is shifting toward alternative feed sources, with microalgae emerging as a promising option.

The market for microalgae-based aquafeed was valued at US $239.70 million in 2022, and is projected to grow from US $257.20 million in 2023 to US $433.08 million by 2030, reflecting a compound annual growth rate of 7.73%. The Asia Pacific region leads the market with a 72.05% share. Technological advancements are enhancing microalgae cultivation, with new methods like photobioreactors and closed systems improving both the effectiveness and environmental sustainability of production. For example, Vaxa, a global technology startup, has developed a cutting-edge platform that enhances microalgae growth, particularly increasing omega-3 fatty acid levels. This technology is part of the EIT Food project and utilizes a modular, biologically safe production unit near the Hellisheidy geothermal power station (Fortune Business Insights, n.d.; Polaris Market Research, n.d.; Persistence Market Research, 2023).



11 Economic Viability of Microalgal Products

The economic viability of microalgal products is influenced by several key factors impacting their commercial production. High costs associated with cultivation, including energy, labor, and raw materials, affect feasibility. Cultivation, harvesting, and dewatering processes contribute to operational and capital expenses, necessitating advancements in cultivation techniques and bioreactor designs to improve cost efficiency (Maehle & Skjeret, 2022).

Market pricing and economic potential are driven by demand for microalgal products like biofuels, nutraceuticals, and cosmetics. The profitability of these products depends on market acceptability and competition with traditional sources (Jesse & Quinn, 2019). Understanding customer preferences and market trends is crucial for aligning production with demand (Bhatt et al., 2022). Technological improvements in farming and manufacturing can enhance economic sustainability. Advances in photobioreactor design, nutrient delivery systems, and extraction methods can increase biomass yield, product quality, and reduce costs (Maehle & Skjeret, 2022). Ongoing research is vital to keep pace with technological advancements and market demands. Sustainability is becoming increasingly important, with practices like utilizing CO_2 from industrial sources improve both cost efficiency and environmental impact. Emphasizing sustainable practices can attract investors and align with the global trend toward eco-friendly products. Government regulations significantly impact the economic environment for microalgal products (Bhatt et al., 2022). Supportive legislation, such as subsidies for renewable energy or tax incentives, can improve feasibility, while strict regulations might increase costs. Diversifying income sources by producing multiple products, such as biofuels and high-value chemicals, can enhance profitability and resource utilization (Maehle & Skjeret, 2022; Jesse & Quinn, 2019). Overall, the economic feasibility of microalgal

products requires a comprehensive approach considering production costs, market trends, technological progress, sustainability, regulatory support, and potential co-products (Maehle & Skjeret, 2022). Key factors include energy efficiency, downstream processing, strain selection, market demand, economies of scale, and technological innovations (Bhatt et al., 2022; Elisabeth et al., 2021). Addressing these factors can drive the growth of a sustainable bioeconomy (Elisabeth et al., 2021).

11.1 Cost Analysis of Production and Processing

Conducting a cost analysis for microalgal production and processing is essential for evaluating its economic viability. This analysis involves various steps, including cultivation and downstream processing, and considers both fixed and variable costs (Srimongkol et al., 2022). Costs can vary significantly based on factors such as cultivation method and production scale, impacting operational efficiency and economic feasibility (Fortune Business Insights, n.d.). The choice between open ponds and closed photobioreactors plays a crucial role in production expenses. Open ponds typically have lower construction and operational costs but face issues like contamination and reduced biomass yield due to environmental conditions. Conversely, photobioreactors, though more expensive to build and maintain, offer better growth condition control and higher biomass yields, which may reduce costs per unit in the long term. Research indicates that certain configurations of photobioreactors can result in production costs of approximately 69 €/kg, primarily due to labor and depreciation (Wikipedia contributors, 2024b; Saratale et al., 2022).

The efficiency of nutrient utilization also depends on the cultivation system. Heterotrophic systems require additional carbon sources, potentially increasing operational costs, while phototrophic systems use sunlight and CO_2, which may lower nutrient requirements if combined with waste streams. Advanced systems, including co-cultivation with other microbes, can enhance nutrient recycling and biomass production, improving overall economic feasibility (Fig. 6).

Cultivation systems also impact the environmental footprint of microalgal production. Open ponds require large land areas and more water, whereas closed systems are more resource-efficient but require more energy (Nagi et al., 2021). Integrating CO_2 from industrial sources can reduce the carbon footprint and provide economic benefits through carbon credits or reduced waste disposal costs. Technological advancements further enhance economic viability. Innovations in bioreactor design, such as flat-panel and tubular reactors, improve light penetration and gas exchange, leading to increased productivity and reduced costs. Advanced monitoring systems that regulate growing conditions can boost biomass production efficiency and minimize operational risks, thus enhancing economic feasibility (Global Seafood Alliance, 2020).

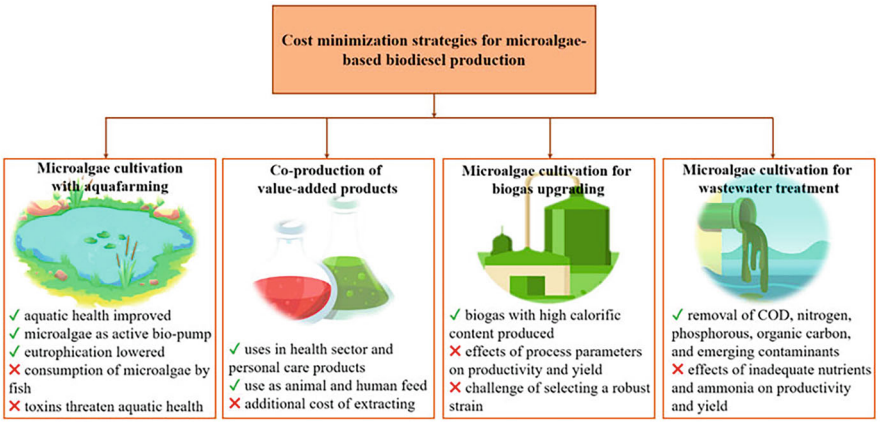


Fig. 6 Cost-minimization methods for biodiesel production from microalgae (Saratale et al., 2022)

11.2 Major Cost Components

Capital investment in microalgal production is significant, particularly for facilities utilizing photobioreactors. Initial costs encompass infrastructure, including cultivation systems, and equipment such as bioreactors, harvesting systems, and processing apparatus (Cabrera-Capetillo et al., 2023). Operational costs are also substantial, with labor being a major expense. Costs related to monitoring, harvesting, and processing are significant, though automation and efficient management can reduce these expenses. Skilled labor is essential for overseeing and regulating cultivation processes (Elisabeth et al., 2021; Pieter et al., 2020). Energy expenses are a crucial factor, especially for photobioreactor systems that require constant illumination and temperature control. Innovations that enhance energy efficiency are vital for reducing overall production costs. Energy usage for lighting, mixing, and temperature regulation can be considerable (Saratale et al., 2022; Nagi et al., 2021). Nutrient costs vary, affecting production expenses. The composition and amount of nutrients like nitrogen and phosphorus in the growth medium impact costs. Effective nutrient recycling and sourcing strategies can help mitigate these expenses (Sari et al., 2021; Bhatt et al., 2022).

Harvesting and processing also contribute significantly to costs. Techniques such as centrifugation, filtration, and flocculation have different cost implications. Centrifugation is energy-intensive and can add up to 50 €/kg in tubular photobioreactor systems, while filtration is more cost-effective but may require additional steps. Flocculation is less energy-intensive but involves the use of chemicals (Global Seafood Alliance, 2020; Cabrera-Capetillo et al., 2023; Hill et al., 1984). Post-harvest processing, including drying and extraction, further impacts costs. The choice of drying method (sun drying, freeze drying, spray drying) and extraction techniques (solvent extraction, supercritical CO_2 extraction) significantly influences expenses, especially for high-value products like omega-3 fatty acids or pigments (Polaris Market Research, n.d.; Branco-Vieira et al., 2020).

11.3 Strategies for Cost Reduction

Cost reduction in microalgal cultivation is vital for economic viability and market competitiveness. Expanding from small-scale setups, such as 25 m^2, to larger facilities, like 1500 m^2, can cut production costs by up to 90%, reducing expenses from €290/kg to around €43/kg with the use of tubular photobioreactors (TPBR) (Doshi et al., 2017; Garbowski et al., 2020). Implementing artificial illumination can further decrease costs by 35% to 36% and enhance biomass production. Optimizing conditions like luminosity, nutrient availability, temperature, and pH is crucial for maximizing efficiency (Acién et al., 2012). Efficient harvesting techniques, such as flocculation or advanced filtering, can lower energy use and operational expenses. Combining different harvesting methods and using low-cost substrates, such as agri-industrial effluents, can reduce nutrient costs and support sustainability initiatives (Elisabeth et al., 2021; Gabriel et al., 2024). Centralizing production facilities can lead to significant cost savings, potentially reducing manufacturing expenses by 60–80% compared to smaller systems (Garbowski et al., 2020). Advances in bioreactor technology and automation can further improve production efficiency and reduce labor costs (Acién et al., 2012).

The pricing strategies for microalgal products must account for production costs, market demand, and competition to ensure profitability. Production costs can vary widely, from €1/kg to €69/kg, with small-scale operations ranging from €290/kg to €587/kg, while larger, optimized facilities can lower costs to as low as €43/kg. Key factors affecting costs include labor, energy use, nutrients, and harvesting and processing methods (Elisabeth et al., 2021; Doshi et al., 2017; Garbowski et al., 2020; Acién et al., 2012).

11.3.1 Pricing Strategies

Optimal pricing strategies for microalgae products are crucial for maximizing profitability and ensuring market competitiveness. Pricing based on the total cost of production plus a profit margin guarantees cost coverage but may not align with market conditions or consumer willingness to pay (Garbowski et al., 2020; Acién et al., 2012). Alternatively, setting prices based on the perceived value of high-value microalgal products, such as astaxanthin or omega-3 fatty acids, allows for premium pricing that reflects the product's benefits and uniqueness. Market penetration pricing, where initial prices are set lower to attract customers and gain market share, can also be effective but requires careful management as prices are gradually increased (Garbowski et al., 2020; Acién et al., 2012). Adjusting pricing in response to real-time market demand and competitor pricing can optimize revenue. During high demand periods, prices can be raised to increase profit margins. Offering products in bundles, implementing promotional campaigns, and introducing subscription services can enhance customer loyalty and streamline sales (Doshi et al., 2017; Garbowski et al., 2020; Acién et al., 2012). Utilizing cost-saving measures,

such as growing microalgae on low-cost substrates and investing in advanced technologies, can reduce production expenses, allowing for competitive pricing while maintaining profitability. Highlighting the environmental benefits of microalgal products can justify higher prices and appeal to eco-conscious consumers (Garbowski et al., 2020; Acién et al., 2012; Gabriel et al., 2024).

11.3.2 Profitability Analysis

Conducting a profitability analysis is essential for assessing the financial sustainability of microalgal operations. Calculating Return on Investment (ROI) helps compare initial investment costs with anticipated profits from sales, while the payback period measures how quickly an investment can be recovered through earnings. Analyzing various pricing scenarios, such as adjusting astaxanthin prices, can provide insights into potential outcomes and guide decision-making. Implementing cost-reduction strategies, such as scaling up production to achieve economies of scale (reducing costs by 60–80%), investing in advanced technologies, and utilizing waste streams like CO_2, can enhance efficiency, lower per-unit costs, and improve overall profitability (Doshi et al., 2017; Garbowski et al., 2020; Acién et al., 2012; Gabriel et al., 2024). Microalgal products' economic feasibility depends on efficient manufacturing techniques, pricing strategies, and market access. Despite initial costs, technology improvements, economies of scale, and government incentives can increase profitability. Expanding product ranges and using byproducts can reduce risks and improve profit margins. Despite competition, sustainable alternatives offer profitable opportunities. Strategic planning and innovation can contribute to sustainable development.

12 Consumer Acceptance

Market surveys and consumer behavior studies reveal growing awareness and acceptance of microalgal products due to their health and environmental benefits (Lafarga et al., 2021; Vitafoods Insights, n.d.). Educating consumers is key to expanding market acceptance and improving purchase intentions. Environmental concerns and personal values heavily influence consumer preferences, with social proof and influencer marketing enhancing product acceptance (Lafarga et al., 2021; Hung et al., 2023). Consumers are willing to pay a premium for microalgae-based products perceived as health-enhancing and sustainable. Effective communication of these benefits is crucial for market success. While microalgae products are currently popular among health-conscious individuals, increasing awareness and integrating microalgae into familiar foods could address aversions and boost consumer acceptance (Lafarga et al., 2021; Vitafoods Insights, n.d.; Hung et al., 2023; Olsen et al., 2024; Garcia-Segovia et al., 2020).

12.1 Branding and Marketing Strategies

Effective branding strategies for microalgal products should emphasize their unique health benefits, such as high protein content, omega-3 fatty acids, and antioxidants, while also highlighting their environmental advantages like minimal resource use and CO_2 capture (Vitafoods Insights, n.d.; Rumin et al., 2021). Transparent communication about production standards and certifications can build consumer trust and credibility. Targeted marketing, including educational efforts through social media and partnerships with health influencers, can boost awareness and acceptance (Vitafoods Insights, n.d.; Rumin et al., 2021). Offering samples and promotional discounts may encourage trial and repeat purchases. Product diversification and innovation, such as integrating microalgae into familiar foods and exploring new formulations, can appeal to various customer segments (Chu, 2017). Strategic collaborations with food companies and research institutions can enhance market entry, product development, and quality [232,238]. Additionally, conducting market research and adhering to regulatory compliance are crucial for understanding consumer preferences, improving offerings, and building brand loyalty through transparent labeling and local sourcing (Chu, 2017; Doucha & Lívanský, 2014). Microalgal products can be effectively branded by highlighting their health benefits, sustainability, and transparency. These strategies should focus on health-conscious consumers, highlighting their nutritional value and functional foods. Consumer education, social proof, and innovative product development can build trust and enhance brand loyalty. Targeting specific consumer segments, such as health-conscious consumers, vegans, and superfood enthusiasts, and optimizing digital marketing can also be effective. Continuous innovation and strategic partnerships will strengthen market positioning and consumer loyalty.

13 Next-Generation Products in the Pipeline and Novel Applications in Various Industries

The current use of algae in industry is primarily limited to specific applications, but recent research highlights a shift toward high-value bioproducts and the concept of algae-based biorefineries (Doucha & Lívanský, 2014; Laurens et al., 2017). Advances in biotechnology are enhancing microalgae's potential as a source of nutrients and bioactive compounds, paving the way for innovative health products. Microalgae offer significant potential in commercial food markets due to their high protein, carbohydrate, and lipid content, with microalgal proteins, such as those from *Spirulina*, showing promise as sustainable alternatives to soy-based proteins (Koyande et al., 2019b; Kent et al., 2015; Grahl et al., 2018; Palanisamy et al., 2019; Montero-Lobato et al., 2018). Other species like *Nannochloropsis* and *Isochrysis*, along with various strains like *Pavlova* and *Phaeodactylum*, are being explored for their biomass generation and applications in aquaculture (Olasehinde

et al., 2017; Enzing et al., 2014; Surzycki et al., 2009; Yan et al., 2016). These advancements emphasize the diverse benefits and applications of microalgae in nutrition and health. Research on alternative microalgal species beyond *Spirulina* and *Chlorella,* such as *Nannochloropsis* and *Isochrysis*, has highlighted their potential for biomass generation. These species, including *Pavlova*, *Phaeodactylum*, and others, are available in various forms—live, fresh, frozen, and freeze-dried—and are used in starter cultures, aquaculture concentrates, and formulated meals (Yan et al., 2016).

Advancements in recombinant protein technology have enabled microalgae, like Dunaliella, to be used for producing high-quality proteins due to their unique chloroplasts, which support complex protein synthesis (Márquez-Escobar et al., 2018). Microalgae such as *Chlamydomonas reinhardtii* and *Schizochytrium* sp. are being explored for producing medicinal proteins and innovative vaccines, showing promising results in immune response and clinical efficacy (Miquel-Clopés et al., 2019; Merchant, 2001; Karkos et al., 2011). Clinical trials have also validated the health benefits of powdered dietary supplements from *Spirulina* and *Chlorella*, including improvements in lipid profiles, immune function, and antioxidant properties (Mao et al., 2005; Park et al., 2008; Ryu et al., 2014; Kim et al., 2019; Garcia et al., 2017; Begum et al., 2016; Guerin et al., 2003).

Microalgae are significant sources of pigments such as chlorophylls, carotenoids, and phycobilins. *Haematococcus* sp. and *Dunaliella* sp. are notable for accumulating bioactive pigments like astaxanthin and β-carotene, respectively (Guerin et al., 2003; Tafreshi & Shariati, 2009; Dewi et al., 2018; Talero et al., 2015). Research is underway to explore the health benefits of microalgae-derived chemicals, including their potential in disease prevention. Carotenoids, including violaxanthin, have shown antiproliferative effects on cancer cells (El-Hack et al., 2019; Cha et al., 2008; Pasquet et al., 2011; Soontornchaiboon & Kim, 2011; Amaro et al., 2013; Grudzinski et al., 2016; Patias et al., 2017), while zeaxanthin, produced by various microalgae, holds promise as a dietary supplement and dye (Sajilata et al., 2008b; Zhang et al., 2018a; Chung et al., 2017; Gong & Bassi, 2016). Lutein, an important carotenoid with antioxidant and anti-inflammatory properties, is found in microalgae such as *Chlorella vulgaris* and *Scenedesmus* sp. (Fernández-Sevilla et al., 2010; Araya et al., 2014; Chen et al., 2017; Xie et al., 2017; Marcati et al., 2014; Pletikapić et al., 2011). Microalgae offer advantages over traditional sources like calendula, including faster growth and higher lutein concentrations. Additionally, microalgae produce high-molecular-weight polysaccharides with applications in immunomodulation, antioxidation, and antimicrobial activities (Chen et al., 2017; Xie et al., 2017). Sulfated polysaccharides from species like *Porphyridium* spp. and *Aphanothece sacrum* are valuable for their water-binding and thickening properties, beneficial in various industries (Marcati et al., 2014). Sulfated exopolysaccharides (sPS) from microalgae are complex polymers, primarily composed of xylose, glucose, and galactose (Sajilata et al., 2008b). These sPS, produced by species such as *Chlorella stigmatophora*, *Tetraselmis* sp., and *Dunaliella tertiolecta*, show promise for use in functional foods, cosmetics, and medicines due to their bioactive

properties (Zhang et al., 2018a; Chung et al., 2017; Gong & Bassi, 2016; Fernández-Sevilla et al., 2010; Araya et al., 2014). However, their commercial application is limited by their low concentrations in microalgae (Chen et al., 2017).

Microalgae also produce phenolic compounds, which have shown antioxidant, anti-inflammatory, and neuroprotective effects, making them potential treatments for cancer and neurodegenerative diseases (Xie et al., 2017). Additionally, microalgae are key sources of polyunsaturated fatty acids (PUFAs), including DHA and EPA, which are beneficial for health. Despite progress in using microalgae for DHA, development for EPA and other essential fatty acids remains less advanced (Marcati et al., 2014; Pletikapić et al., 2011; Guzman et al., 2003). Arachidonic acid is essential for cellular membranes and functions, impacting the neurological system, skeletal muscle, and immune responses, and acts as a precursor to prostaglandins and eicosanoids. Its potential use in newborn formulas highlights its role in the central nervous system and retinal development. Arachidonic acid can be sourced from diet or synthesized from linoleic acid through metabolic processes (Guzman-Murillo & Ascencio, 2000).

The microalgal industry, though providing valuable biomass for food and nutrition, faces limitations due to technological challenges and restricted algae types. Advances in phenomics and culture technologies are necessary to optimize production and improve economic viability. Algae can produce high-value compounds either naturally or through genetic engineering, but research is mainly focused on model species. Expanding the study to wild-type algae and integrating them into versatile biorefineries could enhance their application as plant biostimulants and other uses (Soanen et al., 2016; Balti et al., 2018; Goo et al., 2013). In addition to well-established microalgae-based products, emerging areas of development include biofuels, polyhydroxyalkanoates (PHAs), enzymes, and various bioactive compounds such as violaxanthin, zeaxanthin, prebiotics, phenolics, glutathione, and sterols. Phytosterols from algae, which include compounds like stigmasterol, are currently obtained through inefficient plant-based extraction methods. Algae offer a more efficient alternative for producing these sterols, including novel types like gymnodinosterol and brevesterol, with potential yields comparable to or exceeding those from plants (Patel et al., 2013; Ratledge et al., 2010; Ryckebosch et al., 2014).

Microalgae are being explored for their potential to produce PHAs, which could replace petroleum-based plastics due to their lower cultivation costs (Wen & Chen, 2003; Tallima & El Ridi, 2018). Additionally, algae are emerging as sources of valuable enzymes, such as cellulases and lipases, which could be used in various industrial applications. Although commercial production of microalgae-based enzymes is still in its early stages, laboratory research shows promise (Wells et al., 2017; Sasso et al., 2012). Furthermore, microalgae are a sustainable source for biofuels, including biodiesel, bioethanol, and biogas, which could reduce greenhouse gas emissions and provide an alternative to traditional fossil fuels (Chiaiese et al., 2011; Ras & Geleijnse Trautwein, 2014).

Ethanol, a long-recognized fuel source, is primarily produced from sugar cane and starch, but environmental concerns arise from its competition with food crops. Microalgae present a sustainable alternative due to their ability to produce ethanol

photosynthetically and tolerate high ethanol, salt, and pH levels. *Chlorella vulgaris* is particularly suitable for ethanol production with conversion efficiencies above 65.0%. The production process also allows for the extraction and purification of ethanol and the reuse of CO_2, making microalgae a promising source for bioethanol (Batta et al., 2006; Piironen et al., 2003). Biomethane, or syngas, is generated from the anaerobic digestion of microalgal biomass through a series of processes including hydrolysis and fermentation. This method is energy-efficient compared to other systems as it bypasses extraction steps, utilizing the entire algal structure—proteins, lipids, and sugars—and converting residual nutrients into reusable minerals. Microalgae's growth in liquid media and their three-dimensional structure make them ideal for this process, with the resulting gas being a valuable energy source and the biomass residue serving as fertilizer (Cassuriaga et al., 2018). Biohydrogen production from renewable sources addresses energy sustainability challenges. While biohydrogen is environmentally friendly, being non-toxic and producing only water and heat, its production remains costly compared to traditional energy sources. Microalgae are particularly suited for biohydrogen production due to their rapid growth, minimal nutrient requirements, and efficient fermentation processes. They can generate hydrogen through biophotolysis or dark fermentation, though large-scale commercial production is still lacking. Scaling up photobioreactor designs and integrating wastewater in industrial biorefineries could reduce costs and enhance production efficiency (Rahman & Miller, 2017). Bioplastics, derived from renewable resources, offer ecological and sustainable alternatives to traditional plastics. Algae, with their high biomass, rapid growth, and ease of cultivation, are promising candidates for bioplastic production. They provide a potent alternative to other microbial sources and can be used to manufacture biocomposites with desirable qualities (Brasil et al., 2017; Yong et al., 2016; Demirbas & Karslioglu, 2007).

13.1 Novel Application of Microalgal Products in Food and Feed Market

Microalgal biomass is rich in essential nutrients such as carbohydrates, vitamins, proteins, and lipids, making it a valuable additive in various foods including pasta, dairy products, and baked goods, with approvals from the US Food and Drug Administration (FDA) (Alam et al., 2015). These nutrients also contribute to the production of omega-3 polyunsaturated fatty acids, notably docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids, which are used to boost the nutritional value of eggs, meat, and milk. Microalgae are a significant source of high-quality protein and are increasingly used in the health food industry due to their health benefits and practicality. Key species like *Spirulina, Chlorella, and Dunaliella* are known for their high protein content (55–70% dry weight). Spirulina supports immune function and helps prevent viral infections and cancer, Chlorella extracts boost hemoglobin levels and lower blood sugar, and *Dunaliella salina* thrives in brackish environments

(Khan et al., 2018b; Hernández et al., 2014; Batista et al., 2014). The functional food sector is expected to expand rapidly, with microalgal extracts being used in products like *Dunaliella* carotenoid-enriched oils, Chlorella health drinks, and Spirulina CO_2 extracts. Additionally, microalgae can enhance food items by improving their bioactive content. Research into the use of spray-dried microalgae such as *Nannochloropsis oculata, Porphyridium cruentum*, and *Diacronema vlkianum* in ice cream production shows that microalgae can improve color and functional attributes, though precise optimization of their quantities is essential (van den Oever et al., 2017; Zheng & Suh, 2019).

Microalgae, such as *Chlorella, Scenedesmus*, and *Spirulina*, enhance animal health and are vital in aquatic feed for marine and freshwater species, zooplankton, crustaceans, and finfish. Selection for feed additives requires considerations of toxin levels, biological component availability, cell wall digestibility, and cultivation ease. Over 40 microalgae species are used globally, with *Isochrysis galbana* and *Tetraselmis suecica* improving growth in saltwater, while drum-dried *Scenedesmus* is ideal for Artemia, and Spirulina and Chlorella are used in fish feed (Zhu et al., 2016; Becker, 2007b; Gutiérrez-Salmeán et al., 2015; Gouveia & Rema, 2005).

Microalgae pigments like astaxanthin and phycocyanin benefit farmed animals, enhancing growth and color. Spirulina and Chlorella are used in feed for prawns and crustaceans, respectively, while astaxanthin from *Haematococcus pluvialis* improves salmonid meat color. In pet food, microalgal biomass enhances well-being, resulting in glossy fur and attractive feathers (Zhu et al., 2016; Becker, 2007b; Gutiérrez-Salmeán et al., 2015; Gouveia & Rema, 2005). Microalgae are utilized in producing nutraceuticals due to their nutritional and functional benefits. Chlorella, cultivated for algal nutraceuticals since 1960 in Japan, provides bioactive compounds like lutein, astaxanthin, and beta-carotene that benefit health (Gouveia et al., 2007). The cosmetics industry values microalgae for their antioxidant properties, aiding in skin regeneration, UV protection, and treatment of aging and pigmentation issues (Mobin & Alam, 2017; Lorenz & Cysewski, 2000). Over 350 commercial cosmetic products use microalgal components for skin care, including UV protection and skin whitening by inhibiting melanin production (Berthon et al., 2017; Christaki et al., 2015; Jimenez-Lopez et al., 2021). Microalgae biomass is rich in bioactive compounds with therapeutic potential for treating cancer, viruses, and microbial infections (Santiesteban-Romero et al., 2022; Wang et al., 2015). *Spirulina, Chlorella, Dunaliella,* and *Haematococcus* are commonly used for drug production, with some species showing cytotoxic effects on various cancer cells (Mayer et al., 2013; Lauritano et al., 2012; Fabris et al., 2020; Singh et al., 2005). *Spirulina platensis* produces neuroprotective compounds that may aid in treating Alzheimer's and Parkinson's diseases. Additionally, microalgae offer antiallergenic and immune-regulating molecules and show promise in developing oral vaccines for influenza A, Zika, and HIV viruses (Wang et al., 2015; Ramos et al., 2011; Raposo et al., 2013b).

13.2 Environmental Applications

Microalgae are effective at capturing and reusing atmospheric CO_2 through photosynthesis, with a sequestration efficiency 10–50 times greater than terrestrial plants (Guzmán et al., 2003). They can absorb CO_2 from industrial flue gases and convert it into organic compounds like lipids, pigments, proteins, and carbohydrates (Morales-Sánchez & Rodríguez-Hernández, 2016). However, real-world scale-up studies are needed to evaluate long-term effectiveness and address operational challenges, such as flue gas cooling, before integrating microalgae into power generation and chemical production.

Microalgae are highly effective for remediating industrial and mining effluents due to their nutrient removal, adsorption capacity, and regenerative properties, offering advantages over chemical treatments. They utilize pollutants for growth and can accumulate heavy metals, providing a cost-effective solution for bioremediation. Their ability to survive in harsh conditions, adapt to various habitats, and form symbiotic relationships enhances their industrial application, though their impact is limited to aquatic environments and they require more time for cleanup compared to chemical methods (Schmidell & Pinto, 2016; Moussavi & Ghasemi, 2020). Heavy metal contamination from anthropogenic activities poses severe environmental and health risks. Phycoremediation, using microalgae, is an eco-friendly method for removing these contaminants from polluted effluents. Microalgae use biosorption and bioaccumulation to extract metals and are also effective in reducing nitrogen, phosphorus, and organic matter in wastewater, which helps prevent eutrophication and ammonia toxicity (Kumar & Srivastava, 2017). They achieve high carbon fixation rates and biomass productivity, surpassing terrestrial crops. However, challenges such as culture contamination, cell harvesting, and limited large-scale processing experience remain (Chen & Zhang, 2011).

13.3 Research and Development Initiatives

Microalgae are potential organisms for fuel generation, waste processing, therapeutic uses, and food components. They can produce lipids, proteins, vitamins, and convert carbon dioxide and nitrogen into usable forms. However, their use in industry is limited due to production costs. Microalgae offer sustainable solutions in energy, medicine, food, and environmental management. Recent research focuses on genetic engineering, optimizing bioprocesses, ensuring environmental sustainability, high-throughput screening, and digital technologies.

Genetic engineering is crucial for enhancing microalgae productivity, stress tolerance, and metabolic efficiency. Techniques such as CRISPR-Cas9 and TALENs are employed to modify microalgal genomes, introducing beneficial traits or removing detrimental ones. These advanced methods have successfully created algal strains with improved characteristics, such as increased lipid content, which is

vital for biofuel production. For instance, genetic modifications in *Nannochloropsis* species have led to significant enhancements in lipid content by overexpressing genes related to fatty acid biosynthesis (Kumar & Kumar, 2013). Similarly, the amplification of key enzymes in lipid production pathways has improved lipid yields in *Chlorella vulgaris* and *Nannochloropsis gaditana*, while genetic alterations in *Haematococcus pluvialis* have boosted the synthesis of valuable compounds like astaxanthin, enhancing the economic viability of these processes (Cecchin et al., 2020). Synthetic biology is advancing microalgal research by allowing scientists to design and manipulate metabolic pathways, which enhances the production of valuable compounds like terpenoids and astaxanthin. This approach has led to significant improvements in the yield of these compounds, which are used in various applications such as medicine, biofuels, and cosmetics (Khoo et al., 2019). The progress in genetic engineering and synthetic biology is paving the way for more efficient microalgal biofactories with enhanced yields and functionality.

Optimizing microalgal cultivation and processing is crucial for scaling up production and achieving economic viability. Advances in photobioreactor (PBR) design, such as flat-panel and helical-tubular models, improve light dispersion and gas exchange, enhancing biomass productivity (Fig. 7). Innovations in downstream processing, including supercritical fluid and ultrasonic-assisted extraction, have

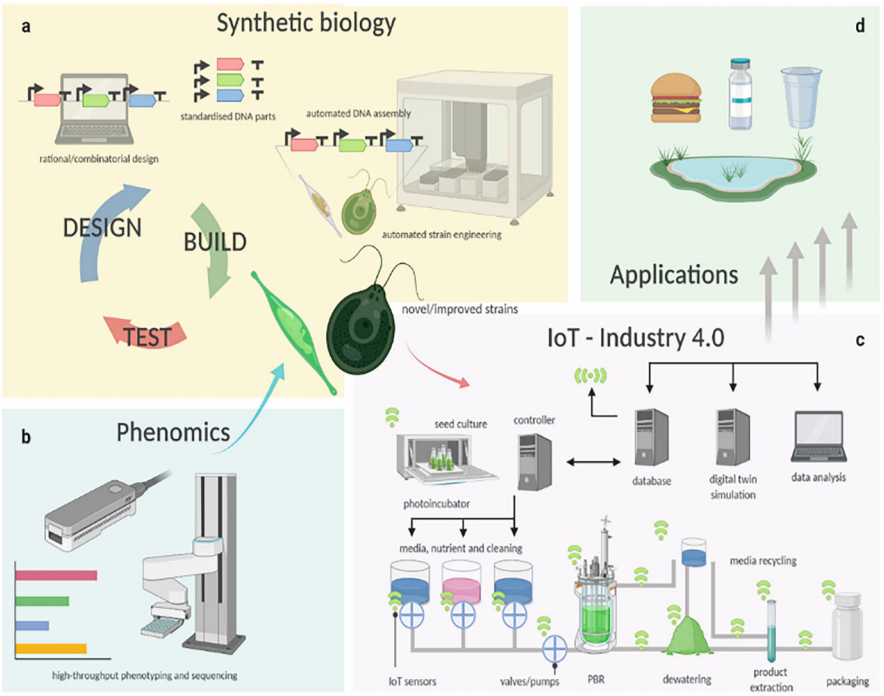


Fig. 7 Technology developments in algal cultivation and harvesting, automation, phenotyping, and synthetic biology (Chen et al., 2022)

improved efficiency and reduced costs (Penloglou et al., 2024; Deconinck et al., 2018). High-throughput screening (HTS) technologies and omics approaches provide rapid strain identification and insights into metabolic processes, respectively, further advancing microalgal research. Digital technologies, including IoT and AI, optimize cultivation conditions through continuous monitoring and data analysis, enhancing process efficiency (Bradley et al., 2023; Zhang et al., 2023; Song et al., 2024).

Data analytics are crucial for enhancing both predictive maintenance and operational efficiency in microalgal production. Predictive maintenance leverages historical and real-time data to foresee equipment failures, reducing downtime and maintenance costs, while data-driven decision-making aids in scaling and sustaining microalgal production systems economically (Song et al., 2024). Incorporating Industry 4.0 technologies into microalgal cultivation can significantly boost productivity and sustainability. Environmental sustainability drives microalgal product development, with Life Cycle Assessment (LCA) being essential for evaluating the ecological impacts of production processes. Recent LCAs highlight microalgae's potential to mitigate climate change and treat wastewater by sequestering CO_2 and recycling nutrients (Deconinck et al., 2018). Adopting circular economy principles, such as using wastewater for algae growth and repurposing byproducts like biomass, enhances sustainability and minimizes waste (Fabris et al., 2020). Collaborative research networks, such as the Horizon 2020 program and the Algae Biomass Organisation, are pivotal in advancing microalgal technologies by fostering interdisciplinary cooperation and funding opportunities (Wang et al., 2022).

14 Sustainability and Innovation Through Enhanced Cultivation Techniques and Integrated Circular Economy Models

Microalgae are a nutritious source of proteins, carbohydrates, lipids, vitamins, and antioxidants, making them valuable for producing high-value goods like biofuels, animal feed, biofertilizers, and bioactive compound (Asaduzzaman et al., 2024; Hsia & Yang, 2015). Enhanced cultivation techniques and circular economy models are driving sustainability and innovation in microalgal products, with cultivation systems categorized into open and closed systems.

14.1 Open System

Open pond systems, used widely for large-scale microalgal production, are simple and cost-effective but susceptible to environmental fluctuations and microbial contamination (Mérida & Padrón, 2023). Innovations such as adjustable paddle wheel

designs and improved CO_2 injection techniques (e.g., microbubbles and direct infusion) have enhanced energy efficiency, mixing uniformity, and CO_2 absorption, thereby boosting biomass productivity (Costa & De Morais, 2014). Additionally, using wastewater as a nutrient source aligns with circular economy principles, providing both growth nutrients and waste treatment (Yadala & Cremaschi, 2016). Recent advancements address challenges in open systems, including contamination, nutrient management, and environmental conditions. While traditional monoculture techniques face issues with microbial contamination and reduced productivity, co-culturing microalgae with other microorganisms has emerged as a promising solution. Co-cultures leverage the flexible metabolism of microalgae, potentially improving outcomes in various sectors such as food, pharmaceuticals, and biofuels (Abdelfattah et al., 2023).

14.1.1 Photobioreactor Designs

Photobioreactors (PBRs) are advanced systems designed to cultivate microalgae in a controlled environment, offering significant advantages over open pond systems in terms of light utilization, gas exchange, and productivity (Rasheed et al., 2023). Although initially more costly, PBRs provide up to 10 times higher yields per unit area by optimizing nutrient and energy use within a confined space. LED lighting in PBRs enhances photosynthesis and biomass production, with red and blue LEDs boosting lipid accumulation for biofuel production. Automated systems integrated into PBRs allow real-time adjustments of conditions like pH, temperature, and nutrients, increasing stability and reducing operational costs (Hsia & Yang, 2015; Rasheed et al., 2023; Richmond, 2004). Recent innovations in PBR designs include flat-panel and helical-tubular configurations. Flat-panel PBRs enhance light penetration and photosynthetic efficiency with large surface areas and internal light guides, while helical-tubular designs improve mixing and gas exchange through turbulent flow, ensuring uniform exposure of algae to light and nutrients (Wang et al., 2016; Saravanan et al., 2021). Hybrid systems that combine elements of both designs offer flexible production methods and improved efficiency in diverse environmental conditions. Advances in materials, such as UV-resistant and transparent components, further enhance PBR performance by increasing light transmission and reducing maintenance needs.

Co-cultivation and polyculture systems involve growing multiple microalgae species together to leverage their beneficial interactions, leading to enhanced nutrient utilization, reduced contamination risks, and increased biomass production (Abdelfattah et al., 2023). These systems allow certain species to secrete compounds that boost the growth of other species, fostering more robust and productive cultures. In wastewater treatment, polyculture systems excel by enabling different algae species to target various contaminants, resulting in a more comprehensive cleanup. The diverse biomass from these systems also provides multiple potential uses, adding overall value (Saravanan et al., 2021; Padmaperuma et al., 2018).

14.1.2 Cultivation Systems and Polyculture

In microalgal cultivation, there are two main types of cultures: axenic cultures, which contain only one species, and non-axenic cultures, which include multiple microorganisms. The choice between these cultures depends on the specific goals, such as production efficiency, substrate consumption, or biomass accumulation (Yao et al., 2019). Co-cultivation systems, involving both microalgae and bacteria, can optimize nutrient cycling and establish beneficial interactions, leading to symbiotic or synergistic relationships. These systems are crucial for sustainable energy production, bioremediation, and applications in various industries (Yao et al., 2019; Dao et al., 2018; Makut et al., 2020; Gou et al., 2020; Vraspir & Butler, 2009; Yong et al., 2014; Owolabi et al., 2012).

Microalgae-bacteria consortia offer several advantages, such as enhanced growth through mutual exchange of substances and oxygen-carbon dioxide cycling (Gou et al., 2020; Vraspir & Butler, 2009; Yong et al., 2014). Various co-cultivation techniques, including direct mixing, pelletization, and biofilm creation, each have distinct benefits and limitations (Novoveská et al., 2016). While monocultures are commonly used in commercial cultivation, co-cultivation addresses issues like contamination and resource efficiency, offering increased stability and productivity. Additionally, microalgae biofilms and mixed cultures are promising for wastewater treatment and renewable energy production, supported by advancements in omics and genetic engineering (Kuo et al., 2022; Lamba et al., 2023).

14.2 Integration with Circular Economy Models

The concept of the Circular Economy (CE) focuses on reducing consumption and increasing resource efficiency, closely aligning with the Sustainable Development Goals (SDGs). By integrating algal biomass into CE models, environmental benefits are realized through reduced greenhouse gas emissions and improved wastewater treatment. CE principles, symbolized by the 6Rs (reduce, redesign, remove, reuse, recycle, recover), advocate for minimizing waste and optimizing resource use (Geissdoerfer et al., 2017; Alhawari et al., 2021; Faruk et al., 2023; Singh & Ahluwalia, 2012). Applying these principles to microalgal cultivation involves using waste materials like CO_2 and nutrient-rich wastewater as resources to promote algal growth, creating a regenerative cycle that enhances sustainability. Microalgae support CE by converting waste streams into valuable products while reducing reliance on artificial nutrients (Handayani et al., 2023; Barik et al., 2024; Van Den Hende et al., 2014). They can utilize various waste sources for growth, aiding in wastewater treatment and CO_2 sequestration, which contributes to reducing industrial emissions (Chen et al., 2011; Milledge, 2013). The integration of microalgae into circular models not only optimizes resource use but also creates renewable products, such as biofuels and biofertilizers, enhancing overall sustainability

(Guarnieri et al., 2011; Katiyar et al., 2017). Advances in technology, including improved bioreactor designs and genetic engineering, have further boosted the efficiency of microalgal production and processing, reinforcing the role of microalgae in a circular economy (Abdel-Raouf et al., 2012; Roy & Mohanty, 2021).

Using food waste as a substrate for cultivating microalgae and employing polygeneration strategies are key to enhancing the sustainability of microalgal-based biofuel production. Microalgae, beyond biofuels, are utilized for generating high-value products such as medicines, bioplastics, and dietary supplements, aligning with circular economy principles to reduce costs and maximize waste utilization (Abo et al., 2019). The circular economy aims to minimize environmental impacts and resource consumption, with microalgae offering benefits like high protein content and applications in food supplements and livestock feed. To address high production costs, leveraging nutrient-rich wastewater for algae cultivation is a promising strategy, potentially lowering expenses and advancing the sector (Fu et al., 2019). Expanding focus to include de-oiled microalgal biomass (DMB) for producing additional chemicals and biomaterials enhances economic value and supports a sustainable economy (Carino & Vital, 2022).

15 Supply Chain Dynamics of Microalgal Products

15.1 Strain Selection and Cultivation

The supply chain dynamics in microalgae farming encompass various stages from strain selection to the final product delivery, including cultivation, harvesting, processing, and distribution. Microalgae are pivotal in addressing food and fuel security due to their efficient commercial production. These single-celled photosynthetic organisms are extensively studied for their sustainable production of vitamins and carotenoids (Diaz et al., 2023; Subashchandrabose et al., 2021). They can grow using carbon dioxide and minimal saline medium through photosynthesis or a variety of organic compounds via heterotrophy (Boussiba et al., 1987). Compared to higher plants, microalgae are more suitable for mass production in resource-limited areas due to their faster growth rates, higher biomass productivity, and ability to grow using marginal resources without competing for arable land and freshwater (Diaz et al., 2023; Subashchandrabose et al., 2021; Belay, 2002).

Microalgae are utilized across various sectors, including textiles, poultry farming, biofertilizers, medicines, and eco-friendly fuels like bioalcohols, biogas, and biodiesel. However, achieving cost-competitive algal products remains challenging, as current costs do not match those of fossil fuels. Key research areas include strain selection, cost-effective growth media, optimized biomass production, product-specific cell stoichiometry, commercialization success, and reduced operational costs, particularly in cultivation and harvesting. Strain selection, focusing on factors like biomass productivity, nutrient content, and stress tolerance, is crucial for maximizing the production of valuable substances like biofuels, nutraceuticals,

and bioplastics. For example, *Chlorella vulgaris* and *Spirulina platensis* are favored for high biomass and nutrient content, while *Nannochloropsis* sp. and *Scenedesmus* sp. are noted for their lipid content, essential for biofuel production (Lorenz & Cysewski, 2000; Shah et al., 2022). The composition of microalgae, including lipids, proteins, carbohydrates, and pigments, plays a critical role in strain selection for various products. For instance, *Nannochloropsis* is chosen for its high lipid content, essential for biodiesel production, while Spirulina is selected for its protein-rich profile, ideal for nutraceuticals (Markou & Nerantzis, 2013; Wang et al., 2021). Additionally, certain strains like *Haematococcus pluvialis* are valued for their ability to produce high-value compounds such as astaxanthin under stress (Kim et al., 2022; Kumar et al., 2021). The adaptability of strains like *Chlorella sorokiniana*, which increases lipid synthesis under nitrogen deprivation, enhances their potential for biodiesel production. Furthermore, the resilience of strains like *Scenedesmus* against infections and pollutants is vital for maintaining stable and uncontaminated cultures, especially in large-scale outdoor production (Rawat et al., 2011; Mata et al., 2022).

The capacity of microalgal strains to thrive under varying temperature conditions is crucial for consistent year-round production, particularly in outdoor systems. For example, *Dunaliella salina* is known for its thermotolerance, making it suitable for open ponds in diverse climates (Chaudhary et al., 2014). High photosynthetic efficiency is also vital for biomass production, as demonstrated by *Chlorella pyrenoidosa,* which efficiently converts light into biomass, making it ideal for biofuel generation (Gouveia & Oliveira, 2009). Additionally, strains like *Scenedesmus* and *Chlorella* can utilize wastewater, offering dual benefits of biomass production and bioremediation (Sun et al., 2021). Economically feasible large-scale production favors strains like *Spirulina* in open pond systems due to lower operational costs (Shen et al., 2009; Tredici, 2004). Efficient biomass harvesting, as seen with *Chlorella* and *Spirulina*, further enhances economic viability. Market demand, particularly for omega-3 fatty acids, drives the selection of strains such as *Nannochloropsis* and *Isochrysis* for commercial production, based on economic potential.

15.2 Cultivation Techniques for Microalgae

Microalgae are a fast-growing, efficient crop with potential industrial applications, wastewater bioremediation, and carbon dioxide reduction. However, challenges include limited biomass production, small cell sizes, and expensive harvesting and processing procedures. Optimal culturing systems should have sufficient light, efficient material transfer, simple operation, minimal contamination, low costs, and high land efficiency (Cultivation of Algae – Open Pond – Oilgae – Oil from Algae, n. d.). Techniques include open culture systems and controlled closed cultivation systems using bioreactors (Fig. 8).

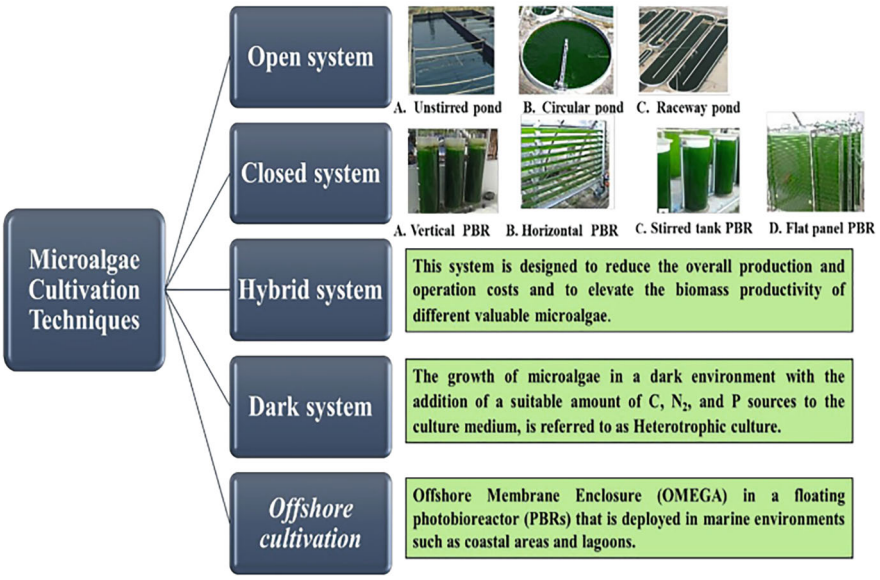


Fig. 8 Microalgal cultivation techniques (Shen et al., 2009)

15.2.1 Open Pond

Open pond cultivation is a widely used and cost-effective method for large-scale microalgae production, with advantages such as low capital and operational costs and reduced energy needs for mixing (Tan et al., 2020). However, it requires significant space and is prone to contamination and adverse weather conditions. Various open pond types include natural water bodies like lakes and man-made structures such as circular and raceway ponds. Raceway ponds, which account for over 90% of global microalgae production, are the most popular due to their economic efficiency, though they typically yield low biomass concentrations (below 1.0 g/L) and are susceptible to contamination. To counter these issues, robust and fast-growing strains like *Spirulina, Chlorella,* and *Dunaliella* are commonly used. Circular ponds, characterized by their round shape and central agitator, offer effective mixing but are limited by size due to increased water resistance and high energy consumption during agitation (Hamed, 2016; Rogers et al., 2014). Despite their simplicity, open pond systems face challenges like culture contamination, excessive water evaporation, and site selection requirements for optimal sunlight exposure. Additionally, these systems are constrained by factors such as pH, temperature, and dissolved oxygen, making them less efficient in achieving high biomass concentration compared to closed photobioreactor systems (Ruangsomboon et al., 2020).

15.2.2 Photobioreactor

Photobioreactors are specialized closed systems designed to cultivate phototrophs like microalgae, offering significant advantages over open pond cultures by preventing contamination and optimizing land use (Sierra et al., 2008). These systems ensure a controlled environment for the culture, leading to increased biomass yield and metabolic efficiency, though they involve high initial and operational costs. Tubular photobioreactors feature transparent elongated tubes that can be arranged in various configurations to maximize sunlight absorption. While effective for certain microalgae strains, these reactors face challenges with mass transfer inefficiency due to the lengthy tubes, which can cause variability in substrate and product concentrations (Posten, 2009; Tredici et al., 2010; Torzillo & Zittelli, 2015; Tamburic et al., 2011; Rinanti et al., 2013). Flat panel photobioreactors use a rectangular compartment to enhance light exposure and photosynthetic efficiency, achieved through a recirculating airlift system that reduces oxygen buildup (Tan et al., 2018; Narala et al., 2016). However, their aeration setup can harm microalgae cells. In contrast, vertical column bioreactors use a cylindrical tube and sparger for efficient gas-liquid mass transfer but suffer from inadequate light supply and high construction costs. Thin-layer reactors, like tubular designs but with reduced water depth, maximize light availability and improve mixing but lack pH control and CO_2 transfer optimization (Zakariah et al., 2015; Pulz, 2001).

15.2.3 Hybrid Cultivation System in Two-Stage Growth

The two-phase hybrid cultivation system offers a solution to the limitations of both open- and closed-culture systems by reducing production costs and increasing microalgae biomass productivity (Villanova et al., 2023). This system involves two stages: initially, microalgae are cultivated in a photobioreactor under tightly controlled conditions to ensure optimal cell growth and minimize contamination. In the second phase, these cells are transferred to open ponds where they face nutrient depletion and environmental stresses, which enhances lipid accumulation and helps them outcompete other microorganisms in the culture (Villanova et al., 2023; Liyanaarachchi et al., 2021) (Table 3).

15.3 Technologies and Methodologies for Harvesting and Processing

15.3.1 Harvesting and Dewatering

Harvesting and dewatering of microalgae involve removing water from the culture to achieve an appropriate solid content, a challenging process due to the large volume of diluted water and low biomass concentration (Uduman et al., 2010; Soomro et al.,

Table 3 Comparison of open, closed, and hybrid microalgae photobioreactor-facilitated cultivation systems (Narala et al., 2016)

Parameter	Open pond (raceway pond)	Closed photobioreactors	Hybrid system
Space required	High	Moderate	High
Setup cost	Low	High	Moderate
Contamination risk	High	Low	Low
Evaporation loss	High	Almost none	Moderate
CO_2-losses	High	Almost none	Not given
Biomass quality	Not susceptible	Susceptible	Susceptible
Culture quality	Low	High	High
Growth rate	Low	High	High
Scaling-up feasibility	Easy	Difficult	Moderate
Reproducibility of production parameters	–	Possible within certain tolerances	–
Operation type	Batch	Batch	Continuous
Energy input for mixing	Low	High	Moderate
Maintenance	Easy	Difficult	Moderate
Standardization	Not possible	Possible	–
Weather dependence	Absolute, cultivation is impossible during the raining season	Insignificant as closed configurations allow cultivation even during bad weather	–
Efficiency of treatment processes	Low, time-consuming, large volume flows due to low concentrations	High, short time, relatively small volume flows	–

2016). This step is crucial and costly, representing 20–30% of the total production expenses, primarily due to the high energy and capital costs involved dewatering aims to reduce the water content, which can be as high as 99%, using methods such as filtration and centrifugation to concentrate the microalgal biomass for further processing.

Various techniques, including filtering, centrifugation, flocculation, and flotation, are employed to collect microalgae biomass, often in combination to improve efficiency. The choice of method depends on factors such as the microalgae species, cell density, and the specific end product requirements. The small size and low concentration of microalgae cells, along with their strongly electronegative membrane surfaces, make harvesting energy-intensive and costly, often exceeding the energy content of the biomass itself. Effective harvesting and dewatering are essential for reducing volume and costs in subsequent processing stages (Barros et al., 2015; Show & Lee, 2014; Singh & Patidar, 2018; Yin et al., 2020; Ananthi et al., 2021; Milledge & Heaven, 2013; Suparmaniam et al., 2019; Hwang et al., 2013).

Filtration is crucial for separating microalgae from liquids, utilizing techniques like microfiltration, ultrafiltration, and vacuum filtration, tailored to the size and characteristics of the microalgae. Membrane filters, with pore sizes ranging from 100 nm to 0.5 nm, are made from materials such as polyvinyl chloride and polyethersulfone. Innovations like polyvinyl alcohol-coated membranes enhance performance by improving hydrophilicity and flow rates. Filtration processes include dead end filtration (DEF), which leads to rapid fouling, and tangential flow filtration (TFF), which minimizes fouling and maintains cell viability, making it suitable for smaller microalgae. For larger, filamentous species, vacuum filtration and pressure methods, such as plate-and-frame filter presses, are employed for efficient harvesting (Huang & Kim, 2013a; Gerardo et al., 2015; Bjommalm et al., 2015; Pragya et al., 2013; Christenson & Sims, 2011). Microalgal biomass collection often involves **flocculation,** where algal cells are aggregated and precipitated using various chemicals or bio-flocculants. Chemical flocculation, employing iron and aluminum salts, is widely used due to its effectiveness and low cost. However, these chemicals are toxic and require additional treatment to remove, which raises overall production costs. Bio-flocculation is a more environmentally friendly alternative, using bio-polymers like chitosan and acrylic acid to aggregate microalgae. This method is safer and more cost-effective compared to chemical flocculants. Chitosan, in particular, is noted for its effectiveness in achieving high cell recovery rates and its biodegradable nature. Despite its advantages, chitosan is expensive, leading to research into other bio-flocculants to reduce costs (Granados et al., 2012; Gerde et al., 2014; Harith et al., 2009; Lee et al., 2009; Bleeke et al., 2015; Salim et al., 2011; Rashid et al., 2013). **Flotation** is an effective method for separating microalgae from liquid solutions by generating bubbles that adhere to solid particles, causing them to float to the surface. This method is categorized into various types based on bubble size, including dissolved air flotation, dispersed air flotation, and electro-flotation. Flotation systems offer advantages such as high efficiency, simple operation, and low cost. Dissolved air flotation, which involves saturating the culture with compressed air before releasing it at atmospheric pressure, is widely used but can be costly due to high energy consumption and chemical use. Dispersed air flotation, which creates bubbles with a sparger, is more energy efficient. Electro-flotation uses electrolysis to produce small bubbles and can also disrupt cells with alternating current, though it is energy-intensive and requires frequent electrode replacement. The buoy-bead flotation method is noted for optimizing harvesting efficiency and reducing chemical use (Laamanen et al., 2016; Niaghi et al., 2015; Alhattab & Brooks, 2017; Baierle et al., 2015; Chen & Chen, 2010).

Centrifugation is an effective technique for separating microalgae cells from culture fluid using centrifugal force, which relies on the density and size of particles. This method is highly effective for harvesting cells but is both time-consuming and energy-intensive. The strong gravitational forces involved can potentially damage delicate cells and compromise sensitive nutrients. Various centrifugal systems, including disc stack, perforated basket, imperforated basket centrifuges, decanters, and hydro-cyclones, are employed for this process. While centrifugation offers high recovery efficiency (exceeding 90%) and does not require chemicals, its high energy

consumption and potential for cell damage limit its economic feasibility, particularly for large volumes. To enhance efficiency, centrifugation is often combined with sedimentation to further separate the supernatant (Singh & Patidar, 2018; Dassey & Theegala, 2013; Rawat et al., 2013; Heasman et al., 2000). **Sedimentation** separates particles from liquids based on their physicochemical properties, concentration, and size distribution. While this method is cost-effective and simple, it is generally suitable only for microalgae larger than 70 μm. For smaller microalgae (5–20 μm), flocculants are needed to enhance settling velocities. Gravity sedimentation involves allowing a slurry to settle under gravity, creating a clear liquid supernatant. However, this method is not universally applicable to all microalgal species and can be inefficient for those requiring significant electrical power for enhanced sedimentation (Show & Lee, 2014; Singh & Patidar, 2018; Yin et al., 2020; Ananthi et al., 2021).

15.4 Post-Harvesting Processing

Microalgal biomass, after harvesting, retains moisture that needs to be eliminated to stabilize and conserve it. Different drying techniques are used based on the operation's size and desired use. Drying enhances subsequent procedures and concentrates the biomass to dry solids content. Efficient dewatering techniques reduce processing costs and improve the effectiveness of subsequent drying and extraction processes. The biomass undergoes several processing stages to convert it into useful products.

15.4.1 Drying of Harvested Algal Biomass

After harvesting, microalgal biomass typically requires drying using methods such as solar drying, convection drying, spray drying, or freeze drying, based on the specific needs of the end products. Drying is crucial for maintaining the chemical composition, extending shelf life, reducing shipping costs, and minimizing microbial contamination. The choice of drying method influences the final product quality and costs, with drying accounting for 2–3% of the total system capital cost. **Solar drying** is a cost-effective method where biomass is spread in thin layers and dehydrated by sunlight. While economical, it is reliant on weather conditions, has slow drying rates, and may be contaminated by dust and insects. **Convective drying**, including oven drying, is more efficient than solar drying and retains similar oil yield and free fatty acid content as other methods. **Spray drying** produces dry powder with retained nutrients but may lead to protein loss and more oxidation of carotenoids compared to freeze drying. **Freeze drying** preserves bioactive substances and is used for high-value products, though it is energy-intensive and costly. **Drum drying** involves distributing biomass on a heated drum and is suitable for large-scale drying but can cause heat-induced damage to sensitive chemicals. Advances in temperature control and inert gases have improved its effectiveness (Bilad et al., 2014;

Mathimani & Mallick, 2018; Bharte & Desai, 2018; Brennan & Owende, 2010; Prakash et al., 1997; Balasubramanian et al., 2013; Guldhe et al., 2014; Oliveira et al., 2009, 2010; Desmorieux & Decaen, 2005; Dissa et al., 2014; Ryckebosch et al., 2011; Cherubini et al., 2009; Dong et al., 2016; Xu et al., 2011).

15.4.2 Cell Disruption

Microalgae biomass extraction involves several steps to recover valuable chemicals, with mechanical-based methods being crucial (Karemore & Sen, 2016; Ghasemi Naghdi et al., 2016; Salinas-Salazar et al., 2019; Corrêa et al., 2020; Wang et al., 2020; Günerken et al., 2015; Toursel et al., 2019; D'Hondt et al., 2017). After reaching the stationary growth phase, the biomass can be processed either as dry or as wet. For **dry biomass**, a dewatering procedure like centrifugation is followed by thermal drying, which produces biomass with over 85% dry weight. **Cell disruption** is necessary to break cellular walls and release components that are otherwise not easily extractable. This process may involve mechanical, physical, or non-mechanical techniques to enhance extraction yields. Different cell disruption methods vary in effectiveness depending on the cell wall properties of the microalgae species (Balasubramanian et al., 2013; Guedes et al., 2013; González-Delgado & Kafarov, 2013; Bernaerts et al., 2019; Elain et al., 2020).

15.4.3 Mechanical and Physical Methods

Cell lysis of microalgae can be achieved through various mechanical, physical, and thermal methods, each with distinct advantages and challenges (Piasecka et al., 2014; Zghaibi et al., 2019; Adam et al., 2012; Lafarga, 2020). Mechanical techniques include bead milling, which uses agitation with beads (0.3–0.5 mm in diameter) to disrupt cell walls. This method is effective and scalable but can be energy-intensive and varies in efficiency based on bead density and biomass flow. High-speed homogenization (HSH) employs shear forces and hydrodynamic cavitation for cell disruption, suitable for industrial applications despite high energy consumption. High-pressure homogenization (HPH) uses high pressure to generate turbulence and shear stress, effective at industrial scales but may produce undesirable cell debris and is less effective for harder cell walls. Microwave irradiation provides a simple and scalable method, effective for lipid extraction by heating polar molecules, though it may not be suitable for volatile components. Ultrasonication uses high-frequency sound waves to create cavitation bubbles that disrupt cell walls, suitable for selective protein release but often combined with chemical treatments for improved efficiency. Pulsed electric fields (PEF) offer energy-efficient, scalable cell disruption through electroporation, though it has high operational costs and is influenced by medium conductivity. Thermal treatments like autoclaving and steam explosion use heat to disrupt cells but may generate unwanted debris and consume significant energy. Each method's effectiveness and suitability depend on specific microalgae species and desired outcomes.

15.5 Non-mechanical Methods

Chemical methods for microalgae cell lysis include acid or alkaline treatments, detergents, osmotic shock, and enzymatic treatments. **Chemical methods** involve using solvents, acids, alkalis, hypochlorites, antibiotics, and detergents to deform cell walls and induce disruption. While effective, these methods pose environmental and economic challenges, particularly at an industrial scale. Surfactants, which interact with cell membrane phospholipids, can facilitate cell disruption and biomass harvesting by altering membrane properties, improving intracellular component release, and aiding in cell aggregation. **Osmotic shock** uses high solute concentrations (e.g., salt, dextran, polyethylene glycol) to cause cell wall damage by reducing osmotic pressure. This increases cell wall permeability and allows the escape of internal chemicals. Though simple, osmotic shock is slower compared to methods like autoclaving. **Enzymatic lysis** employs commercial enzymes such as cellulases, proteases, lysozymes, and glucanases to selectively disrupt cells under mild conditions. Enzymes are used in immobilized form to enhance stability and longevity. Despite their efficiency, enzymatic methods have drawbacks including long processing times, lower production capacity, potential product inhibition, and high costs. The effectiveness of these cell-disruption techniques varies based on microalgae type and product characteristics. Combining methods can improve disruption efficiency and bioproduct recovery, impacting subsequent purification processes (Toepfl et al., 2006; Zhang et al., 2020; Lorente et al., 2015, 2017; Nurra et al., 2014; Al hattab & Ghaly, 2015; Singh et al., 2019; Castro et al., 2015; Juárez et al., 2016; Huang & Kim, 2013b; Gim & Kim, 2018; Liang et al., 2012; Zhang et al., 2018b; Zheng et al., 2016; Gomes et al., 2020; Patel et al., 2018; Liu et al., 2016; Chew et al., 2017).

15.5.1 Extraction

Microalgal cells contain valuable chemicals that require isolation. Techniques like solvent extraction, cellular disruption, and supercritical fluids can be used to extract these valuable compounds (Molina Grima et al., 2013; Bensalem et al., 2018; Sharif et al., 2014; Reverchon & De Marco, 2006; Esquivel-Hernández et al., 2016; Rajan & Barron, 2019). Organic solvents are widely used for extracting biomolecules from microalgae, generally following a cell disruption process to improve solvent penetration and extraction efficiency. Various techniques, derived from plant oil extraction methods, include Organic Solvent Extraction, which involves solvents penetrating and rupturing cell walls to separate intracellular components (Urh et al., 2009; Mangold, 1983; Yang et al., 2018; Shen et al., 2016). The Folch Method uses a 2:1 chloroform-methanol mixture to create a biphasic system, though it is labor-intensive and requires large volumes of solvents. The Soxhlet Extraction method involves the repeated refluxing of organic solvent over biomass, which is effective but also solvent-intensive and laborious. The Bligh and Dyer Method

(B&D) is similar to Folch but uses different phase separation approaches, focusing on lipid extraction from aqueous systems. Choosing an appropriate solvent depends on factors such as polarity, lipid solubility, water miscibility, and toxicity. In addition to traditional solvents, alternative bio-based solvents such as terpenes, ionic liquids (ILs), deep eutectic solvents (DES), and liquid polymers are being explored. ILs and DES are noted for their reduced environmental and health impacts compared to conventional solvents. DES, formed through hydrogen bond interactions, offers cost-effective solutions with diverse starting materials and addresses some of the limitations of ILs, such as high cost and toxicity.

Supercritical fluid extraction (SFE) is an environmentally friendly technique used primarily in the food and pharmaceutical industries for its contamination-free and high-purity results. SFE operates by using supercritical fluids (SCFs), which are substances at conditions above their critical point, displaying properties of both liquids and gases (Dyńska-Kukulska & Ciesielski, 2012; Lage & Gentili, 2018; Sun et al., 2019; Żyszka-Haberecht et al., 2019; Jaime et al., 2005; de Jesus et al., 2019). This method is advantageous as SCFs have lower viscosity compared to liquids and better solubility characteristics than gases, making them effective for extracting microalgae biomolecules such as polyunsaturated fatty acids, pigments, and vitamins. Supercritical CO_2 (sc-CO_2) is the most commonly used solvent in SFE due to its low critical point near room temperature and low pressure (30.9 °C and 73.9 bar), which also makes it safe and environmentally friendly. The efficiency of SFE is influenced by several adjustable factors, including solvent type, temperature, pressure, extraction time, solvent flow rate, sample size, and particle size. SFE-CO_2, in particular, is recognized for its sustainability and safety, as CO_2 is generally regarded as safe (GRAS) by the FDA and avoids the use of harmful solvents.

15.6 Analysis, Separation, and Purification

The analysis, separation, and purification of microalgal biomolecules require various specialized methods tailored to proteins, lipids, carbohydrates, and pigments. Each method has distinct advantages and limitations, depending on the biomolecule's characteristics, desired purity level, and operation scale. Supercritical fluid chromatography (SFC) integrates benefits from both gas chromatography (GC) and high-performance liquid chromatography (HPLC), making it ideal for analyzing compounds that are temperature-sensitive or contain functional groups challenging to detect with traditional methods. Column chromatography, ion-exchange chromatography (IEX), and affinity chromatography utilize different principles of adsorption and charge characteristics to separate biomolecules, with IEX focusing on charged molecules and affinity chromatography offering high specificity through ligand interaction. Thin-layer chromatography (TLC) is effective for lipid separation but may result in oxidation issues, while HPLC provides high separation resolution for a variety of microalgal components such as carotenoids and fatty acids. GC is commonly used for isolating volatile compounds and separating fatty acid methyl esters

(FAME) in lipid extracts (Harris, 2007; Aluç et al., 2018; Cerón-Garcia et al., 2018; Soares et al., 2016, largely dependent on output scale and the 2019; Morowvat & Ghasemi, 2016).

For purifying recovered biomolecules, additional techniques like electrophoresis, ultrafiltration (UF), electro membrane filtration (EF), and ultracentrifugation are employed. Electrophoresis separates proteins and macromolecules based on charge, while UF concentrates macromolecules but lacks selectivity and is prone to fouling (Jones et al., 2012; Jerez-Martel et al., 2017; Rose, 1959; Chaiklahan et al., 2011). EF offers a more selective separation based on charge and molecular weight without the need for pressure, and ultracentrifugation separates molecules by density but is time-consuming and requires expensive equipment. These methods are often combined to enhance the purification process, ensuring high-quality and contaminant-free biocomponents.

15.7 Efficiency and Cost Implications of Different Cultivation, Harvesting, and Processing Methods

Microalgae cultivation, harvesting, and processing are crucial for biofuels and dietary supplements. However, economic feasibility remains a challenge due to the high costs associated with harvesting and processing techniques. Harvesting expenses account for 20–30% of production costs (Barkia et al., 2019; Costa et al., 2019b; Tan et al., 2020; Kumar et al., 2020; Richardson et al., 2014; Gross et al., 2015). Various unit activities, from established to advanced, can be implemented for harvesting and dewatering, with the effectiveness of each operation influencing the subsequent units chosen and their effectiveness in the dewatering process. Open pond systems are the most cost-effective method for cultivating microalgae due to their low initial investment and ease of operation. However, they face challenges like pollution and limited environmental regulation, which can negatively impact biomass output. Photobioreactors offer a highly regulated environment for microalgae cultivation, but they have higher capital and operating costs. PBRs can cost up to $100/kg, making them less feasible for large-scale applications. The growing season duration also impacts production costs, with short periods leading to cost increases and protracted seasons reducing costs. The selection of microalgae species also affects costs, with certain species like *P. tricornutum* resulting in higher net present value (Tan et al., 2020; Kumar et al., 2020; Richardson et al., 2014; Gross et al., 2015; McGrath et al., 2024). The net present value (NPV) of microalgae growing systems is highly sensitive to parameters like productivity, operating expenses (OPEX), and capital expenses (CAPEX). For example, a 20% increase in productivity coupled with a 20% reduction in both OPEX and CAPEX could lower biomass production and nutrient removal costs by approximately 44%. Open pond systems generally offer a more cost-effective option for large-scale cultivation, while photobioreactors (PBRs) deliver higher production at increased costs. The efficiency

and cost-effectiveness of microalgae farming are influenced by factors such as the growing season duration, species of microalgae, and optimization of production and expenditures. Harvesting methods such as centrifugation, filtration, and flocculation each come with their own cost implications. Centrifugation costs about $12,500 per hectare, filtration around $9884 per hectare, and flocculation approximately $2000 per hectare. Emerging technologies like the revolving algal biofilm system and electro-flocculation show promise in reducing harvesting costs. The revolving algal biofilm system enhances land efficiency and biomass output but has a higher initial cost, while electro-flocculation is energy-efficient and chemical-free but remains costly (Slegers et al., 2020). Converting microalgae into biofuels or other products also incurs substantial costs, though integrated systems can help reduce expenses, such as producing microalgal biodiesel at $0.73 per kilogram when grown in wastewater. However, the economic viability of processing is largely dependent on output scale and the technology used (Nguyen et al., 2024; Tsolakis et al., 2023; McKinsey and Well Company, n.d.).

16 Distribution Networks

As the microalgae sector expands, establishing robust distribution networks and efficient logistics becomes critical for global product distribution, maintaining quality standards, and meeting consumer demand. Future advancements in the sector aim to enhance research, technology, and market feasibility. Effective distribution involves a complex network of manufacturers, processors, distributors, and retailers to ensure timely and reliable delivery of products (Tsolakis et al., 2023; McKinsey and Well Company, n.d.).

To optimize the supply chain, identifying regional distribution hubs in major markets like North America, Europe, and Asia-Pacific is essential for centralizing receiving, storing, and distributing products. Forming local partnerships with distributors and retailers can improve market penetration and product availability. Utilizing multiple distribution channels, including online platforms, retail stores, and direct-to-consumer models, can broaden product reach. Collaborating with biotech and food industry players can further expand distribution networks and leverage established channels, aiding in regulatory navigation and providing valuable market insights (Nodooshan et al., 2018; Chopra & Meindl, 2016) (Fig. 9).

16.1 Logistics and Supply Chain Management

The logistics and supply chain management for microalgal products encompasses cultivation, harvesting, processing, and distribution. Cultivation methods impact yield and environmental impact, with closed systems offering better control and reduced contamination risks. Efficient harvesting and continuous processing

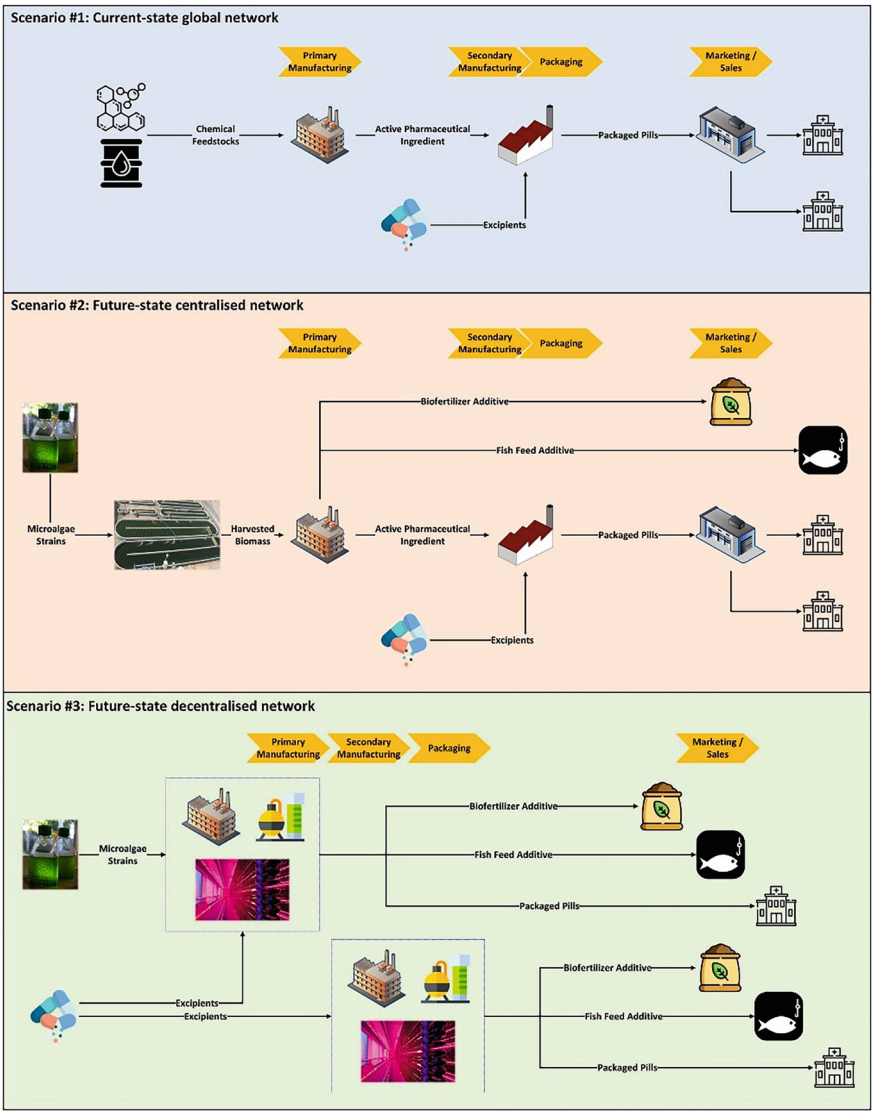


Fig. 9 Network configuration modeling scenarios (Nodooshan et al., 2018)

technologies are crucial for maintaining high-quality biomass and enhancing efficiency. Distribution strategies must handle transportation, temperature control, and inventory management using advanced technologies like IoT and data analytics to improve visibility and responsiveness (Nodooshan et al., 2018; Chopra & Meindl, 2016). For microalgae products such as nutraceuticals and pharmaceuticals, a robust cold chain logistics system is essential to preserve quality and safety. Advanced

logistics software optimizes routing and scheduling, while real-time tracking and demand forecasting improve customer satisfaction and operational efficiency. Lean inventory practices and adherence to regulatory standards ensure effective supply chain management and compliance with food safety, environmental, and trade regulations (Chopra & Meindl, 2016). Microalgal products encounter several challenges in production, processing, and distribution, including contamination risks, complex logistics, and high production costs, especially with advanced systems like photobioreactors. Effective management of the transition from cultivation to harvesting is crucial to maintaining product integrity and minimizing losses. Temperature control during transportation and storage adds further complexity, emphasizing the need for cost-effective solutions to ensure safety and quality. To address these challenges, strategies such as optimizing cultivation systems, advancing genetic engineering, and recycling nutrients can enhance efficiency. Modular and decentralized production systems can lower transportation costs and carbon footprints, while genetic engineering boosts biomass yield. Nutrient recovery through anaerobic digestion and wastewater cultivation improves economic viability. Innovative logistics solutions, including comprehensive planning and efficient harvesting technologies, can reduce processing costs and improve overall sustainability (Womack & Jones, 2003; Christopher, 2016; Richard et al., 2013; Slegers et al., 2015; Market Research Future, n.d.).

16.2 Global Distribution Channels for Microalgal Products

The global distribution of microalgae products involves a complex network of channels and processes designed to effectively reach diverse markets. These channels include both direct and indirect methods, as well as business-to-business (B2B) and business-to-consumer (B2C) approaches (Slegers et al., 2015). Key strategies for optimizing distribution include understanding regional demand, navigating regulatory requirements, and employing various market entry strategies such as direct and indirect exporting, joint ventures, and leveraging emerging channels like e-commerce platforms. Efficient logistics and supply chain management are essential for successful global distribution. This includes selecting appropriate shipping methods, managing cold chain logistics for temperature-sensitive products, and integrating systems for real-time visibility and process optimization. Effective risk management involves diversifying suppliers and maintaining safety stocks, while building a global brand requires consistent messaging, adapting marketing to local cultures, and participating in international trade events to enhance visibility and forge partnerships (Market Research Future, n.d.; Kuo & Chen, 2010; European Commission, n.d.).

16.2.1 Innovations in the Distribution Channels for Microalgal Products

The distribution of microalgal products is evolving to enhance efficiency, accessibility, and sustainability, driven by growing demand in sectors like nutrition, cosmetics, and animal feed (European Commission, n.d.). E-commerce and direct-to-consumer (DTC) models are expanding market reach and improving customer engagement. Subscription models and advanced logistics, including cold chain and blockchain technology, are being adopted to enhance service and transparency. Sustainable packaging solutions and refillable systems are also being developed. Direct distribution allows manufacturers to sell directly to consumers or businesses, offering control over pricing and branding but facing challenges such as high production costs, regulatory compliance, and consumer education (Global Microalgae Market Research Report, 2024–2030, n.d.; Slegers et al., 2020). Indirect distribution, using intermediaries like wholesalers and retailers, helps reach broader markets and reduces operational burdens, with a focus on cost-effectiveness (Slegers et al., 2020; Microalgae Market by Type (Spirulina, Chlorella, Dunaliella Salina, Nannochloropsis), Production Technique (Ponds, Fermenters, PBR), Distribution Channel (B2C, B2B), Application (Nutraceuticals, Food and Beverages), and Geography – Global Forecast to 2030, n.d.). Business-to-business (B2B) distribution caters to industries needing raw materials, while business-to-consumer (B2C) focuses on direct sales to end-users through various retail and online channels (Microalgae Market by Type (Spirulina, Chlorella, Dunaliella Salina, Nannochloropsis), Production Technique (Ponds, Fermenters, PBR), Distribution Channel (B2C, B2B), Application (Nutraceuticals, Food and Beverages), and Geography – Global Forecast to 2030, n.d.). Both distribution models play crucial roles, influenced by factors such as product type, target market, and strategic goals. To enter the global market, businesses need to conduct thorough market research, tailor their strategies to local conditions, and implement effective export strategies. Consistent branding and marketing can enhance product visibility and differentiate the company from competitors. Microalgae products, with unique attributes like sustainability and health benefits, can attract global consumers. Localization can improve market penetration by modifying product formulations and packaging.

16.2.2 Conclusion

Microalgae, including cyanobacteria, are photosynthetic organisms that convert sunlight into chemical energy and produce oxygen. They are found in areas where sunlight can penetrate the ocean and are a vital component of aquatic food webs. Microalgae have gained renewed interest due to concerns about sustainability, climate change, and resource depletion. They are grown for biofuels, medicines, and nutraceuticals, producing valuable compounds like omega-3 fatty acids and antioxidants. They are also used in various industries, including food,

pharmaceuticals, and agriculture, to monitor water contamination and generate renewable energy. Microalgae, such as Chlorella and Spirulina, are gaining attention for their rich nutritional content and potential for human consumption. Spirulina is a cyanobacterium with spiral-shaped filaments that converts sunlight into energy and oxygen, while Chlorella is a unicellular green alga found in freshwater environments. Microalgae products are increasingly used in various industries due to their nutritional profiles and functional properties.

Microalgae, rich in protein, vitamins, and minerals, are gaining popularity in various industries. Nutritional supplements, such as Spirulina and Chlorella, are used in wellness for immunity and energy. Functional foods enriched with microalgae offer health benefits, including antioxidants and cardiovascular benefits. Microalgae are also used in the feed industry, providing essential amino acids and vitamins. In the pharmaceutical and cosmetic industries, microalgae produce therapeutic compounds with anti-inflammatory, antioxidant, and anticancer properties. In the cosmetic industry, microalgae are used for skin benefits and are a promising source of biofuels like bioethanol and biodiesel. Research is focused on improving cultivation techniques and economic viability of microalgae biofuels. Microalgae biofuels are being developed using genetic engineering, photobioreactor design, and bioprocessing technologies, making them economically viable. The microalgae-based products market is expected to grow in 2024 due to the growing demand for sustainable alternatives. The market is worth around US $3.2 billion and is expected to grow at a CAGR of 9–11% over the next 5 years. Key players include Cyanotech Corporation, DIC Corporation, Algenol Biofuels Inc., and AlgaEnergy. The market is driven by consumer acceptance, with increased awareness of the health and environmental benefits of microalgae-based products. The industry is also being used in food, cosmetics, agriculture, wastewater treatment, and carbon capture.

Microalgae-based products are being developed through research and development initiatives, focusing on genetic engineering and synthetic biology techniques. Collaboration between academia, industry, and government is fostering the development of scalable cultivation and harvesting technologies. Investment in R&D is crucial for optimizing growth conditions and reducing costs. Enhanced cultivation techniques, such as closed photobioreactors and hybrid systems, are improving efficiency and yield while minimizing environmental impact. The integration of microalgae production with circular economy models is also being explored. The efficiency and cost implications of harvesting and processing techniques directly impact the overall cost and viability of microalgae production. Distribution networks are crucial for the distribution of microalgae products, which are distributed through various global channels. The industry faces technical and operational challenges, such as high costs and complexity of scaling up production. Optimal cultivation systems and efficient harvesting and processing methods are essential for maintaining high-yield outputs. Government policies and regulations play a significant role in shaping the microalgae market, with subsidies for sustainable practices and incentives for renewable energy sources boosting growth.

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Bio-green Evolution: The Journey of Microalgae Products from Lab to Market—Considerations and Recommendations—Part II



Ambika H. D. and Hema Vijayan P. U

Abstract Microalgae, a diverse group of photosynthetic microorganisms, are gaining attention as a sustainable and versatile resource with applications across various sectors. Innovations in cultivation systems, including open ponds and advanced photobioreactors, have addressed challenges like contamination, nutrient management, and light optimization, enabling scalable production. Moreover, microalgae play a vital role in carbon dioxide sequestration and bioremediation, contributing to environmental conservation. The expanding global market for microalgal products reflects growing consumer interest in sustainable, natural, and health-focused alternatives. Advances in genetic engineering and bioprocessing technologies further enhance their industrial feasibility. Despite hurdles such as high production costs and scalability issues, the integration of microalgae with circular economy models and emerging technologies positions them as a transformative solution to pressing challenges like climate change, resource scarcity, and food security.

Keywords Microalgae · Photobioreactor · Open ponds · Genetic engineering · Renewable energy · Bioremediation · Carbon dioxide sequestration · Bioprocessing · Sustainability

1 Challenges and Opportunities

1.1 Technical and Operational Challenges

1.1.1 Scaling Up Production

Transitioning from laboratory to commercial microalgal production requires innovative solutions like contamination management, resource optimization, efficient light capture, genetic engineering, and environmental and economic analysis. This could lead to sustainable, commercially feasible technology applications like

A. H. D. (✉) · H. Vijayan P. U
Research and Development, ProAlgaeTech, Stuttgart, Baden-Württemberg, Germany

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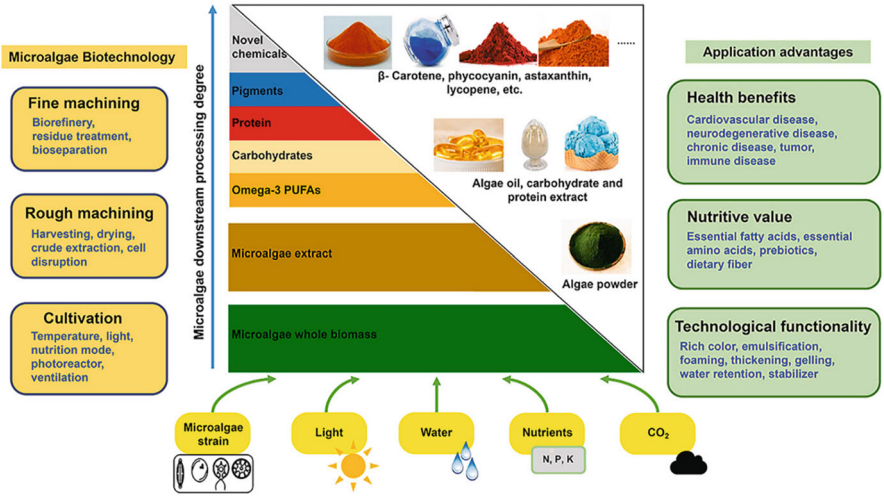


Fig. 1 The most common challenges associated with large-scale cultivation systems. (Rai et al., 2016)

bioremediation and renewable energy production (Chisti, 2007; Pulz & Gross, 2004; Rai et al., 2016) (Fig. 1)

Management of Contamination Large-scale microalgae cultivation is heavily impacted by contamination from other microorganisms, which can compromise production efficiency and product quality. Maintaining culture purity is essential to prevent the introduction of unwanted species, which can lead to culture collapse, reduced yields, and inconsistent product quality. The challenge of sustaining axenic cultures increases with scale, particularly in outdoor systems, where contamination risks are higher. Contamination diverts resources from the target microalgae, escalating costs and affecting yields. Detection of contaminants is complex and often requires advanced methods like flow cytometry for accuracy. To manage contamination, strategies include using closed photobioreactors, rigorous cleaning protocols, selective strain management, and optimizing nutrient supply (Richmond & Cheng-Wu, 2001).

Optimizing the Supply of Nutrients To optimize microalgae cultivation, various strategies are employed, including tailored nutrient formulations, continuous nutrient supply systems, and the use of wastewater as a nutrient source (Carney & Lane, 2014). Balancing macronutrients and micronutrients is crucial to avoid growth limitations, while continuous nutrient addition enhances growth rates and yields (Chisti, 2008). Wastewater offers a sustainable and cost-effective nutrient source, contributing to environmental sustainability (Rodolfi et al., 2009). Advanced monitoring and control systems enable real-time tracking and adjustment of nutrient levels, improving efficiency. Genome-scale metabolic modeling helps predict nutrient needs under different conditions, and nutrient recycling from biomass residues

reduces costs and supports sustainability. Adjusting growth conditions, selecting suitable strains, and employing genetic engineering can further optimize nutrient uptake and metabolic efficiency. Implementing a biorefinery approach creates a closed-loop system for resource utilization, enhancing economic viability and sustainability in large-scale microalgae cultivation (Koller et al., 2014; Christenson & Sims, 2011).

Efficiency of Light Utilization Light is critical for photosynthesis in microalgae, yet uneven light penetration in large-scale systems like open ponds often hampers growth. To enhance productivity, it's important to design systems that maximize light utilization by optimizing photobioreactor configurations and using reflective materials. Factors affecting light efficiency include self-shading, which reduces light penetration to deeper layers, natural light variability due to seasonal and weather changes, and mixing dynamics that cause inconsistent light exposure (Markou & Georgakakis, 2011; Janssen, 2003). Strategies to improve light utilization include implementing light guide systems, using artificial lighting such as LEDs, and designing photobioreactors to minimize self-shading with shallow depths and transparent materials. Additionally, genetic engineering to optimize photosynthetic machinery and dynamic light management techniques, such as automated systems and flashing light, can further boost biomass production and efficiency (Zhu et al., 2008; Hawrot-Paw & Ratomski, 2024).

Temperature Control Microalgae are highly sensitive to temperature variations, which can affect their growth rates and metabolic processes. Managing ideal temperature conditions, particularly in large-scale outdoor systems, poses significant challenges and requires effective temperature control solutions (Simionato et al., 2013). Temperature influences how microalgae absorb and utilize light, with excessive light and high temperatures potentially causing photooxidative damage and reducing productivity. Therefore, maintaining proper temperature control is crucial to prevent overheating and ensure optimal growth (Tredici, 2010).

Carbon Dioxide Supply Optimal carbon availability is crucial for photosynthesis in microalgae, which requires strategies like utilizing industrial flue gases or optimizing aeration systems to maintain sufficient CO_2 levels (Wang et al., 2012). Challenges include the variability in CO_2 concentrations from sources like atmospheric air or diluted flue gases, typically lower than required for optimal microalgal growth (Brennan & Owende, 2010; Chisti, 2007). Capturing and concentrating CO_2 involves costly and complex technologies, while transporting CO_2 presents logistical issues, especially for large-scale operations. Integrating CO_2 supply systems with cultivation setups demands precise design to ensure effective gas delivery and mixing, and continuous CO_2 monitoring adds to the technical demands (Kumar, 2010). The overall economic viability of CO_2 supply systems is a major concern due to the high costs associated with CO_2 capture, transportation, and integration.

Harvesting and Dewatering Harvesting and dewatering microalgae are energy-intensive processes that pose significant technological and economic challenges, impacting the sustainability of large-scale production. Microalgae cultures are

typically dilute, with biomass concentrations between 0.02% and 0.06%, necessitating effective dewatering methods to reduce energy use and costs (Wang et al., 2008). Current technologies often fall short in efficiency for large-scale applications, highlighting the need for innovative, cost-effective solutions that minimize environmental impact. Life cycle assessments (LCA) are crucial for evaluating the sustainability of harvesting methods and ensuring they have a minimal carbon footprint (Gouveia & Oliveira, 2009). The variability among microalgal species further complicates the development of universal harvesting techniques, underscoring the need for ongoing research into scalable and efficient methods to enhance the economic viability of microalgae production (Molina Grima et al., 2003).

Strain Selection and Genetic Engineering Selecting microalgal strains with high resilience and productivity is essential for optimizing cultivation, but enhancing these traits through genetic engineering presents challenges. The vast genetic diversity among microalgae offers opportunities for strain improvement but complicates the selection process due to limited genomic resources and insufficient understanding of desirable traits (Tan et al., 2020). Genetic engineering can accelerate growth and increase product output, yet it faces hurdles such as inefficient transformation techniques and regulatory concerns associated with genetically modified organisms (GMOs) (Radakovits et al., 2010). Additionally, adapting microalgae to various environmental conditions and achieving stable, predictable outcomes from genetic modifications are complex tasks. Ensuring compliance with safety standards and balancing the costs of genetic engineering with the potential benefits are crucial for commercial success (Fu et al., 2012).

Scale-Up Dynamics Scaling up microalgae cultivation from small to large systems involves complex, non-linear dynamics that significantly impact fluid dynamics, nutrient distribution, and biomass density (Chisti, 2007; Acién Fernández et al., 2001). As production systems increase in size, changes in reaction kinetics, chemical equilibrium, and thermodynamics require careful re-engineering of equipment and processes (Acién Fernández et al., 2001). Challenges such as inconsistent light distribution, nutrient supply, and mixing must be addressed to maintain biomass productivity. The transition to larger systems introduces unpredictable issues due to altered fluid flow, mass transfer, and light penetration (Posten, 2009). Accurate scale-up models are needed, as existing dynamic models often fall short in predicting large-scale challenges. Further research is essential to develop comprehensive models that can effectively guide the scaling process and anticipate potential difficulties (Posten, 2009).

Long-Term Culture Stability Maintaining stable and productive microalgae cultures over extended periods presents significant challenges, especially in large-scale operations. Key issues include genetic instability, which can lead to reduced growth rates and lower biomass yields due to mutations and structural chromosome variations. Contamination risks also increase with prolonged cultivation, complicating efforts to maintain axenic conditions and consistent product quality (Zittelli et al., 2013). Environmental factors, such as nutrient composition and light regime, further

influence the metabolic profile and stability of microalgae cultures. Effective strategies to address these challenges include regular monitoring, maintaining genetic diversity, optimizing cultivation conditions, and robust contamination control measures to sustain long-term culture stability. Microalgae offer promising alternatives for food, animal feed, biomaterials, and biofuels and have potential for wastewater treatment and CO_2 capture (Day & Borowitzka, 2016). Despite their advantages, including high efficiency in solar energy conversion and rapid growth on non-arable land, large-scale microalgae production faces significant obstacles. Production costs remain high, ranging from €290 to €570 per kg of dry weight, due to expenses related to infrastructure, operation, and maintenance (Singh & Sharma, 2012). Additionally, scaling up requires expertise in various fields and overcoming social barriers to acceptance. The success of large-scale systems depends on high-quality inoculum, effective photobioreactor design, and efficient downstream processing (Oostlander et al., 2020; Medeiros & Moreira, 2022; Maroušek et al., 2023; Nezlek & Forestell, 2020). The industry must continue to innovate and address these challenges to enhance the economic viability and scalability of microalgae cultivation.

1.1.2 Quality Control and Standardization

Quality control is crucial for validating microalgae-based products, including nutraceuticals and food supplements, to ensure their safety, effectiveness, and market accessibility (Harris et al., 2018). Proper formulation and the use of appropriate analytical tools are essential to verify the authenticity and quality of raw materials. Addressing challenges such as strain variability, environmental impacts, and setting standardized protocols is vital for ensuring consistency and reliability in microalgal products (Borowitzka, 2013). Standardization is key to maintaining product quality, meeting safety and efficacy standards, and fostering consumer trust. It also aids regulatory compliance and market acceptance by ensuring consistent quality and adherence to regional requirements (Richmond & Hu, 2013; Grobbelaar, 2010; Elisabeth et al., 2021; Moreno-Garrido, 2008; Pérez-López et al., 2014).

1.1.3 Key Components

1. **Strain selection and maintenance:** Selecting robust microalgal strains for large-scale cultivation is crucial for maintaining genetic stability and product quality. These strains should have desired traits like high growth rates, lipid content, stress tolerance, and biochemical composition. Regular monitoring, subculturing, and regulatory compliance ensure consistent biochemical composition, reduce contamination risk, and enhance reliability.
2. **Prevention of contaminants:** Microalgal biomass is susceptible to contamination, making it crucial to implement efficient quality control procedures. To minimize risks, use closed photobioreactor systems, filtration systems, and

sterilization techniques. Select naturally resistant algal strains and manage cultivation conditions. Regularly monitor cultures for signs of contamination and implement early warning systems. A multi-layered approach combining cultivation design, strain selection, environmental control, monitoring, and hygiene is most effective. Identify contaminants using microscopy techniques, molecular techniques like PCR and qPCR, and spectral imaging, flow cytometry, automated monitoring systems, genetic barcoding, and chemical analysis techniques. Combining multiple detection techniques enhances reliability and provides a comprehensive understanding of culture health (De Farias Neves et al., 2020; Lam et al., 2018; Wang et al., 2013; Zhu et al., 2020).

1.1.3.1 Analytical Characterization

Analytical characterization is the process of describing the properties and characteristics of a substance or material using advanced techniques. It is crucial for evaluating the biochemical makeup of microalgal products, including proteins, lipids, carbohydrates, vitamins, and pigments. Standardization of analytical methods is essential for consistency in quality control. Techniques include HPLC, GC-MS, Spectrophotometry, NMR spectroscopy, Elemental Analysis, and ELISA. These techniques ensure the safety and quality of microalgal products for various applications, including food, feed, and biofuels. Regular validation is essential for maintaining accuracy and reliability in quality control (Pleissner et al., 2020; Davani et al., 2022; Biotechnologies for Microalgal Biomass Production, n.d.).

1.1.3.2 Process Monitoring

Regular monitoring of growing parameters is crucial for ensuring product quality. Automated systems can optimize growth conditions in real time. Techniques like nephelometry, spectroscopy, flow cytometry, microscopy, and analytical methods can be used to monitor microalgae cultivation. Standardized protocols enhance the reliability and reproducibility of process monitoring. By combining real-time monitoring, analytical characterization, and standardized protocols, microalgae producers can maintain control over biomass quality, ensuring successful commercialization (Laurens & Wolfrum, 2013; Blanken et al., 2016).

1.1.3.3 Quality Management Systems

Microalgal producers can enhance the safety, efficacy, and consistency of their products through quality control measures, such as regular audits, ongoing training, and integrating quality management systems (QMS) that incorporate principles like Hazard Analysis and Critical Control Points (HACCP) (Davani et al., 2022). These practices help maintain commercial success in the industry by identifying potential

hazards, ensuring consistency in operations, providing clear guidelines for staff, and maintaining quality standards. Comprehensive documentation of production processes, including strain information, growth conditions, and quality control results, enhances traceability and regulatory compliance. Regular quality assessments using analytical techniques help monitor the biochemical composition of microalgal biomass, establishing reference standards for key metabolites. Environmental monitoring, such as light, temperature, pH, and nutrient levels, is essential for optimal growth conditions. Implementing a QMS benefits consumers by ensuring product safety, consistency, reliability, regulatory compliance, and continuous improvement. Acquiring certifications like GRAS and USDA Organic is crucial for adhering to regulations and market acceptance (Sudas et al., 2022; Elisabeth et al., 2021). Quality control and standardization of microalgal products are essential for market acceptance and regulatory compliance. These protocols involve frequent screening for impurities, nutritional composition, and biologically active substances, ensuring product dependability, customer trust, regulatory approvals, and global market competitiveness.

1.1.4 Regulatory and Policy Considerations

1.1.4.1 Compliance with Global Standards and Impact of Policies on Market Entry (Microalgae Visual Quality Check, n.d.; Cruz & Vasconcelos, 2023; De Oliveira & Bragotto, 2022; Younes et al., 2019; Kose & Oncel, 2015; Deprá et al., 2020; Regulatory Framework for Microalgae-Based Products, 2023; The EU Novel Food Regulation and Microalgae, 2021; FDA's Regulatory Requirements for Microalgae; The Role of Intellectual Property in Microalgal Biotechnology, 2016; Environmental Regulations and Sustainability Certifications, 2023)

The field of algae biotechnology holds immense promise for diverse applications, including sustainable biofuel production and pharmaceutical development. However, its growth and commercialization are subject to a complex regulatory and ethical framework that varies by region and application. This regulatory landscape ensures the safety, efficacy, and environmental sustainability of microalgal products while also fostering innovation and market expansion. Key regulatory areas include health and safety standards, environmental regulations, intellectual property rights, and market access. Microalgal products intended for human consumption, such as food supplements and nutraceuticals, must comply with stringent safety standards enforced by agencies like the FDA in the United States and EFSA in the European Union. These products must adhere to Good Manufacturing Practices (GMP) and undergo thorough safety evaluations to ensure their efficacy and safety. Additionally, animal feed additives derived from microalgae require proof of safety and efficacy through toxicity studies and nutritional assessments. Accurate labeling is crucial for informing consumers and meeting regulatory standards. Regulations

concerning genetically modified microalgae are also significant, with directives in the EU and regulations by the EPA and USDA in the US overseeing their use. Environmental regulations address issues like discharge permits, impact assessments, and sustainability certifications, aiming to minimize ecological impacts and promote responsible practices. The use of microalgae in agriculture as biostimulants and biofertilizers is also regulated to prevent negative effects on soil and crop health.

In addition to regulatory concerns, intellectual property and innovation policies play a crucial role in algae biotechnology. Patents protect advancements in strains, processes, and technologies, while government grants and public-private partnerships support research and commercialization efforts. Market access and trade regulations, including export/import controls and quality standards, are vital for global market entry. International organizations like the WHO and OECD provide global guidelines to harmonize regulatory practices and facilitate international trade. Algal-derived goods undergo safety evaluations to assess potential health hazards, including toxicity, allergenicity, and nutritional composition. Environmental impact assessments are necessary for biotechnology initiatives involving genetically modified algae. Risk mitigation measures, such as controlling genetically modified algae, monitoring negative impacts, and developing contingency plans, are based on safety and environmental studies.

1.1.4.2 Impact of Policies on Market Entry of Microalgal Products (Deprá et al., 2020; Regulatory Framework for Microalgae-Based Products, 2023; The EU Novel Food Regulation and Microalgae, 2021; FDA's Regulatory Requirements for Microalgae; The Role of Intellectual Property in Microalgal Biotechnology, 2016; Environmental Regulations and Sustainability Certifications, 2023)

The market entry of microalgal products is influenced by a complex interplay of regulatory, economic, and technical factors. Regulatory frameworks vary significantly across regions, impacting the manufacturing, commercialization, and consumer acceptance of microalgae-based products. In the European Union, for example, the Novel Food Regulation necessitates extensive safety assessments for new food products, including those derived from microalgae, which can lead to delays and increased costs. In the U.S., obtaining Generally Recognized as Safe (GRAS) status requires rigorous scientific documentation, adding to the regulatory burden. International standards and local regulations on labeling and import processes further complicate market entry, affecting the accessibility of microalgal products across different markets.

Economic and technical barriers also present challenges to the growth of the microalgae sector. High production costs, coupled with intense competition from low-cost producers and established agricultural products, hinder the economic viability of microalgae-based products. Insufficient funding and support can impede the scaling up of production and technological advancements. Technological gaps in cultivation, harvesting, and extraction processes further affect production efficiency

and product quality. Addressing these barriers through sustainable practices, improved technologies, and supportive government policies can enhance market competitiveness and facilitate entry into new markets. Promoting consumer awareness of the health benefits and sustainability of microalgae can drive demand and increase market acceptance, offering opportunities for growth in the sector. The successful commercialization of microalgal products requires adhering to global standards, understanding market access, and addressing regulatory complications. By implementing favorable policies and strict adherence, the sector can enhance competitiveness and sustainable development.

1.2 Strategic Opportunities

1.2.1 Collaborative Ventures and Partnerships (Impact of Trade Regulations on Microalgal Products, 2014; Qualitas Health's Cooperation with Grupo Indukern and Gullspång ReVC; Algalíf and ImmunRise Partnership, 2023; Green Aqua and Algikey Collaboration, 2023; AlgaEnergy's Partnership with Laboratoire M2; DSM's Partnership with Evonik; National Renewable Energy Laboratory (NREL) Collaborations, n.d.; European Algae Biomass Association (EABA) Collaborations, 2021; U.S. Department of Energy's Bioenergy Technologies Office (BETO); EU Horizon Europe Initiative, 2022; InvestEU Blue Economy Fund/BlueInvest Programs, 2023)

Strategic collaborations in the microalgae sector significantly enhance the capabilities and market potential of the enterprises involved. These partnerships enable firms to leverage each other's expertise, technology, and resources, which accelerates product development and fosters innovation. For example, Qualitas Health's collaborations with Grupo Indukern and Gullspång Re: food VC grant access to advanced facilities and market insights, expediting the creation of new algae-based products. Similarly, the alliance between Algalíf and ImmunRise aims to develop novel biocontrol products from microalgae, utilizing shared resources to overcome regulatory challenges and innovate faster. These partnerships also facilitate entry into established distribution networks and navigate complex regulatory environments, essential for gaining market acceptance and accelerating approvals.

In addition to enhancing market capabilities, strategic partnerships address significant economic and technical barriers. High production costs and intense competition can limit the growth of microalgae-based products, particularly in regions with established agricultural practices. Partnerships, such as those between Green Aqua and Algikey, focus on developing a range of algae-based products for the aquaculture industry, enhancing manufacturing capabilities and product variety. Collaborations can also improve the scalability of production by addressing technological gaps in growth, harvesting, and processing. Joint funding and shared responsibilities

further alleviate financial pressures, facilitating research and development in the microalgae sector. Moreover, strategic alliances offer environmental benefits and contribute to sustainability goals. Collaborations can advance the development of eco-friendly biocontrol products, enhance wastewater treatment processes, and promote biodiversity through microalgal cultivation. For instance, partnerships with academic institutions and organizations like the National Renewable Energy Laboratory (NREL) can drive research on genetic improvements and sustainable practices. These collaborations not only support environmental health by reducing pollution and resource consumption but also contribute to climate change mitigation through sustainable biofuel production. By integrating microalgae into various applications, these strategic partnerships bolster both ecological benefits and market competitiveness. Microalgal products offer strategic advantages through collaboration, market expansion, and various finance sources. These opportunities can enhance technological prowess, market presence, and sustainability in the microalgal industry.

1.2.2 Predictions and Trends in the Microalgal Industry

The microalgae sector is undergoing substantial growth, driven by its diverse applications and sustainability benefits. Microalgae's ability to produce sustainable biofuels is a major trend, as they offer an eco-friendly alternative to traditional crops used in biofuel production. Unlike conventional crops, microalgae can be cultivated in various aquatic environments with minimal land use and have high lipid content suitable for biodiesel production. As global efforts to reduce carbon emissions intensify, the demand for microalgae-based biofuels is expected to rise significantly (European Algae Stakeholder Forum (EU4Algae Forum), n.d.).

In addition to biofuels, microalgae are becoming increasingly important in nutrition and functional foods. Rich in essential nutrients like proteins, omega-3 fatty acids, and vitamins, microalgae such as spirulina and chlorella are gaining popularity for their health benefits. They are also used to enhance the nutritional value and shelf life of food products. The growing health and wellness trend is fueling interest in microalgae-derived ingredients, which are valued for their ability to fortify foods and supplements (European Algae Stakeholder Forum (EU4Algae Forum), n.d.). Microalgae also play a critical role in aquaculture as a sustainable feed source, providing essential nutrients for aquatic species and utilizing waste products for cultivation. Their potential extends to biopharmaceuticals and nutraceuticals, with research focusing on their therapeutic properties and antioxidant benefits. Moreover, microalgae's capabilities in carbon capture and bioremediation, alongside the development of biodegradable bioplastics, highlight their role in addressing environmental challenges. Urban farming innovations further support microalgae cultivation, integrating it into city infrastructures and enhancing sustainability. As the sector evolves, microalgae are positioned to address key global issues such as climate change, food security, and pollution (European Algae Stakeholder Forum (EU4Algae Forum), n.d.).

1.2.3 Market Projections and Potential Game Changers in the Microalgal Industry

The global microalgae market, valued at USD 11.86 billion in 2023, is expected to reach around USD 25.72 billion by 2033, growing at an 8.05% CAGR from 2024 to 2033. The market is driven by the increasing use of marine microalgae components and proteins in aquaculture, pharmaceutical, and nutraceutical products, driven by government initiatives to standardize cultivation techniques. The market is expected to experience steady positive growth from 2024 to 2031, driven by factors such as increasing consumer demand, technology advancements, and shifting customer preferences. Health and well-being awareness has led to a growing demand for microalgae products, which are considered healthier alternatives. Technological advancements in the industry have resulted in more efficient and environmentally friendly production techniques, enhancing market expansion (Cohen, n.d.). Key industry players are investing in the development of new products and enhancing distribution networks to increase future demand. The microalgae market is expected to experience substantial growth in the coming years due to these factors.

1.2.4 Microalgae Market Drivers

Microalgae are microorganisms that offer numerous health benefits, including protection against cardiovascular diseases, cancer prevention, antioxidant properties, obesity reduction, and blood pressure reduction. They are also a source of bioenergy and food, promoting environmental sustainability and promoting human health. The growing demand for sustainable and natural products, driven by environmental concerns and a trend towards sustainable practices, has led to the use of microalgae in various sectors such as food, feed, cosmetics, and biofuels (Precedence research, n.d.; Global Microalgae Market Research Report, 2024–2030, n.d.; prophecymarketinsights, n.d.; Microalgae Market by Type (Spirulina, Chlorella, Dunaliella Salina, Nannochloropsis), Production Technique (Ponds, Fermenters, PBR), Distribution Channel (B2C, B2B), Application (Nutraceuticals, Food and Beverages), and Geography - Global Forecast to 2030, n.d.). Advancements in biotechnology and cultivation techniques have led to increased productivity, reduced manufacturing costs, and improved product quality. Novel agricultural techniques, genetic engineering, and improved harvesting methods have increased productivity and cost-effectiveness. The industry is also focusing on improving production and exploring new uses. Large-scale cultivation of microalgae is driven by its abundant protein content and bio-active components, including antioxidants, anticarcinogens, and antihypertensives. Companies are introducing new products containing microalgae to increase public awareness and product sales.

The growth of microalgae in a diluted environment relies on the progress of the microalgae itself and its constituents. Challenges include selecting suitable strains, improving strains, limiting sunlight availability, and water removal for concentrates.

Technological advancements and scientific ingenuity are needed to cultivate microalgae and transform it into desired products. The use of enhanced cultural techniques and innovative technologies in microalgae production is fueling the growth of the microalgae market. The microalgal industry is expected to experience significant growth due to technological advancements, growing eco-conscious demand, and favorable government regulations, with the potential for genetic manipulation and other applications.

1.2.5 Strategic Recommendations for the Microalgae Industry

The growth of the microalgae industry hinges on robust investment in research and development (R&D). Enhancements in cultivation techniques, such as advanced photobioreactors (PBRs), are critical for boosting productivity and scalability. Emphasizing automation and real-time monitoring can significantly improve efficiency and reduce labor costs. Additionally, optimizing open pond systems to better control environmental variables and reduce pollution is crucial. Genetic engineering and selective breeding can further enhance microalgal strains, making them more productive, resilient, and nutrient-rich, which is essential for specialized applications like biofuels and nutraceuticals (Microalgae Market by Type (Spirulina, Chlorella, Dunaliella Salina, Nannochloropsis), Production Technique (Ponds, Fermenters, PBR), Distribution Channel (B2C, B2B), Application (Nutraceuticals, Food and Beverages), and Geography—Global Forecast to 2030, n.d.). Diversifying the range of products is essential to meet the varied demands of different markets. The microalgae sector can supply biofuels, bioplastics, nutraceuticals, pharmaceuticals, and cosmetics, with emerging applications such as bioremediation and biofertilizers offering new opportunities. Tailoring products to specific market needs—such as high-protein supplements for the food industry or customized pigments for cosmetics—can improve market appeal and consumer acceptance (Di Caprio et al., 2020).

Sustainability remains a key focus for the industry's development. Integrating microalgae cultivation with carbon capture and utilization (CCU) systems can reduce industrial CO_2 emissions and produce valuable biomass. Collaborating with industries like power plants and cement manufacturers to use CO_2 emissions for microalgae growth can drive environmental and economic benefits. Additionally, using microalgae for wastewater treatment addresses pollution and water scarcity, while nutrient recycling can lower reliance on synthetic fertilizers and reduce environmental impact (Chew et al., 2018). Engaging in partnerships with academic institutions and industry stakeholders, adhering to regulatory standards, and implementing effective marketing strategies are crucial for advancing the sector and ensuring its long-term sustainability and market success (Microalgae Market by Type (Spirulina, Chlorella, Dunaliella Salina, Nannochloropsis), Production Technique (Ponds, Fermenters, PBR), Distribution Channel (B2C, B2B), Application (Nutraceuticals, Food and Beverages), and Geography—Global Forecast to 2030, n.d.; Efroymson et al., 2021).

1.2.6 Best Practices for Microalgal Industry Stakeholders

The microalgae industry is expanding rapidly due to its potential to address global challenges such as climate change, resource scarcity, and food security. To capitalize on this potential, stakeholders including researchers, manufacturers, legislators, and investors must adopt best practices that optimize production efficiency, ensure product quality, and promote environmental sustainability. Key strategies involve advancing Photobioreactor (PBR) technologies and open pond systems, enhancing genetic engineering techniques, and developing a diverse range of products. Effective use of automation, real-time monitoring, and advanced PBR designs can improve productivity, while improved pond systems can mitigate contamination risks and optimize growth conditions (Di Caprio et al., 2020; Ubando et al., 2015).

Genetic engineering methods, such as CRISPR, are essential for developing microalgal strains with high productivity, stress resistance, and improved nutritional profiles. These advancements have the potential to significantly enhance the efficiency of microalgae-based biofuels and other products. Conventional breeding methods also play a role in refining strains adapted to specific environmental conditions, further boosting their effectiveness (Mata et al., 2010; Wijffels & Barbosa, 2010). Additionally, a diverse product portfolio including biofuels, bioplastics, nutraceuticals, and pharmaceuticals can meet varying market demands and foster innovation. Microalgae's applications in bioremediation and biofertilizers also present new opportunities for addressing environmental issues and generating revenue (Scaife et al., 2015; Batista et al., 2015).

Sustainability is integral to the industry's growth, with microalgae offering solutions for carbon capture and bioremediation. Integrating microalgae cultivation with carbon capture and utilization (CCU) systems can reduce CO_2 emissions and produce valuable biomass. Collaborations with industries like power plants and cement manufacturers can enhance these efforts. Additionally, utilizing microalgae for wastewater treatment and nutrient recycling can address pollution and reduce the reliance on synthetic fertilizers (Chew et al., 2018). Employing water and energy-saving techniques and ensuring sustainable raw material sources are crucial for minimizing environmental impacts (Harun et al., 2010). Collaboration with academic institutions, research groups, and industry partners is vital for advancing R&D and expanding market reach. Strategic alliances can foster innovation and create interconnected supply chains, while engagement with government and non-governmental organizations can secure funding and support policy changes (Barros et al., 2015). Compliance with regulatory standards, obtaining certifications, and implementing traceability systems are essential for product credibility and marketability. Targeted marketing strategies and robust distribution networks will enhance consumer awareness and access to microalgae products, supporting long-term industry growth (Pulz & Gross, 2004; Milledge & Heaven, 2014; Chisti, 2007).

1.2.7 Road Map for Future Developments

The microalgae industry is poised for significant growth due to advancements in technology, increased demand for eco-friendly solutions, and its potential to address critical global challenges such as food security and climate change. Positioned at the crossroads of biotechnology, renewable energy, and environmental sustainability, microalgae offer solutions for various applications, including biofuels and nutraceuticals. To harness these opportunities effectively, it is essential to develop a strategic plan focused on innovation, sustainability, and market expansion (Caporgno & Mathys, 2018). The proposed strategy for advancing the microalgae industry includes a detailed roadmap outlining key milestones. This plan emphasizes accelerating research and development, assessing technology readiness, increasing funding, and establishing a regulatory framework. It also aims to enhance market diversification and foster stakeholder collaboration. By addressing technical deficiencies, evaluating current technologies, and attracting investments, the roadmap seeks to expand market opportunities across various sectors such as food, feed, cosmetics, and biofuels (https://thefishsite.com/articles/startup-publishes-digital-roadmap-for-microalgae-carbon-sequestration-ocean-visions, n.d.; Barroca et al., 2023).

Challenges facing the microalgae sector include regulatory uncertainties, low consumer acceptance, high production costs, and technological limitations. Addressing these issues requires a clearer regulatory framework, educational initiatives to boost consumer awareness, and advancements in production technologies. Implementing effective contamination management, optimizing nutrient supply, and integrating carbon capture technologies are crucial for improving production efficiency and environmental sustainability. By focusing on these strategic goals and embracing innovations such as advanced cultivation systems, genetic engineering, and waste stream utilization, the microalgae industry can enhance its viability and contribute significantly to global sustainability goals (Rumin et al., 2021; Ruiz et al., 2016). The microalgal industry roadmap aims to overcome challenges and expand opportunities by researching and implementing innovative solutions, prioritizing sustainability, and minimizing environmental impact. It prioritizes market development, customer interaction, and health benefits. Regulations are essential for commercialization, and efficient harvesting and processing techniques are developed. Long-term objectives include expanding manufacturing capacity, broadening applications, and improving value chains.

1.2.8 Reflections on the Future of Microalgae Products

Microalgae, photosynthesis-capable organisms, are being explored for their potential to address global issues like food security, renewable energy, and environmental conservation. They can be used in biofuels, dietary supplements, pharmaceuticals, and bio-derived commodities, contributing to sustainable development and environmental stewardship due to their minimal land use and carbon dioxide collection capabilities.

1.2.9 The Potential of Microalgal Products

Nutraceuticals and Functional Foods Microalgae, rich in essential components like proteins, vitamins, minerals, and omega-3 fatty acids, are valuable for nutraceuticals and functional foods. Species like Spirulina and Chlorella are popular for dietary supplements due to their health benefits, including immune system boost and inflammation reduction. The future of microalgal products is promising due to growing consumer demand for natural, health-promoting ingredients (Khan et al., 2018a; Gross, 2004).

Biofuels and Renewable Energy Microalgae can revolutionize renewable energy by producing biofuels, offering a sustainable and environmentally friendly alternative to fossil fuels. However, large-scale production remains challenging due to manufacturing costs and energy requirements. Advancements in genetic engineering, strain selection, and bioreactor design are expected to improve efficiency and cost-effectiveness (Gouveia et al., 2008; Chisti, 2007).

Environmental Applications Microalgae have significant environmental applications, particularly in bioremediation and carbon capture. They can absorb pollutants from wastewater and capture carbon dioxide from industrial emissions through photosynthesis. Integrating microalgae into CCU systems could transform CO_2 into valuable commodities like biofuels and bioplastics, potentially revolutionizing industrial processes (Li et al., 2008; Mata et al., 2010).

1.2.10 Challenges and Barriers

Microalgal products have great potential for the future, but they face challenges such as scaling up production, refining cultivation techniques, and maintaining consistent product quality. High production costs raise concerns about their economic viability. Technological advancements like artificial intelligence and machine learning can enhance production processes and reduce expenses. Collaborations between industry players, research institutions, and government agencies can promote knowledge exchange, financial resources, and policy assistance (Slade & Bauen, 2013). Economic viability is a significant challenge for microalgal products, as they require high energy consumption and specialized growth conditions. Innovations in cultivation approaches and efficient techniques for collecting and treating biomass are needed to compete economically. Regulatory and market acceptance are crucial for the widespread use of microalgal products. Ensuring safety and efficacy through rigorous testing and compliance with regulatory standards is essential (Li-Beisson et al., 2019). Technological advancements in genetic engineering, synthetic biology, and metabolic engineering can enhance the efficiency and capacities of microalgae. Genetically engineered algae with enhanced lipid content can augment biofuel output and enhance the nutritional content of food products. Advancements in bioreactor design can improve growth conditions and reduce production costs. Collaborative research efforts and significant investment in R&D are essential for driving technological progress in the microalgae sector.

1.2.11 Strategic Recommendations

Microalgae, rich in nutrients and bioactive substances, is gaining global attention for its potential to address resource scarcity and environmental sustainability. The industry is experiencing growth due to strategic partnerships, consolidations, and investments in research and development. Advancements in cultivation methods, harvesting techniques, and processing technologies are making it easier to produce a wider range of products made from microalgae, known for their improved quality and efficiency. Emerging sectors such as bioplastics, water treatment, and carbon capture are exploring the potential of microalgae as an environmentally friendly alternative to conventional materials and methods (Radakovits et al., 2010).

Investing in research and development is essential for capitalizing on the potential of microalgal products. Collaborations across academia, business, and government can accelerate innovation and facilitate the conversion of scientific discoveries into commercial applications. Establishing supportive laws and regulations can provide a favorable environment for the growth and development of the microalgae industry. The global market for products generated from microalgae is projected to reach a value of US$ 2.81 billion by 2028, with a compound yearly growth rate (CAGR) of 7.9% from 2020 to 2028. Europe is expected to continue being a major participant in the market, with the Asia-Pacific and Latin America regions expected to have substantial market expansion due to the rising demand for food and nutraceuticals made from microalgae (Wijffels et al., 2010). To establish markets for microalgal goods, targeted marketing techniques and customer awareness are necessary. Companies must commit resources to branding and advertising efforts that highlight the unique benefits of microalgae, such as sustainability, health-enhancing characteristics, and flexibility.

1.2.11.1 Sustainable Solutions for a Greener Future

Microalgae-derived products offer sustainable and adaptable solutions, particularly in industries seeking to reduce their ecological impact. They can efficiently absorb carbon and produce sustainable energy sources like biofuels. The global market for microalgae-based products is expected to grow due to the growing focus on sustainable practices across various industries. Microalgae have been crucial in addressing issues such as nutrient-rich supplements, biofuels, important compounds, and wastewater remediation. Sustainable manufacturing procedures are essential for the long-term sustainability of the microalgae industry, including minimizing ecological impact, optimizing resource efficiency, and ethically sourcing raw materials. Closed-loop systems, renewable energy use, and biodegradable packaging can enhance sustainability. Certification systems and eco-labeling can improve transparency and customer trust. The potential of microalgae products in various industries, including nutraceuticals, biofuels, and environmental applications is promising. With continuous research, investment, and successful collaboration, the microalgae industry can make a significant impact on the world stage (Li et al., 2008).

1.2.12 Call to Action for Industry and Academia

Microalgal products have the potential to address global concerns and drive sustainable innovation in various industries. They are being explored for nutrition, energy production, and environmental restoration. However, a coordinated effort between industry and academia is needed to fully utilize their potential. The microalgal industry is a rapidly evolving sector that requires continuous investment in research and development to discover new applications. Collaboration with research institutions and educational institutions can accelerate the investigation and transformation of microalgal products into commercially viable products. Advanced technologies like automation, artificial intelligence, and bioreactors can improve efficiency and scalability. Diversifying product portfolios to include food additives, nutraceuticals, biofuels, bioplastics, and medications can strengthen the business's resilience against obstacles (Radakovits et al., 2010). Strategic alliances with academic institutions, government agencies, and other industry players can expedite research and development initiatives. Public-private partnerships can combine academic expertise with corporate resources, fostering innovation and attracting finance and investment. This can help overcome challenges related to increasing production and ensuring consistent product quality. The microalgal industry should prioritize sustainability, using ecologically sustainable and efficient production techniques. This includes using sustainable energy sources, reusing water, and reducing waste. Comprehensive life cycle assessments can identify areas for improvement and ensure the environmental impact of microalgal products (Li et al., 2008). Consumer education and marketing are crucial for the sector's growth. Companies should launch marketing campaigns that highlight the nutritional value, ecological sustainability, and unique features of microalgae. Developing impactful branding strategies can help differentiate products in the market and build consumer trust (Gross, 2004).

The microalgae industry faces numerous challenges, and a transdisciplinary approach is crucial for addressing these issues. Researchers should focus on integrating biology, engineering, chemistry, and environmental research to develop innovative solutions and enhance the scalability and economic viability of microalgal technology. They should also focus on advancing state-of-the-art technologies for microalgae cultivation, harvesting, and processing (Slade & Bauen, 2013). Industry collaboration is essential for aligning research aims with commercial needs, accelerating the conversion of scientific advancements into economically feasible solutions. Pilot-scale efforts can increase industry confidence and attract investment. Close collaboration with industry partners can provide valuable market trends and customer preferences, guiding academic research toward practical implementations (Radakovits et al., 2010). Developing a skilled workforce is crucial for the growth of the microalgae industry. Academic institutions should provide specialist training programs and courses in algal biotechnology, bioengineering, and sustainable production techniques. Internships and apprenticeship programs can provide hands-on experience and keep industry specialists updated with the latest technologies (Wijffels et al., 2010). Lastly, researchers can advocate for legislation and regulations that support the growth of the microalgae industry. By providing

evidence-based recommendations and engaging in active dialogue, academics can create a conducive environment for innovation and commercialization (Gouveia et al., 2008). The microalgae sector can overcome challenges, stimulate innovation, and ensure sustainable growth through collaboration between business and academia. The potential of microalgae products to address global issues and promote sustainable development is vast. To fully exploit this potential, planned efforts should involve industry and academia, focusing on innovation, partnerships, sustainability, and consumer awareness. This call to action encourages both sectors to harness the potential of microalgae for a healthier and more sustainable future.

2 Conclusion

The microalgae industry presents strategic opportunities for growth and innovation through collaborative ventures and partnerships between industry players, academic institutions, and government agencies. The global market for microalgae-based products is expected to reach several billion dollars by 2030, driven by the growing interest in sustainable and health-oriented products. Stakeholders should focus on best practices, such as investing in research and development, building strategic partnerships, and emphasizing sustainability in operations. Large-scale microalgae production offers substantial benefits across various industries, but achieving success requires careful consideration of factors such as the selection of appropriate cultivation techniques, efficient harvesting and processing methods, and strategic recommendations for growth and efficiency. Investing in advanced technologies, developing robust supply chains, and prioritizing research and development, sustainability, and strategic partnerships can unlock the full potential of the microalgae industry.

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Large-Scale Microalgae Production: Considerations and Recommendations



Astri Rinanti, Melati Ferianita Fachrul, Astari Minarti, and Thalia Sunaryo

Abstract Air pollution is a current public concern, and anxiety is an alarming environmental issue. Industry is the second largest contributor of carbon dioxide (CO_2) emissions after the power generation sector. Two hundred fifteen million tons of CO_2 have been released (37% of total emissions in Indonesia—2021 data). One of the contributors to pollution in big cities comes from the production of CO_2 generated from industrial activities. Utilizing trees to absorb carbon dioxide (CO_2) emissions is one of the solutions to absorb carbon dioxide (CO_2) emissions. However, this solution has shortcomings, considering that the activity of trees to absorb carbon dioxide emissions takes a long time from tree seedlings to become medium trees, so exhaust gases from industry are still released into the atmosphere, while the forest location is far from industrial sources. Therefore, a smart solution is needed to solve this pollution problem. Microalgae production, on a pilot or industrial scale, needs to consider many things, starting from strain improvement, contamination control, daily maintenance, process control in microalgae cultivation, processing unit operations, tools, socio-economic considerations, regulations, environment, and innovation. Challenges and opportunities related to these aspects are discussed to improve high-quality production yields and tight contamination control for efficient and sustainable microalgae production. A case study of a start-up in Indonesia focusing on tropical green microalgae production will be discussed as an example of a community's efforts to undertake large-scale microalgae production within limited space and resources. With a focus on current research and future trends, this book chapter provides a comprehensive view of large-scale microalgae production and recommendations for moving the industry forward.

Keywords Culture conditions · Contamination control · Scale-up · Pilot-scale systems · Biomass production

A. Rinanti (✉) · M. F. Fachrul · A. Minarti · T. Sunaryo
Environmental Engineering Department, Faculty of Landscape Architecture and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia
e-mail: astririnanti@trisakti.ac.id

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1 Introduction: Addressing Air Pollution Through Microalgae Production

In recent decades, air pollution has become an increasingly pressing environmental issue, with carbon dioxide (CO_2) emissions as one of the main causes. Various sectors contribute significantly to the increase of CO_2 concentration in the atmosphere, among which the industrial sector is one of the major contributors to Greenhouse Gas (GHG) emissions and, consequently, global climate change. Production activities involving the burning of fossil fuels, the use of electrical energy, as well as chemical processes in the manufacture of goods can produce large amounts of GHG emissions such as carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O) (Awolesi et al., 2019; Sunaryo et al., 2023). Heavy industries, such as the energy sector, manufacturing and raw material processing, are often the main cause of these emissions, as their production processes require high energy and generate significant waste (Vargas & Halog, 2015; Wei et al., 2021). As a result, the phenomenon of climate change is increasingly evident and is characterized by rising global temperatures, changing weather patterns, and a range of adverse environmental impacts. The Intergovernmental Panel on Climate Change (IPCC) has stated that if no action is taken to prevent the continued increase in GHG emissions, the Earth's temperature could reach as high as 64 °C during the twenty-first century (IPCC, 2013). GHG emissions can cause a greenhouse effect that impacts climate change and global warming and has an impact on human health (Kristanto & Koven, 2019).

Tree planting has been widely recognized as an effective strategy to reduce GHG emissions and mitigate the impacts of climate change. The photosynthesis process performed by trees allows them to absorb carbon dioxide (CO_2) from the atmosphere, store it in biomass, and contribute significantly to carbon sequestration. Reforestation and tree planting practices can increase carbon storage in soil and biomass and help stabilize the local climate (Redjeki et al., 2021; Suprayitno et al., 2024). Research conducted by Suprayitno et al. (2024), for example, showed that neem trees (*Azadirachta indica*) can provide carbon reserves of 239.34 kg or 41% of the total carbon reserves in the Sunan Bonang University Tuban Campus area, which is 581.27 kg or equivalent to 2.1 tons of CO_2 absorbed from the atmosphere.

While tree planting is a long-recognized strategy for reducing greenhouse gas (GHG) emissions, recent studies have shown that using liquid microalgae can provide more significant results in emission reduction. Microalgae have the ability to sequester CO_2 at a much higher rate than trees, thanks to their rapid growth and high photosynthetic efficiency. Microalgae can absorb up to 10–50 times more CO_2 per hectare compared to plants. In addition, microalgae can be produced in controlled systems that allow optimization of growth conditions, thereby increasing yields (Rusdiani et al., 2016). Several previous studies have proven that microalgae have the potential to produce high-value products such as biofuels, which not only contribute to the reduction of GHG emissions but also provide a more sustainable energy alternative (Ayuwaningsih et al., 2018; Minarti et al., 2024; Rinanti & Purwadi, 2019). Thus, while tree planting remains integral to climate change

mitigation efforts, liquid microalgae offers a more efficient and rapid solution to reducing GHG emissions.

2 Microalgae as a Carbon Sequestration Solution

To reduce GHG emissions resulting from various activities, a range of physical, chemical, and biological methods can be used to absorb GHG. This absorption strategy is known as carbon capture and storage/utilization (CCS/U). GHG emission reduction in CCS is carried out through several stages, i.e., capture, separation, transportation, utilization, and storage of CO_2 via various approaches (Fig. 1). The main methods of carbon emission capture include: (i) pre-combustion, where CO_2 is separated before combustion and the fuel is broken down into synthesis gas (a mixture of CO_2 and H_2), (ii) post-combustion, where CO_2 is captured after fuel combustion through chemical absorption, (iii) oxy-fuel, where the fuel is burned with pure oxygen to produce high amounts of CO_2, and (iv) combustion with chemical circulation, where oxygen-carrying particles (metal oxides) react with the fuel to produce metals, CO_2, and H_2O (Leung et al., 2014; Osman et al., 2023; Shreyash et al., 2021; Suzuki et al., 2013). Separation of CO_2 from flue gas is also

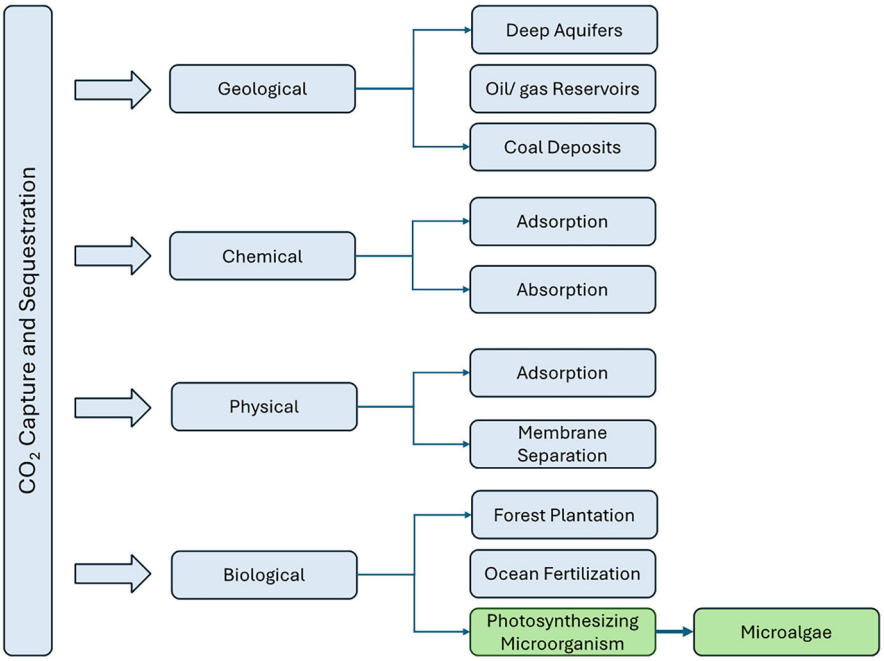


Fig. 1 Alternative Methods of CO_2 Capture and Sequestration

essential in CCS technology through different separation techniques such as adsorption/absorption, membrane separation, and cryogenic distillation.

Physical methods for reducing carbon emissions, such as CCS/U, have been explored extensively. However, there are several technical and economic limitations with the current technologies, making it imperative to improve existing technologies as well as develop suitable alternatives. Among various other methods, biological sequestration of CO_2 emissions is a relatively cost-effective and environmentally friendly approach compared to physical and chemical methods (Prasad et al., 2021).

Among CCS/carbon emission technologies, the biological approach is considered the most economical and environmentally friendly, as it relies solely on sunlight and photosynthetic organisms such as aquatic and terrestrial plants (Ashour et al., 2024). Utilizing microalgae is one of the most promising options for capturing atmospheric carbon emissions due to its high efficiency, scalability, and potential to produce economically valuable byproducts. Through photosynthesis, microalgae are capable of sequestering about 50 gigatons of CO_2 per year or more than 50% of global photosynthetic activity (Ashour et al., 2024).

Microalgae are known to possess a Carbon Concentration Mechanism (CCM) that improves photosynthetic efficiency and CO_2 absorption. A set of specialized plastid structures in microalgal cells called pyrenoid can increase the concentration of CO_2 around the thylakoid membrane, thereby increasing the efficiency of the Rubisco enzyme in the absorption of carbon emissions. However, the mechanism of CCM varies among microalgal species. For example, *Chlorella* vulgaris has a simpler CCM than Chlamydomonas reinhardtii and Nannochloropsis oceanica (Guan et al., 2013). This diversity indicates differences in efficiency in CO_2 uptake and biomass production (Yu et al., 2020).

The varying CCM mechanisms among microalgal species also affect the effectiveness of CO_2 sequestration in various cultivation systems. The selection of an appropriate cultivation system, such as an open pond or closed photobioreactor, is crucial to maximizing the carbon sequestration potential of microalgae. The system used affects not only the efficiency of CO_2 sequestration but also the productivity of the biomass produced, so it is important to consider the compatibility between the microalgae species and the cultivation method used.

There are two main systems in microalgae cultivation, namely open pond (OP) and photobioreactor (PBR). The open pond system faces challenges like low fixation efficiency of between 10% and 40%, low solubility, and significant losses during cultivation. Meanwhile, PBR has the advantage of increasing microalgae productivity through the regulation of environmental factors and optimal volume utilization—some microalgae species, such as *Chlorella* sp. KR-1, *Chlorella* ZY-1, and *Chlorella* vulgaris, are able to tolerate up to 90% vol of CO_2, while *Chlorella* sp. T-1 can tolerate up to 100% vol of CO_2 with a cultivation system using PBR (Tazkiaturrizki et al., 2024). PBR is known to increase microalgae productivity by 2–5 times compared to normal conditions (Rusdiani et al., 2016).

In their studies, Tazkiaturrizki et al. (2024) and Minarti et al. (2024) highlighted some of the advantages of the PBR system for microalgae cultivation, which include: (1) better environmental control, allowing optimal regulation of temperature, pH,

nutrients concentration, and light intensity to improve productivity and yield quality; (2) Lower risk of contamination, making it ideal for the production of microalgae used in food, cosmetic, or pharmaceutical products; (3) higher light capture efficiency, which increases microalgae productivity; and (4) more efficient water use as it reduces water loss through evaporation. However, they also noted some of the disadvantages of the PBR systems, including (1) higher investment and operational costs, as it requires sophisticated equipment and greater energy consumption; (2) difficulty in scalability as the increased size and complexity of the closed system leads to higher costs and technical challenges; and (3) higher challenges in maintenance, including cleaning, sanitization, and more complex monitoring compared to open systems.

As documented by Rinanti et al. (2024) and Rinanti and Purwadi (2019), cultivation of microalgae in the PBR system begins with the selection of an appropriate photobioreactor type, such as tubular, flat-plate, or hybrid, according to the needs and purpose of cultivation. Microalgal inoculum, derived from existing cultures or isolated from the natural environment, is prepared by isolation methods such as sampling, separation, inoculation, colony isolation, purification, and species identification. After the inoculum is ready, the next step is to prepare the culture media, whether natural or artificial, each of which is adjusted to the cultivated microalgae species. Inoculation is done by adding the inoculum to the culture medium in the photobioreactor to initiate microalgae growth.

Environmental parameters such as temperature, pH, light intensity, and gas concentration (CO_2 and O_2) can be adjusted to optimize growth. The photobioreactor is also equipped with a stirring and aeration mechanism to maintain an even distribution of microalgae, oxygen, and CO_2, aiding nutrient absorption and light exposure. During the cultivation process, regular monitoring and maintenance are required to ensure optimal conditions, including adding nutrients if needed and keeping the system clean to avoid contamination. Once the microalgae have reached the desired density, harvesting can be done using methods such as centrifugation, filtration, or flocculation. The resulting microalgal biomass is then further processed into products like biodiesel, protein, pigments, or animal feed (Fig. 2).

The absorption process of CO_2 emissions in PBR is evidently influenced by several environmental factors, such as:

(a) pH

The pH of the media during microalgae cultivation has a vital role in microalgae growth, namely in the process of nutrient absorption, photosynthetic activity, and carbon absorption efficiency. Microalgae generally show optimum growth in a cultivation medium with a pH of 6.5–8.5. pH that sways too far from the neutral range can reduce the growth rate. Various cultivation strategies such as autotrophic, mycotrophic, and photoheterotrophic affect pH on carbon fixation and carbohydrate accumulation in *Chlorella* vulgaris JSC-6 (Cheng et al., 2022). Therefore, monitoring and controlling pH is essential in microalgae cultivation for carbon sequestration, as pH affects photosynthetic rates and cellular activities that enable effective CO_2 utilization.

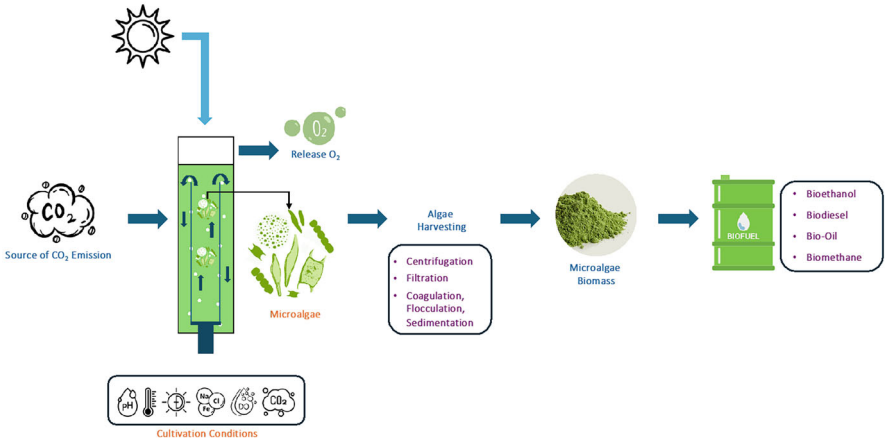


Fig. 2 Process of Absorption of CO_2 emissions by Microalgae to the final product made from microalgae raw materials

(b) Temperature

Generally, temperatures between 25 °C and 35 °C are considered ideal for *Chlorella* sp.'s growth and carbon fixation (Politaeva et al., 2023). Temperatures under moderate conditions affect the Rubisco enzyme, which is essential for carbon fixation, so it binds oxygen instead of carbon dioxide, resulting in photorespiration that reduces the CO utilization rate by up to 30%. A temperature that is too high can damage the photosynthetic apparatus and lower the overall carbon fixation efficiency. Therefore, temperature regulation during cultivation is essential to ensure adequate dissolution of CO_2 without damaging the enzymes and preventing photorespiration.

(c) Light Intensity

Lighting conditions play an important role in regulating photosynthetic efficiency and CO_2 fixation capacity in microalgae. Optimal light intensity directly influences photosynthetic rate and biomass productivity (Souliès et al., 2016). Although higher light intensity can increase the growth rate initially, if light intensity exceeds the saturation point, photoinhibition can occur and reduce CO_2 fixation. A minimum lighting intensity of about 10–30 μmol m^{-2} s^{-1} is required to ensure microalgae are able to absorb CO_2 and convert it into biomass through photosynthesis.

(d) Inorganic Nutrient

Microalgae require sufficient macro and micronutrients to achieve optimal growth and CO_2 fixation. Macronutrients such as nitrogen and phosphorus are essential for microalgal growth and development. At the same time, micronutrients such as vitamins and trace metals are also required in small amounts but are essential for carbon fixation and optimal growth (Li et al., 2022a, b). Nitrogen is an essential macronutrient that plays a role in synthesizing

proteins, nucleic acids, and chlorophyll, all essential for photosynthesis and biomass accumulation.

(e) Dissolved Oxygen

Dissolved oxygen (DO) levels also affect the efficiency of CO_2 fixation and the overall productivity of microalgae. Although oxygen is an essential byproduct of photosynthesis, excess oxygen accumulation above 25 ppm can inhibit the rate of CO_2 fixation. Research shows that reducing DO levels can significantly increase carbon sequestration efficiency, with a 30-fold decrease in DO being able to triple the rate of CO_2 fixation in *Chlorella* sp. Therefore, understanding and managing DO and CO_2 levels is critical to optimize the rate of CO_2 biofixation in Carbon Capture and Utilization (CCU) approaches (Gaurav et al., 2024).

(f) Source and Concentration of CO_2

The effectiveness of CO_2 biofixation by microalgae is strongly influenced by the carbon source and substrate used in the culture system. CO_2 concentrations in aerated gas below 0.5% vol. may limit microalgal growth due to low solubility and limited affinity of carbon fixation enzymes such as Rubisco (Kazbar et al., 2019; Mustafa et al., 2021). However, excessively high CO_2 concentrations above 6–12% vol. can also reduce growth by disrupting the Carbon Concentrating Mechanism (CCM) (Kazbar et al., 2019). For example, high CO_2 concentrations above 30 g m^{-3} can cause up to 30% reduction in biomass productivity in *Chlorella* vulgaris, as high levels of dissolved CO_2 inhibit the carbonic anhydrase enzyme that plays a role in the conversion between CO_2 and bicarbonate ions (Abomohra et al., 2016).

Studies have previously conducted CO_2 emission absorption testing using microalgae in various ideal conditions required for absorption (Chiang et al., 2011; Kassim & Meng, 2017; Li et al., 2011; Xie et al., 2014), as can be seen in Table 1.

3 Contamination Control in Large-Scale Microalgae Production

Microalgae cultivation systems on an industrial scale need to consider the risk of contamination they are susceptible to, as contamination can significantly impact productivity and yield quality. In microalgae cultivation, contamination can come from a variety of sources, including other microorganisms (such as microalgae, bacteria, and fungi), as well as environmental materials such as water and nutrients used in the culture (Ji et al., 2018). Therefore, effective strategies to prevent and control contamination must be implemented to ensure successful microalgae production.

Table 1 Microalgae's ability to remove CO_2 and the capture rate under different growth conditions

Microalgae	CO_2 Removal Efficiency (%)	CO_2 Capture rate (g L d)$^{-1\ -1}$	Cultivation Conditions			
			CO_2 (vol%)	pH	T °C	Light Intensity
Chlorella vulgaris	18	–	30	7.2	30	1800[b]
Chlorella vulgaris	18	2.22	–	–	30	2783[b]
Chlorella vulgaris	18	2.66	25	7–8.2	25	4050[b]
Chlorella vulgaris	40	0.51	10–20	8.2	8	4540[b]
Chlorella vulgaris	–	0.25–1.7	0.03	18	18	6000[b]
Chlorella sp.	10	–	10	–	26	16,000[b]
Chlorella sp.	5	0.35	5	7.1	–	5400[b]
Chlorella sp.	10	–	10	6	25	4050[b]
Chlorella sp.	40	0.097	15	7.5–9	30	450[a]
Chlorella sp.	40	2.33	26	6.3–9	26	100[a]
Desmodesmus sp.	100	1.58	30	–	30	60[a]
Anabaena sp.	10	1.01	5–15	–	20–25	127–250[a]
Scenedesmus obliquus	18	0.252	13.8	7	25	5.496[b]
Scenedesmus obliquus	18	–	26–28	–	26	12,000[b]
Botryococcus braunii	10	–	5.5	–	25	150[a]

[a]In µmol ms^{-2-1}
[b]In lux

Biological contamination that occurs during microalgae production on an industrial scale can be controlled using various methods, including physical, chemical, biological, and environmental control (Bhusan Makut & Samantaray, 2021).

(a) Physical Method

Equipment disinfection, sonication, and ultraviolet sterilization are commonly used physical methods. Sonication or physical disruption using ultrasound is considered more feasible in preventing the survival and growth of bacteria, phytoplankton, and zooplankton. Meanwhile, ultraviolet sterilization and pasteurization are also used to keep microalgae cultures sterile. However, these physical methods are generally only effective in laboratories or on small scales and are expensive when applied on a large scale (Cheng et al., 2022).

(b) Chemical Method

Various chemical agents such as antibiotics, fungicides, pesticides, salts, aldehydes, and peroxides are used to control biological contamination. However, the concentration of chemicals used must be in the right amount. For example, cytochalasin is an anti-grazing pesticide that is effective against protozoa. In contrast, quinine sulfate (anti-grazing pesticide) at a concentration of 10 mg/L can destroy ciliates in D. salina cultures. Although effective, the large-scale use of chemicals is often expensive and can inhibit microalgal growth, negatively impacting the environment, human health, and aquatic life.

(c) Biological Method

Biological control is a new approach that is safer for microalgae than conventional chemical agents. Plant-based pesticides, such as celangulin, matrine, and toosendanin, have effectively controlled rotifers in microalgae cultivation (Cheng et al., 2022; Li et al., 2022a, b).

(d) Environmental Method

Environmental conditions, such as pH, temperature, and light, need to be adjusted to the needs of microalgae in their growth. Some microalgae and cyanobacteria strains are resistant to extreme environmental conditions, such as pH, temperature, and light intensity. For example, Spirulina grows well at high pH and *Dunaliella* in high salinity conditions. Touloupakis et al. (2016) found that alkali-resistant Synechocystis strains grew well at pH 11, while Poterioochromonas strains did not survive at the same pH. The addition of carbonate in the culture can also increase the pH and remove biological contaminants. In addition, light, salinity, and temperature shock can also affect the survivability of contaminants within microalgae cultures.

One of the important efforts in maintaining a successful microalgae cultivation process on an industrial scale is to ensure the process is free from contamination. Contamination can affect the productivity and quality of the microalgae harvest. One effective method to minimize the risk of contamination is to start cultivation from pure stocks of microalgae on a small scale, conducted in a controlled environment. In a laboratory, microalgae growth conditions, including light, nutrients, temperature and pH, can be monitored and regulated with precision (Fig. 3). This way, microalgae stocks remain pure before they are further propagated for large-scale use in industrial facilities. This process also allows for better management of specific microalgae strains of interest, while maintaining culture health and stability.

4 Opportunities and Challenges in the Microalgae Market

The microalgae market is showing significant growth potential, fueled by the increasing demand for sustainable and eco-friendly products. Growing global awareness around the issues of climate change and environmental pollution has encouraged consumers to turn to more sustainable sources of food and energy, including



Fig. 3 Controlled cultivation of microalgae on a laboratory scale

those that are offered by microalgae. Factors such as the increasing need for plant-based proteins and natural raw materials in the food and supplement industry have arguably driven the demand for microalgae products.

Key market opportunities in the microalgae industry include the production of biofuels, bioplastics, biofertilizers, nutraceuticals, pharmaceutical products, and cosmetics. In addition, two rapidly developing technology areas are driving the evolution of the market: microalgae harvesting and extraction technologies and the production of genetically modified microalgae. These market opportunities demonstrate the immense potential of microalgae as a multifunctional biological resource (Rumin et al., 2020).

Microalgae-based biodiesel has many promising future opportunities. First, as a carbon-neutral fuel source, algae can help in reducing GHG emissions by absorbing carbon dioxide during growth. Algae are also a renewable and sustainable energy source that can reduce dependence on fossil fuels. Biodiesel from algae has diverse applications, from transportation to power generation, and can be grown on unused land. Continued research and development could potentially make it a more affordable fuel alternative. In addition, algae can be used as a fertilizer or protein source for animal feed and can utilize industrial waste such as wastewater and carbon dioxide, making it a more economical and environmentally friendly solution (Abomohra et al., 2016; Brown et al., 2018; Rahpeyma & Raheb, 2019; Vieira de Mendonça et al., 2021; Yuan et al., 2023).

Notwithstanding the opportunities, we also identified a few challenges in entering the microalgae market, particularly in regard to intense competition with other environmentally friendly products. Innovation in microalgae cultivation and processing technology is critical to gain a significant market share. Developing more efficient and cost-effective methods for microalgae production, such as using integrated culture systems or using industrial waste as growth media, will provide a competitive advantage.

In the production of biodiesel from microalgae, some of the challenges faced are related to high production costs compared to fossil fuels, limited scalability to meet global market needs, and energy-intensive harvesting. In addition, production efficiency is still low as it requires a lot of time and resources, while in water-sensitive

regions, the extensive water usage of microalgae production is indeed an issue (Barsanti & Gualtieri, 2018; Bošnjaković & Sinaga, 2020; Chia et al., 2018). In addition, the risk of contamination by other species can reduce biodiesel yield and quality. There are also technical challenges in developing efficient cultivation and harvesting technologies and the need to build adequate infrastructure, which requires significant investment. All of these obstacles point to the need for innovation and support to make microalgae a more economical and environmentally friendly biodiesel solution (Anto et al., 2020; Min et al., 2022).

5 Regulatory, Environmental, and Socio-economic Considerations

Microalgae production and distribution cannot be separated from the influence of applicable regulations and policies. Governments in various countries have implemented regulations to protect the environment and ensure the safety of microalgae-based products. For example, in the European Union, regulations related to food safety and biotechnology products, such as EU Regulation No. 2015/2283, require all new products, including those based on microalgae, to meet strict safety standards before they can be marketed.

The European Union has adopted an ambitious strategy for the development of the bioeconomy, in which microalgae are considered an important biological resource. Microalgae are currently promoted for applications in areas such as environmental technology, bioenergy production, as well as a valuable source of nutrients for humans and animals. The microalgae sector is expected to proliferate in the EU's blue bioeconomy, especially in coastal areas. The blue algae industry in the EU generates annual revenues of EUR 1.5 billion, with indirect activities such as research contributing an additional EUR 240 million (European Commission, n.d.; Zhao et al., 2022). However, the decline in macroalgae populations due to global warming, overexploitation, and ecosystem degradation necessitates the construction of specialized production facilities (Bourgougnon et al., 2021; Li et al., 2022a, b). Microalgae have competitive advantages in efficient photosynthesis, rapid biomass growth, and the ability to absorb pollutants, so they can reduce pressure on wild populations through microalgae aquaculture that supports biomass demand and provides environmental benefits (Bourgougnon et al., 2021; Yang et al., 2015).

6 Innovation and Case Study: Pioneering Tropical Microalgae Production in Indonesia

As a tropical archipelago rich in natural resources, Indonesia has the potential to develop its microalgae industry. Climate diversity and the availability of abundant water resources make Indonesia an ideal location for microalgae cultivation. Innovations in microalgae cultivation technologies and methods in the country can help meet the growing global demand for sustainable products as well as contribute to the reduction of GHG emissions.

Start-ups engaged in the microalgae utilization industry are growing rapidly. These start-ups utilize the great potential of microalgae to become products of economic value for various sectors, including food, animal feed, cosmetics, and bioenergy. Not only for economic value products, the microalgae industry is also present as one of the environmentally friendly solutions to global challenges such as climate change. The start-ups provide various bio-material-based products and services made from biofatty oils, clove oil, and biomass-based furfural. These products are suitable for various industries, such as PVC, adhesives, paints, and coatings. To illustrate, one start-up has designed a microalgae aquarium called Photo-tank with an aesthetic design for carbon emission absorption to reduce carbon footprint and convert it into released oxygen. The Photo-tank is also equipped with sensors to monitor the surrounding air quality (Fig. 4).

Overall, innovations in microalgae cultivation and processing, combined with various parties' collaborative efforts, can transform Indonesia's microalgae industry

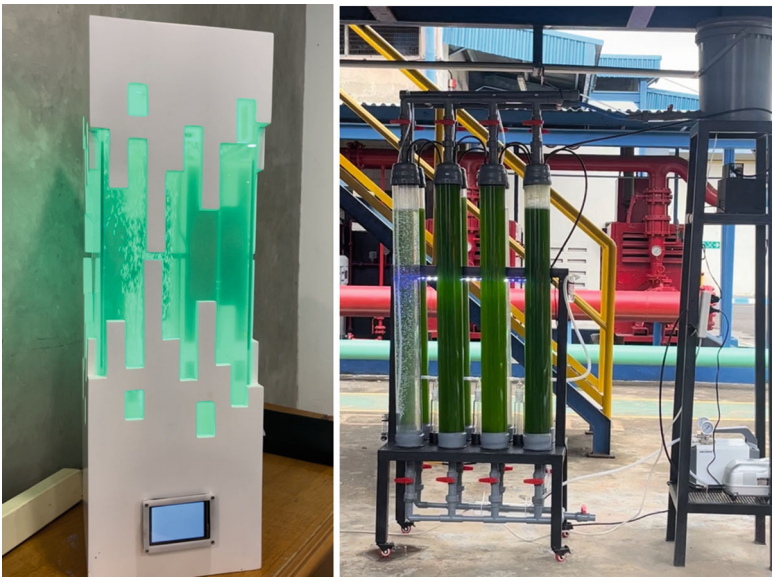


Fig. 4 Microalgae Photo-tank

into an important pillar in achieving economic and environmental sustainability. By capitalizing on its geographical and resource advantages, Indonesia can become a global leader in tropical microalgae production.

7 Recommendations for Advancing the Microalgae Industry

Several important steps need to be taken to promote the growth and sustainability of the microalgae industry. First, investing in research and development (R&D) is essential to improve microalgae cultivation and processing efficiency, productivity, and technology. In addition, the development of adequate infrastructure, including modern and environmentally friendly processing facilities, must be optimized to support the production and distribution of microalgae. At the same time, the government needs to create supportive policies and regulations, including incentives for research and production, as well as regulations related to product safety and sustainability. Increasing consumer knowledge and awareness of microalgae-based products with many nutritional, health, and environmental benefits must be continuously carried out to create greater and more sustainable demand. Consistent marketing and education campaigns will help expand the reach of microalgae products in the global market and increase consumer confidence in these products.

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Part III
Scaling Up Microalgae Production—Strategies, Applications, and Case Studies

Patent Mapping in Microalgae-Mediated Processes



Hissashi Iwamoto, Walter José Martínez-Burgos, Juliana Cardoso, Roberta Pozzan, Guilherme Anacleto dos Reis, Alexander Parody Muñoz, Ariane Fátima Murawski de Mello, Júlio Cesar de Carvalho, and Carlos Ricardo Soccol

Abstract Microalgal production has gained importance as various bioproducts can be derived from biomass processing. Large-scale production growth impacts on the rise of innovations. Microalgae biomass is the main and final product in the market. Techniques for algae production still are followed by extraction of pigments, proteins, carbohydrates, and lipids. Considering the microalgae diversity, non-conventional techniques could be critical for efficient cultivation, biomass processing, and product purification. Therefore, the patent survey includes many focused on industrial cultivation processes applied to microalgae. In this chapter, an overview of the patent survey is presented, from types of reactors, cultivation modes, strategies to increase productivity, reduced cost, biomass harvesting and processing, and final product refinement. It is known that the first patent was registered in 1982 and described the use of microalgae *Chlorella* extract as a plant growth promoter. After that, no patent was registered for 15 years. This scenario changed in 2015. Currently, microalgae-based patents are being registered in various innovative areas. Those technologies are primarily concentrated on "cultivation area" (C12), including "media culture" (C12N), development of "apparatus for culturing" (C12M), and culturing strategies for "bioproduct synthesis" from microalgae (C12P). This group accounts for more than 89% of microalgae patents. Other representative groups are related to "greenhouse gas mitigation" (Y02) and "effluents treatment" (C02) involving nearly 32% of patents. Following, 15% of registered patents are related to bioproducts derived from microalgae, namely: "ingredient for food or feed" (A23), "oil or lipid" (C11), and "protein and amino acid" (C07). Other minor groups, such as fuel-related technologies (C10L), pharmaceuticals purposes (A61K), and

H. Iwamoto (✉) · W. J. Martínez-Burgos (✉) · J. Cardoso · G. A. dos Reis · A. F. M. de Mello · J. C. de Carvalho · C. R. Soccol
Bioprocess Engineering and Biotechnology Department, Federal University of Paraná, Polytechnic Center, Curitiba, Brazil

R. Pozzan
Cellular Biology Department, Cellular Toxicology Laboratory, Federal University of Paraná, Polytechnic Center, Curitiba, Brazil

A. P. Muñoz
Universidad Libre, Barranquilla, Colombia

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unitary process development (B01D) will also be presented. In this chapter, a brief discussion about each group classification (IPCs and CPCs codes) is included.

Keywords *Haematococcus* · *Dunaliella* · *Spirulina* · Patent · Technology · Industry · Bioreactor

1 Introduction

Patents are increasingly a source of science and technology, a collection of recent data about process and products, containing legal and commercial information. So, how patents contribute to scientific knowledge? In simple terms, patents are a form of intellectual property that gives the owner the legal right to protect the invention from others on making, using, or selling an invention for limited time in exchange for publishing an enabling disclosure of it. Regarding scientific development, patents are another way to promote knowledge sharing, as they require the complete disclosure of inventions. As a result, patents can be seen as promoters of the pool of public knowledge (Alba et al., 2021; Kong et al., 2023).

The growing demand for food has been a challenge for agroindustry. Besides, increasing agricultural production while preserving the environment is a constant challenge for scientific research. It prioritizes large-scale production with sustainable technologies and natural inputs over synthetics. In addition, the agroindustry generates large amounts of waste that can cause serious environmental problems if disposed of inappropriately (Murata et al., 2021).

Microalgae are photosynthetic microorganisms, which have great potential as bioresource alternatives for various industries. Microalgal research has increased in academic and private settings due to their varied and versatile nature, growing in both autotrophic and heterotrophic conditions, their adaptability to domestic and industrial wastewater, their simple unicellular structure, and their ability to produce valuable co-products (Murata et al., 2021).

In 2022, the emerging microalgal biomass market reached USD 2.4 billion/year, exceeding the value projected in 2015 for 2024, which was USD 1.143 billion/year. From 2015, USD 608 million/year, the market has grown at a CAGR of 18.7% until 2022, which was higher than the expected in 2015 for 2024 (CAGR of 7.4%) (Transparency Market Research, 2023). As microalgal biomass is a promising source for the increasing food demand, there is a noticeable increase in cultivation technologies for reliable and productive photobioreactors (Kirnev et al., 2020). Various developments have been applied not only in cultivation but also in waste and pollutant treatment.

This chapter will focus on the patent analysis related to microalgal biomass production and its derived bioproducts in large-scale production. A discussion among the different IPC groups of patents will be provided for a better understanding of how these technologies are organized.

2 Database Patent Search and Data Analysis

To start the patent search on microalgae and large-scale production scenarios, the search was conducted using four different databases: Espacenet (European Patent Office search engine), WIPO (World Innovation and Patent Office), INPI (Brazilian National Institute for Industrial Property), and Google Patents from December 2023 to June 2024. The search was narrowed using the keyword "Microalga* AND Industr*". Google Patents was used as a guide for IPC (International Patent Classification) distribution analyses, based on the result an overview of the patent distribution could be done (Table 1).

Most of the microalgae patents fall under the C12 group, related to "*Biochemistry; beer; spirits; wine; vinegar; microbiology…*". This group accounts for more than 89% of the microalgae patents related to "*cultivation development*". Within this group, 36.7% of the total patents are included to "*culturing techniques and mediums*" (C12N); 34.8% are related to "*bioreactor or photo-bioreactor*" development (C12M); and 13.1% are related to "*bioproducts from microalgae*" (C12P).

Microalgae have been widely studied as an alternative for waste and pollutants treatment. Therefore, the next group Y02 that involves patents related to "*contaminants removal from environment*" represents more than 26% of deposited technologies. This group is divided into technologies for "*adaptation to climate changes*"

Table 1 IPC description and patents distribution over the groups

IPC	Patents related to IPC (%)	Description of IPC
C12N	36,7	Medium, microorganism, maintenance
C12M	34,8	Apparatus for cultivation
C12P	13,1	Cultivation process/production
Y02E	9,9	Greenhouse gas reduction (CO_2 production, transport, and distribution)
Y02P	6,9	Greenhouse gas reduction (CO_2 industry and agriculture)
C02F	6,6	Wastewater usages
Y02A	6,2	Technologies for adaptation to climate change
C12R	4,9	Measuring or testing processes
A23K	3,7	Fodder (animal feedings)
Y02W	3,7	Wastewater treatment
C11B	3,6	Oils produced by pressed material
A01G	3,2	Related to vegetable propagation/microalgae propagation
A23L	3	Food (non-alcoholic)
C10L	2,5	Fuels
C07K	2,4	Peptides/Amino acids obtention
A61K	2,4	Technologies for preparation to pharmaceutical products obtention or deodorization or disinfection or sterilization
B01D	2	Unitary process of Separation/Harvesting/Purification/Extraction/Crystallization
A23V	1,8	Food ingredients
A23D	1,7	Edible oils

IPC description was obtained from WIPO (2024).

IPC description was obtained from WIPO (2024)

(Y02A), "*reduction of greenhouse gases*" (Y02E and Y02P), and "*wastewater treatment*" (Y02W). The difference between Y02E and Y02P are gases generated from "*transportation and distribution*" and "*industry and agriculture,*" respectively.

Almost all technologies of the "*medium and nutritional requirements for cultivation*" group (C02) are related to "*wastewater usage*" in microalgae cultivation (C02F). The C02F differs from the Y02W on the contamination removal purpose, as the first is focused on the residue as a source of nutrients for microalgae cultivation, while the other is focused on mitigation of those abundant nutrients and contaminants in the wastewater.

Other technologies are applied to "*feed and food*" group (A23), which represents 10% of the total technologies in microalgae and industrial production. This involves the microalgae biomass destined for "*non-human feeding*" (A23K), "*oil production"* (A23D), the "*preparation and preservation of food*" (A23L), and serving as *"food ingredients"* (A23V).

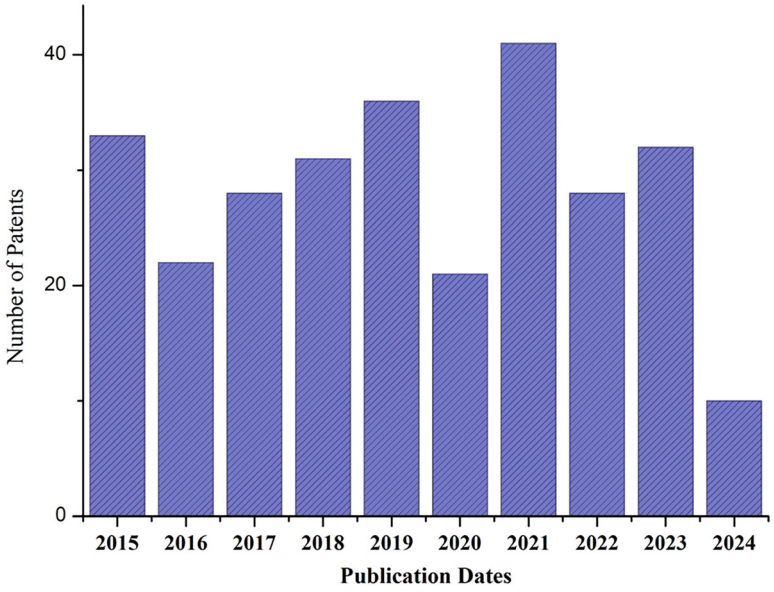
Technologies applied to bioproduct exploration still are minor, and in this search two groups were included: "*oil and lipid production by direct extraction*" (C11B) and "*peptides and amino acids obtain*" (C07K). Group C11B is related to the extraction process and differs from A23D, which relates to edible oil as a final product.

It should be noted that the number of codes exceeds 100% of total documents because many documents fall under more than one classification.

2.1 Historical Perspective of Microalgae Cultivation and Large-Scale New Trends

Patent registrations related to microalgae began in the 1960s in the EPO database, focusing on food products based on *Chlorella*. The first large-scale use of microalgae was also patented with this same microalgae, supported by a Japanese health food company (de la Jara et al., 2016; Khatoon & Pal, 2015; Severo et al., 2021). Among other microalgae of greater commercial interest, the first patents related to *Spirulina* (*Arthrospira*) were filed in 1972, addressing a medium that promoted greater protein production. *Chlamydomonas* was cited for the development of algaecide compounds in patents from 1973 onwards. Patents related to the production of pigments began in 1989, with *Haematococcus* and *Dunaliella* mentioned in 1978 for production of glycerol. The registration of patents related to commercial microalgae has grown considerably over the years, with patents on general microalgae topics being the most published between 2007 and 2015, followed by patents on *Spirulina* and *Chlorella* (de la Jara et al., 2016).

The use of bioreactors to cultivate microalgae has been patented by various companies, with some variations in system conditions, mainly for the purpose of producing nutraceutical products (Christenson & Sims, 2011). A patent filing from 1997 describes a *Spirulina* cultivation plant in an open pond with electricity

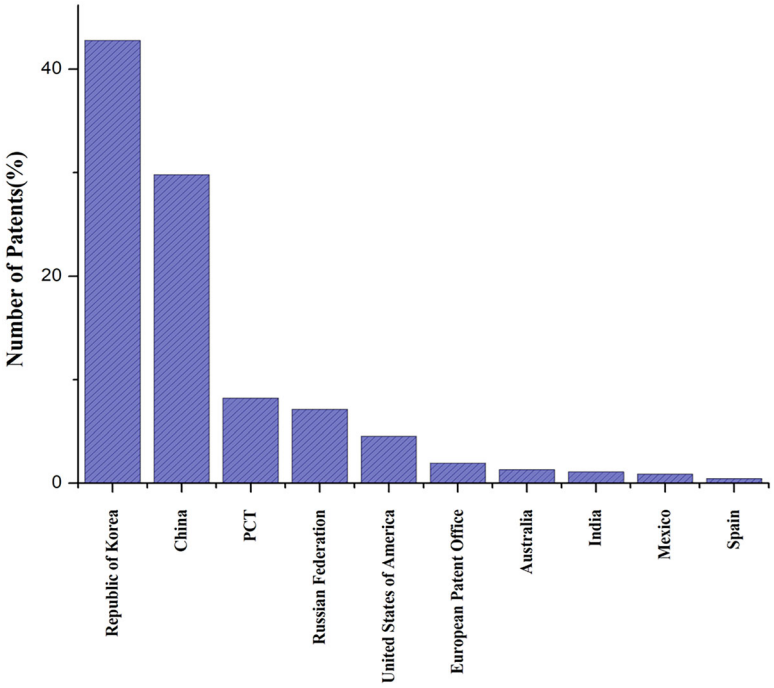


Graph 1 Number of patents by publication date

cogeneration (Christenson & Sims, 2011; Jensen & Reichi, 1996). Patents on closed bioreactors have also paved the way for understanding cultivation conditions in this type of system, especially regarding the benefits of temperature, pH, and contamination control, as well as reduced water loss and more efficient agitation. However, issues such as toxic oxygen accumulation, overheating, biofouling, and high costs are also important topics discussed in these patents. Companies such as Solix Biofuels have filed patents for the cultivation of microalgae in closed reactors, mainly to produce biofuels. Some of these patents seek alternatives to improve thermal regulation, with floating vertical photobioreactors and a patent that incorporates permeable membranes to facilitate the release of O_2 (Christenson & Sims, 2011; Severo et al., 2021; Willson et al., 2017, 2009).

A search on the WIPO platform using the keywords "Microalga* AND Industr*" generated 463 patents filed in the last 10 years. Graph 1 shows the evolution of patent filings by year, peaking in 2021 with 41 patents filed. These results indicate that research into microalgae has produced promising innovations in recent years.

The countries in which institutions file their patents on microalgae are directly related to their governmental objectives, particularly the USA and China, which aim to reduce CO_2 emissions from industries. Additionally, the Republic of Korea and China have had internal incentives for education and research, especially in the sustainability sector, such as the production of biodiesel (Carvalho et al., 2022; Kirnev et al., 2020). A search for patents on photobioreactors conducted by Kirnev et al. (2020) showed an increase in patent filings from 2005 onwards, with the highest level in the United States, followed by China and Korea. This increase was



Graph 2 Number of patents by country in the last 10 years

probably influenced by government agreements on climate change. The overview report on patents related to microalgae developed by WIPO in 2016 showed that the greatest patent filing activity was in Asia, primarily in China, followed by the USA and European countries (Carvalho et al., 2022; Saxon et al., 2022). Results from the most recent WIPO search revealed the highest number of patent publications by the Republic of Korea, with around 43% of patents filed in the last 10 years, followed by China with around 30%, and the United States in fifth place with around 5% of patents filed in the last 10 years (Graph 2).

2.2 Area of Expertise of Microalgae Patent Applications

Patents related to microalgae are filed in different areas of interest, mainly aimed at the food, cosmetics, and pharmaceutical industries, depending on the target of the patent. In general, the microalgae *Chlorella* and *Spirulina* are the most used in patents in the cosmetics, nutraceutical, pharmaceutical, food, biomolecule production, and animal feed industries (de la Jara et al., 2016). Depending on the patented product from microalgae, searches are directed to other areas, such as keywords like lipids and microalgae, which generate most results for the biodiesel (21.24%) and

biofuels (7.96%) industries. In this same search criterion, the resulting IPC codes were mostly (63%) related to processes for the propagation, maintenance, preservation, and composition of unicellular algae (C12N1/12), followed by technologies involving the use of microalgae and the production of fats, oils, and waxes (C12P7/64), with 56% of the resulting patents, and 35% were included in processes using algae (C12R1/89) (Carvalho et al., 2022).

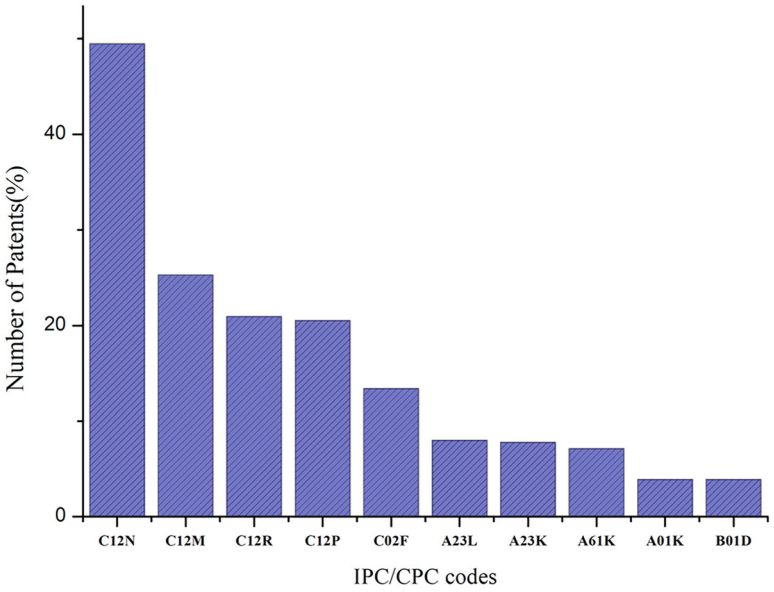
Other classification codes observed in patent searches on microalgae on the Obirt platform are related to photobioreactors associated with combustion processes. Most of the works in this search (52%) were identified as part of the groups YO2E-0050 (on reducing greenhouse gas emissions), C12M-021 (on bioreactors or fermenters), C12N-001 (on microorganisms), and YO2P-020 (technologies for mitigating climate change in the production or processing of chemical industry goods). Additionally, 27% of the patents were classified in other groups related to the capture, sequestration, or disposal of greenhouse gases (Y02C-020) and technologies for adapting to climate change (Y02A-050) (Severo et al., 2021).

A patent search on the Derwent platform using the terms "Microalga* AND Industr*" resulted in a total of 112 patents, primarily classified in the field of chemistry (105), followed by biotechnology applied to microbiology (82), engineering (40), pharmacology and pharmacy (38), water resources (32), and food science technology (23), with smaller numbers of patents classified in instrumentation, polymer science, agriculture, and fuels (considering that a patent can be classified in more than one area). Regarding IPC/CPC codes, 48 patents were classified under C12N-001/12 (unicellular algae, culture media therefor), 27 under C12M-001/00 (apparatus for enzymology or microbiology), 26 under C12R-001/89 (algae), and 15 patents were classified under C12M-001/04 (apparatus for enzymology or microbiology with gas introduction means).

The Derwent platform, Google Patents, and WIPO showed a higher classification of patents related to microalgae under the C12N code (microorganisms or enzymes; compositions thereof; propagating, preserving, or maintaining microorganisms; mutation or genetic engineering; culture media). Specifically, 36.7% of the patents from Google Patents and 49.5% from WIPO fell under this code. In second place was the C12M code (apparatus for enzymology or microbiology), with 34.8% of patents from Google Patents and 25.7% from the WIPO search. Graph 3 shows the results of the current OMPI search for patents on microalgae, classified by their IPC/CPC codes.

2.3 Description of Patents Granted for Large Scale Production

Most patents related to large-scale microalgae cultivation aim to improve cultivation conditions, primarily by modifying factors that reduce the impact of contamination, temperature variations, and production costs, as well as developing new types of



Graph 3 Number of patents by IPC/CPC codes in the last 10 years

reactors. Other patents focus more on recovery/harvesting processes, which can vary greatly depending on the species of microalgae and can be expensive due to energy and equipment costs. Additional work aims to improve the productivity and quality of biomass and microalgal products by altering and supplementing the media. Regarding bioproducts, many patents demonstrate methods to produce oils, biodiesel, biofertilizers, food and nutraceuticals, animal feed, and pigments at lower costs or with new technologies (de la Jara et al., 2016; Saxon et al., 2022).

The patent WO2008083453 (Soccol et al., 2008a, b) aims to produce microalgal biomass with a high protein content on a large scale, using sugarcane husks and CO_2 generated by alcohol industries to supplement the medium. Several species of microalgae are claimed in this patent, including *Spirulina* sp., *Scenedesmus* sp., *Haematococcus pluvialis*, *Dunaliella* sp., *Chlamydomonas* sp., and *Chlorella* sp., among others. The patented production process includes adjusting the pH of the medium containing sugarcane husks by adding water and alkali until the pH reaches a value between 6 and 11. The husks are then distributed in a microalgae cultivation tank, inoculated with an initial biomass concentration of 0.2 g L^{-1}. Pure CO_2 is injected into the cultivation tank, containing 0.1–100% air. Cultivation conditions include an average temperature of 25–35 °C, a duration of around 14 days, and lighting of around 1500 lux (12 h/12 h). The recovery of microalgal biomass involves recycling the supernatant (residual medium) back to the inoculation tank. The benefits of the processes involved in this patent are significant for the development of research related to the production of microalgae and the treatment of industrial waste, achieving high concentrations of microalgal biomass between

2 and 4.4 g L^{-1} and more than 99% reduction in COD and BOD after three cycles of recycling the medium.

Seeking to improve microalgal cultivation, the patent KR1020110100747 (Lee et al., 2012) developed a photobioreactor for large-scale cultivation with light and temperature control, using filtered sunlight. To achieve this, it is suggested that a covered glass or plastic structure should be installed above the cultivation system, with a coating or paint that blocks or transmits unwanted wavelengths. In addition to filtering sunlight, this apparatus allows for internal temperature control and can also remove CO_2 via attached air flaps. This invention is recommended for microalgae such as *Chlorella*, *Haematococcus*, *Spirulina*, and *Chlamydomonas*, among others, primarily to produce pigments, lipids, carbohydrates, and biodiesel.

The patent KR101241393 (Jeong et al., 2013) developed a system for automating the supply of medium to a photobioreactor for growing microalgae. To achieve this, the system automatically takes an optical density reading of a diluted sample and adjusts the medium levels according to predefined parameters. A gas supply tube is also included, injecting gas into the photobioreactors. Based on the crop concentration information captured, an output unit releases a certain volume of the crop into tanks for use in animal feed while simultaneously replenishing the medium in the photobioreactor. Oxygen, pH, and temperature sensors are also attached to the system to control these factors. Many patents related to microalgae have the potential to be applied in various industrial sectors, and most are already used by several large companies in the field.

3 Patents Applied to Innovation on Cultivation (C12)

3.1 Medium, Microalgae Linage, and Cultivation (C12N)

Microalgae are eukaryotic organisms that can reach up to 200 μm and have been gaining interest in research due to their abilities of producing high added value metabolites and photosynthetic fixation of CO_2 (Koller et al., 2012; Vuppaladadiyam et al., 2018). It is estimated that there are up to 8×10^5 species of microalgae available in the nature; however, only 5×10^4 have been described in scientific and technological literature (Vuppaladadiyam et al., 2018). This gap is common since it is often difficult to reproduce all the environmental conditions required for microbial growth and development in laboratory sets. Some examples of microalgae species that are well described in literature are *Chlorella, Botryococcus, Synechocystis, Desmodesmus, Scenedesmus, Haematococcus*, and *Nannochloropsis* (Chew et al., 2018; Zuccaro et al., 2019). New strains of microalgae can be either isolated from strategic locations in the environment or genetically engineered to enhance some characteristic of the original strain.

Microalgae cultivation will invariably depend on which strain is chosen since each microorganism will have specific demands of nutrients, strategies, and physicochemical conditions (e.g., temperature, pH, and media salinity). Cultivation media

is usually composed of essential nutrients such as carbon (which can be organic or inorganic), nitrogen (in the form of nitrates, nitrites, ammonium, urea, or amino acids), phosphorus, and trace elements (e.g., iron, magnesium, potassium, zinc, silicon, among others) (Yousuf, 2019; Zuccaro et al., 2019). There are some synthetic media that are used for microalgae cultivation, such as BG11, BBM, Zarrouk's, and Chu (Chakraborty et al., 2023). Each one of these medias have a specific composition that can be applied for the cultivation of some specific microalgae strains. Despite being balanced and usually guaranteeing a satisfactory biomass development, synthetic media presents a high cost which impacts in overall profitability of microalgae cultivation. To reduce these costs, alternative media based on residues have been proposed. Agro-industrial by-products, wastewater, flue gas, farm waste are some examples of residues that can be applied for further cultivation of microalgae (Chakraborty et al., 2023).

In the sense of using agro-industrial by-products for microalgae cultivation, Soccol and collaborators (2008) described in the patent document WO2008083453 the use of sugarcane husks and CO_2 deriving from sugar and alcohol industries for *Spirulina platensis* cultivation. The microalgae was able to grow with these two substrates along with natural light supply (1500 lux in a 12-hour cycle) reaching a biomass of 4.47 g. L^{-1} after 14 days of cultivation (Soccol et al., 2008a, b). On the other hand, both documents CN109897783 and BRPI0605365A describe methods for microalgae conservation after cultivation. The former, idealized by Wang et al., claims that the water within microalgae cells is absorbed with sandstone particles. With that, cells can be preserved in low temperatures absent from sunlight up to 30 days (Wang et al., 2024). The latter document, proposed by Monteiro (2006), describes the conservation of a *Spirulina* strain under cold temperatures varying from 4 °C to 8 °C (microalgae conserved with the cultivation media) or 0–3 °C (microalgae conserved without the cultivation media) (Monteiro, 2006).

3.2 *Apparatus and Systems Developed for Microalgae Cultivation (C12M)*

There are two main classifications for systems used for microalgae cultivation: open and closed systems. Open systems are vastly applied since they are easy to construct and handle, and cheap to maintain. In these systems, microalgae are grown in static or agitated (circular or raceway) ponds, which are built with concrete or by digging the ground and adding some type of contention to avoid soaking (Yousuf, 2019). Despite being largely employed, these open systems are highly susceptible to environmental and climatic changes along seasons and are not easy to monitor and control. On the other hand, closed systems or photobioreactors (PBRs) are easy to control, do not suffer from seasonal changes, and can provide high biomass productivity and product quality. There are different sets of PBRs such as column, flat panel, tubular, membrane, and plastic bags, each one with specific characteristics

that can guarantee a certain type of cultivation (Yusoff et al., 2019; Zuccaro et al., 2019). The main limitation of PBRs is the high cost associated with construction and handling.

Gong et al. (2014) proposed in the patent document CN103756886 a PBR with a circulation unit, making the cultivation of the microalgae a continuous process which can further enhance productivity up to 30% when compared to the conventional batch process (Gong et al., 2014). In the utility model document CN216039556, Yuan (2021) claims a reaction device comprised of a bend pipe (which assimilates to a raceway PBR) placed inside an illumination box that provides light for photosynthesis by the microalgae (Yuan, 2021). In its turn, Jeong and associates (2011), in the patent document KR101222145, propose a plate-shaped PBR coupled with a light collecting unit which is able to condense the sunlight and distribute it on the PBRs plates (Jeong et al., 2011).

3.3 Cultivation Strategies and Techniques (C12P)

Microalgae can be cultivated under three main strategies. The first one is autotrophic cultivation, in which CO_2 is used as carbon source and light as energy source (through photosynthesis). This type of cultivation is applied mainly in the open cultivation systems. On the other hand, heterotrophic cultivation happens when the microalgae rely on an organic compound for both carbon and energy source. Usually, heterotrophic conditions do not require light and, therefore, pigment production is limited in this type of strategy. Lastly, mixotrophic cultivation happens when light is used as energy source and both inorganic (CO_2) and organic compounds are used as carbon source (Vuppaladadiyam et al., 2018). There is the possibility of applying more than one strategy for microalgae development in multi-step cultivation systems. The choice of strategies will depend both on the microalgae with its metabolic routes and preferences and on the desired product (e.g., autotrophic cultivation leads to a higher pigment titer, nitrogen limitation strategies can lead to a lipid accumulation) (Koller et al., 2012). In the patent document BR 1020170262693, de Morais et al. (2017) describe the co-production of microalgae biomass and metal nanoparticles by using either a Zarouk's cultivation media along with a metal solution or wastewater containing heavy metals. Using wastewater as cultivation media is a great bioremediation strategy, besides, the synthesis of nanoparticles can guarantee enhanced product characteristics. The proposed process, therefore, can present low cost and a great technology alternative for further microalgae cultivation with a co-production strategy (de Morais et al., 2017).

4 Patents Applied to Gas and Effluent Treatment (Y02 & C02)

Environmental pollution and climate and energy crises are serious consequences of the unbridled growth of human population and the uncontrolled exploitation of natural resources and human activities. These issues have been a cause for great concern in recent years, and research for mechanisms to mitigate these problems has been growing (Ukaogo et al., 2020). In this context, microalgae emerge as protagonists of processes that could mitigate these environmental impacts (Mustafa et al., 2021). These organisms can be used in the treatment of sewage and wastewater, since they have the incredible capacity to absorb several substances that are harmful to the environment and that are present in this waste, such as heavy metals and hydrocarbons (Pacheco et al., 2020). Furthermore, as they are photosynthetic organisms, they perform biological fixation of carbon dioxide (CO_2), which can be exploited to reduce greenhouse gases originated in industrial processes (Zhang & Liu, 2021).

Heavy metals are highly toxic and non-biodegradable substances that cause serious consequences to the environment, which creates the need for treatment of wastewater containing such xenobiotics (Yan et al., 2022). Currently, the main methods used to treat water contaminated with heavy metals have several disadvantages, such as high maintenance costs and waste generation that causes secondary pollution (Leong & Chang, 2020). Therefore, it is interesting to develop bioremediation methods that are more sustainable. In this sense, patent CN107473416 describes a method for removing heavy metal ions from liquid microalgae culture through an adsorbent material (Yehua & Derong, 2017). First, the liquid culture medium, after collecting the microalgae, is precipitated in a sedimentation tank and, thus, the insoluble impurities of the culture are removed. After precipitating, the liquid medium is filtered and pumped into a tank containing the adsorbent material (e.g. attapulgite clay, diatomaceous earth, perlite, zeolite, or manganese dioxide, used separately or together). Finally, the adsorbed culture liquid is filtered and identified and can then be adjusted or recycled. This method has proven effective in removing heavy metal ions from solutions and allows the recycling of the microalgae culture medium, reducing costs in water treatment and material filtration in industrial plants.

Industrial wastewater is generally rich in organic matter, and its untreated discharge contributes greatly to the eutrophication and pollution of water bodies (Akinnawo, 2023). Aiming to solve these problems, document CN102863115 describes the invention of a treatment for wastewater from industrial fermentation processes, which also enables the production of algae powder through the use of microalgae (Hailin et al., 2013). The procedure must be carried out in a bioreactor and consists of the following steps: (1) pretreatment of wastewater (dilution and pH correction to enable the growth of microalgae in the waste); (2) addition of microalgae (e.g. *Chlorella vulgaris*, *Nannochloropsis salina*, *Botyococcus braunii*, *Chaetoceros gracilis*, *Halochlorococcum marinum*) to the waste to be treated;

(3) collection of microalgae after treatment is complete (until the ideal level of nitrogen, phosphorus, and other organic components present in the wastewater is reached); and (4) preparation of algae powder. The CO_2 gas released by the industry can also be collected and used in the process, functioning as the main source of carbon for the cultivation of microalgae, and the residual heat from the industrial plant can be used to heat the photobioreactor.

Polycyclic aromatic hydrocarbons (PAHs) are another class of environmentally persistent pollutants that are highly toxic to biota and human health, and most chemical and physical methods for remediating these compounds do not completely remediate them from the environment (Patel et al., 2020). The invention described in patent CN103112993 consists of a promising methodology for treating wastewater from oil extraction, rich in PAHs, while allowing the fixation of CO_2 and the production of biodiesel, using microalgae (Jian et al., 2013). For the cultivation of the microorganisms, it is necessary that the oilfield wastewater has a crude oil content of less than 10 mg L^{-1}, the total organic carbon is less than 40 mg L^{-1}, and the chemical oxygen demand is less than 100 mg L^{-1}. The authors describe the construction of a large-scale cultivation system using five interconnected stepped processing ponds. In the first lagoons, CO_2 gas is introduced directly into the wastewater. In these first stages of the process, the non-CO_2 components of the gas react chemically and physically with some pollutants present in the wastewater that affect microalgae, which allows the preliminary purification of the sewage. The microalgae are then added to this pretreated wastewater and use the CO_2 and the pollutants for their growth, which allows the fixation of CO_2 and the degradation of the hydrocarbons present in the sewage. Microalgae species are selected according to their high stress resistance and hydrocarbon degradation capacity. After the sewage is treated, a silicon carbide microfiltration membrane is used to collect the microalgae; of these, 10–20% are used as culture starters for the next bioremediation process and 70–90% are applied in biodiesel production.

5 Patents for Foods and Feeds Based on Microalgae (A23, C07, & C11)

The application of microalgae as components of food products has also shown to be a promising field. Microalgae are natural sources of biomolecules that are important for human and animal health and can solve some of the difficulties that food industry currently faces (Chen et al., 2022).

A consumer market trend that poses a challenge to the current food industry is vegan diets. Many people have stopped consuming foods containing ingredients of animal origin, which implies the search for non-animal origin substitutes (Alcorta et al., 2021). An example is mayonnaise and salad dressings, which contain eggs as the main emulsifying agent, since the yolk is rich in proteins (He et al., 2021). Plant-based emulsifiers are used, but they have disadvantages, such as the need for

extensive areas for plant cultivation and low emulsion quality (Böcker et al., 2021). In view of this problem, the authors of patent BR 112022011367-6 describe a methodology for obtaining a food product composed of a stable oil-in-water emulsion, of the mayonnaise or salad dressing type, rich in oil (66–87% oil + <1% microalgae oil of weight), which does not contain animal, vegetable, or non-natural emulsifiers, but an emulsifier composed only of microalgae protein (0.01–2% of weight), especially from the species *Chlorella vulgaris* (but may also be from the genera *Arthrospira*, *Dunaliella*, *Tetraselmis*, *Chlamydomonas*, and *Nannochloropsis*) (Bouwens & Draaisma, 2022). The authors indicate the isolation of the protein from the microalgae cells before being added to the product, which gives it a stable texture and a smooth, homogeneous, shiny appearance, free from the typical coloration of microalgae (green or red), giving the product excellent quality.

Another trend in the consumer market is the preference for more natural products, with pharmaceutical or health-benefiting properties, such as dietary supplements (McClements, 2020). In this sense, an example of the production of a completely natural food product is in patent CN103396951, which describes a methodology that enables the production of astaxanthin by *Haematococcus pluvialis*, through its large-scale cultivation (Yong et al., 2013). Astaxanthin is a carotenoid with antioxidant, anti-inflammatory, anticancer, and immunostimulant properties and is used as a dietary supplement for humans and animals (Fakhri et al., 2018). According to the authors of the patent, the growth of *H. pluvialis* is divided into two stages: the green algae stage and the red algae stage. In the green algae stage (10 ~ 20 μm diameter), there is almost no accumulation of astaxanthin in the cells. However, when green algae encounter environments with more hostile physical and chemical conditions (e.g. high temperature, high salt content, strong light, low nutrition), the green algae transform into red algae, increasing in size (40–60 μm diameter). The red algae gradually transform into spores and astaxanthin begins to be produced and accumulates in the spores. The astaxanthin content in *H. pluvialis* reaches about 1.5–3.0% after 10–13 days of microalgae cultivation. Thus, the spores are collected, subjected to an enzymatic hydrolysis treatment to soften the cell wall and then to a wall-breaking treatment through high pressure. The broken spores are then dried (spraying or oven or freeze-drying), becoming a powder. For the granulation of the produced astaxanthin, the methodology uses ethanol, which, according to the authors of the patent, is non-toxic and does not impact the quality of the product, in addition to saving energy. After drying, the granules are subjected to supercritical carbon dioxide equipment to extract the products (90~95% of which are composed of fatty acids and 5~10% of astaxanthin).

Another example of the development of microalgae-based dietary supplements is presented in document BR 112021016556-8. This patent presents a methodology for obtaining a dietary supplement rich in omega-3 fatty acids (50–250 mg/g) and xanthophylls (10–50 mg/g), with the presence also of sterols (1–20 mg/g) and phycoprostrans (2–100 μg/g), from microalgae extracts (Pradelles & Delbrut, 2021). Studies carried out by the authors of the patent show that the use of this supplement can help prevent or reduce the onset of cognitive disorders associated with age or developed by children who suffered prenatal stress. According to the

authors of the invention, the microalgae *Tisochrysis lutea* and *Phaeodactylum tricornutum* are the most promising for this process. After cultivation in photobioreactors, an extract of the microalgae is obtained through various physical and/or chemical processes that allow the elimination of water (e.g. centrifugation, filtration, flocculation), and drying and cell lysis steps may or may not be performed. The extraction of the components of interest is performed using hyper or subcritical fluids, with or without enzymatic, microwave, pressure, and/or ultrasound treatments. The solvents used are subsequently separated from the biomass by means of centrifugation, filtration, and/or evaporation. The formulation of the supplement generally involves the incorporation of the extract in vegetable oil (olive, canola, linseed, sunflower, coconut oil, etc.), in the form of capsules or powder, which can be incorporated into a food product or used in isolation.

The use of microalgae can also be of great value in the process of nutritional enrichment of food products (Gohara-Beirigo et al., 2022). The patented invention BR 102018015732-9 is one of these methodologies. The authors developed a fresh spaghetti-type pasta enriched with *Spirulina platensis*, which provides nutritional improvement to the food, especially in relation to the amount of proteins and micronutrients (e.g. potassium, calcium, iron, magnesium, zinc) (De Melo et al., 2020). The dough is composed of unleavened wheat flour, water, soybean oil, chicken eggs, *S. platensis* powder, and salt. First, the dry ingredients are mixed for 20–60 s, followed by the addition of the liquid ingredients and emulsions. After mixing (3–7 min), the mixture must be kneaded to achieve homogeneity. Finally, the dough is molded in a manual machine to be in a spaghetti shape. Wet cooking (160–200 °C) is carried out to obtain an "al dente" consistency, since it is fresh pasta. Since the procedure is simple, the invention is easy to reproduce and is economically accessible, and the methodology can be applied at home or in industrial scale.

6 Patents on Other Groups: Biomass Recover (B01D), Fuels, Pharmaceuticals Purposes, and Applied to Agriculture (A01N)

6.1 Biomass Recovery (B01D)

In the scientific literature, microalgae have been an emerging subject for a wide variety of applications. Research and development trends include methods for more efficient recovery of biomass, production of biofuels, pharmaceuticals, and inputs to help sustainable agriculture (Liu & Hong, 2021).

The recovery of biomass (B01D) from microalgae (microalgae harvesting) represents an important basic area, as it relates to the processing and recovery of what is generated by microalgae. Currently, in both scientific literature and patent filings, the main recovery methods include gravity sedimentation, centrifugation, filtration, flotation, and flocculation (Caetano et al., 2020; Monte et al., 2018; Morais Junior

et al., 2020). What often determines which method is chosen is the cell density, growth rate, and lipid content (Occhipinti et al., 2024). Of all methods, centrifugation is the most widely used, whether on a small or large scale, due to its speed and average recovery efficiency of 98% (Morais Junior et al., 2020). The main bottlenecks encountered in this area are the low density of microalgae biomass, high energy costs, along with losses caused by shear force (Christenson & Sims, 2011).

None of the patents found by the aforementioned algorithm focus solely on providing an innovation for separating and recovering the biomass produced by microalgae. The inventions also focus on innovating in the separation and purification of bioproducts of interest that are contained in biomass, such as lipids, pigments, biogas, biopolymers, etc. In all cases, the patents seek to apply cheap, efficient, and easy-to-operate separation methodologies.

One example is patent CN114212890-A, filed by Huazhong University of Science and Technology, where a production method has been developed to improve the overall yield of microalgae and therefore reduce the production costs of the process. This method uses the magnetic separation of microalgae, which are magnetized with mineralized nanoparticles. The electrostatic affinity between the positively charged cells and the negatively charged nanoparticles is increased by modifying the cationic polymer. This generates a separation efficiency of 98% (Gong et al., 2022).

6.2 Fuel Production (C10L)

Another application with great potential impact is biofuels. Representing a sustainable and less polluting alternative to fossil fuels, microalgae have gained great prominence in the scientific literature regarding their applicability for generating biogas, biohydrogen, biodiesel, bioethanol, and biobutanol (Abomohra & Elshobary, 2019). What has made microalgae stand out for this biotechnological application is their ability to have high growth rates, photosynthetic efficiency, and cultivation media without special requirements. However, commercial viability is still a challenge, since large-scale production has not been achieved in a way that is acceptable from an economic and productive point of view for the market (Tomar et al., 2023).

Due to the existing bottlenecks, the patents found have sought methodologies that can increase the production of biofuels to a scale that can meet the high demands of society. One of the main approaches is to use microalgae to produce bio-oils that are bioconverted into biofuels. The most applied techniques for this production are the direct use of crude oils, thermal cracking, microemulsions, and transesterification (Sathya et al., 2023).

Of the patents found, the focus is on the production of biodiesel and biogas. One example is EP2283734-A1, filed by Biodiesel del Plata SA, which describes a methodology for producing oil with microalgae (*Botryococcus*) from industrial waste, which can serve as a raw material for biodiesel production. The main

distinguishing feature of the invention is the use of sewage effluents from the dairy and meat processing industries as a production medium, and the transesterification of the bio-oil produced using an acid catalyst that transforms free fatty acids and triglycerides into fatty acid methyl esters (FAME) (Piwko, 2011).

Another example is patent CN106221893-A, filed by Nanning Overseas Chinese Investment Zone Sun Trading Co Ltd., where *Chlamydomonas reinhardtii* strain is used to produce biodiesel. This invention develops a low-cost synthetic cultivation medium made from glucose, sodium nitrate, chitosan, monopotassium phosphate, vitamins, and seawater, which will be applied together with red and blue LED lights. The aim is to produce fatty acids that can be converted into biodiesel through esterification with methanol and a zeolite molecular sieve catalyst (Huang, 2016).

6.3 Pharmaceutical Applications (A61K)

Moving on to applications related to human health, microalgae are a great source for producing bioactive compounds such as antibiotics, vaccines, monoclonal antibodies, hormones, enzymes, among other options with pharmaceutical and therapeutic applications (Rizwan et al., 2018). Scientific literature has delved into how pigments from microalgae can bring health benefits, such as the prevention of cancer, heart disease, neurological disorders, and eye diseases. Due to their high growth rate, simple means of cultivation, and low production costs compared to the chemical industry, microalgae have been considered as great hosts for the application of genetic engineering to produce recombinant proteins (Khavari et al., 2021).

As an example of practical studies, *C. reinhardtii* is the most popular microalgae applied in pharmaceutical biotechnology. It can be used for the most diverse purposes, such as the production of antibodies, vaccines, erythropoietin, and viral protein 28 (VP 28) (Yan et al., 2016). The cosmetics industry with therapeutic properties should also be included in the scope of pharmaceutical applications, where strains such as *Dunaliella salina*, *Haematococcus pluvialis*, and *Scenedesmus* sp. are used in the production of pigments such as β-carotene, astaxanthin, and lutein, which are used as moisturizers, anti-aging, photoprotection, and skin-lightening products (Occhipinti et al., 2024). However, as in other areas of application, there are bottlenecks to be solved for biotechnological products commercialization. One of these shortcomings is the use of large quantities of solvents that are considered toxic to extract and purify these bioactive compounds, along with processing, which is often expensive and limits production profits (Vernès et al., 2015).

Although patents related to applications for pharmaceutical purposes (A61K) are a minority in the search results, it is worth citing examples of inventions that have been filed. Patent WO2015079169-A1, filed by Roquette Freres, sought to develop a methodology for producing protein-rich microalgae flour granules. These proteins can be incorporated into food and animal feed or for applications in the pharmaceutical and cosmetics industries. The pharmaceutical application is specifically to produce oral dispersible tablets, which disintegrate quickly in the oral cavity,

without the need for a liquid to swallow. The microalgae species used is *Chlorella sorokiniana*. The patent's innovation is related to producing biomass with a high concentration of protein (at least 50%) under heterotrophic conditions, which eliminates the need for extractions with toxic and expensive solvents. The microalgae flour is obtained using a vertical sprayer (Passe & Delannoy, 2015).

6.4 Agricultural Applications (A01N)

Finally, there is the application of microalgae for agricultural production. With the problems of climate change, erosion and loss of soil fertility, limited water resources, and an increase in the world's population, the need to increase agricultural production in a sustainable and environmentally friendly way is evident (Chiaiese et al., 2018). Both scientific research and companies in the agricultural sector have sought to develop technologies that can increase food production and combat waste generation through a circular economy system (Pereira et al., 2021).

Based on this, microalgae have emerged as an alternative to help developing inputs and technologies such as growth promoters, biofertilizers, biopesticides, weed control, etc. Several studies have been carried out to evaluate and discover the efficiency of the production of biomass, phytohormones, polysaccharides, antimicrobial compounds, and metabolites of interest from microalgae under different agro-industrial wastes (Murata et al., 2021).

Most of the studies have been carried out in bioreactors, photobioreactors, and open ponds. However, although it is known that microalgae can produce various macromolecules that can be used in a beneficial way for agriculture, their applications are still at an early stage. There are some limitations that hinder their widespread use in agricultural environments (Gonçalves et al., 2023). What still represents a strong limiting factor in research are the high costs related to biomass production, extraction of metabolites, and productivity being considered relatively low (Gonçalves, 2021). Studies have shown that the costs of recovering and purifying biomass along with extraction can amount to approximately 20–30% of total production costs (Barros et al., 2015; Ruiz et al., 2022; Uduman et al., 2010).

Many patents filed seek to create new methodologies to produce metabolites of interest to agriculture, but they do not seek to solve bottlenecks such as high energy, processing, recovery, and purification costs, as well as low yields in the production of biomass or the metabolite of interest (Murata et al., 2021).

Thus, it is worth mentioning as an example patent BR102021012230-B1, filed by Allga Produção De Biomassa De Microalgas e Suas Aplicações Ltda, whose objective was to create a process for obtaining biofertilizer/bio-stimulant from microalgae for use in agriculture. The invention uses a consortium of microorganisms such as *Chlorella vulgaris*, *Scenedesmus dimorphus*, and *Arthrospira platensis*. The methodology uses a synthetic growing medium with controlled temperature and lighting. The patent states that the production system can be made with sterile plastic bag-in-box bags of 1 to 1000 liters in capacity. When reading the document, it is not

possible to know which biofertilizing substance is produced, or what the production yield was in the methodology. Only tests of the bioproduct were carried out on wheat, sugarcane, soya, and corn plantations, which proved to be very promising (Da Silva & Prete, 2023).

Another patent worth mentioning is DE102009030121-A1, filed by Rent a Scientist GmbH. This patent is not directly related to agricultural applications, but it could be a solution to production bottlenecks, since it aims to innovate by providing an additive capable of improving the biomass production of photosynthesizing microorganisms. According to the document, this nanoparticle additive is applicable to a large number of microalgae and algae belonging to the Chlorophyta, Cyanobacteria, Rhodophyta, and Phaeophyceae phyla. The production methodology is said to be applicable both in closed photobioreactors and in large-scale open ponds. The additive consists of nanoparticles of silver, gold, copper, and zinc metals. The patent claims to be able to increase biomass production economically and that this can be applied in a wide variety of areas, including agriculture. Little detail is given on how much biomass production is increased, but it is known that the silver nanoparticles were the ones that obtained the best results, increasing growth and biomass production by 400% (Maier & Schneider, 2010).

7 Final Considerations

Microalgae represent a very rich and diverse subject both in scientific literature and in patent filings. However, the number of studies and discoveries that actually turn into something applicable to be patented is very low, especially in the subjects and sectors discussed in this topic. There are many bottlenecks and gaps to be resolved, and both research and the market are still in the early stages of implementing the production of biofuels, biopharmaceuticals, agricultural inputs, and production and purification methodologies that are economically viable and meet the high demands. All this indicates that there is still room for development and improvement in the use of microalgae.

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Wastewater Treatment in Microalgae Cultivation Systems: Recommendations for Large-Scale Application



Sarah Lacerda Farias, Graziele Ruas, Marc Arpad Boncz, Fábio Rodrigo de Oliveira, Carlos Alexandre Luttemberk, Ênio Leandro Machado, and Fernando Jorge Corrêa Magalhães Filho

Abstract The cultivation of microalgae, prized for their versatile applications, from biofuel to pharmaceuticals, involves selecting cultivation systems like open ponds or closed photobioreactors, considering factors such as application, space, and scale, beyond the potential of combined microalgae with organic coagulants and constructed wetlands (CW). This chapter explores (i) the cultivation of microalgae using (pretreated) domestic effluent as the culture medium in High-Rate Algal Pond (HRAP) reactors and (ii) the use of flocculant (*Acacia decurrens*) associated with CW (French vertical flow) to retain microalgae biomass. Optimization of environmental and operational parameters is crucial for increasing the technology readiness level (TRL) of microalgae-based systems. Pilot systems tested variations in feeding regime, hydraulic retention time (HRT), light intensity, and CO_2 addition. Under the conditions tested in a tropical climate with real primary and secondary wastewater, the main suggestions for process scalability are: operation in semi-continuous mode, HRT of 5–7 days, cultivation without shading, and addition of as much CO_2 as possible, always aiming for the highest biomass productivity while optimizing the removal of pathogens and other emerging pollutants. For the microalgae/organic coagulants/CW system, operational parameters are presented (organic/hydraulic loading, HRT, and conditions for microalgae inoculation), as well as process

S. L. Farias · M. A. Boncz
Federal University of Mato Grosso do Sul (UFMS), Faculty of Engineering, Architecture and Urbanism and Geography (FAENG), Post-graduate Programme of Environmental Technology (PGTA), Campo Grande, MS, Brazil

G. Ruas
São Paulo State University (UNESP), School of Engineering, Bauru, SP, Brazil

F. R. de Oliveira · C. A. Luttemberk · Ê. L. Machado
Postgraduate Program in Environmental Technology, University of Santa Cruz do Sul (UNISC), Santa Cruz do Sul, RS, Brazil

F. J. C. M. Filho (✉)
Federal University of Rio Grande do Sul (UFRGS), Institute of Hydraulic Research (IPH), Porto Alegre, RS, Brazil
e-mail: fernando.magalhaes@ufrgs.br

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performance control to comply with environmental legislation. The system presents significant efficiency in reducing pollutant load and mitigating eutrophication with less need for treatment area, as well as the potential for nutrient recovery for agriculture. The conditions tested guide the applicability of using this technology on a large scale, positioning it as an important tool for bioeconomy advancement.

Keywords Microalgae-bacteria system · Organic coagulants · Constructed wetlands · Wastewater treatment · Circular economy

1 A Short History of Microalgae-Based Wastewater Treatment

The use of microalgae for wastewater treatment goes back several decades; shortly after the first oil crisis in 1973, and based on earlier ideas of Oswald (Oswald & Golueke, 1960), the National Renewable Energy Laboratory (NREL) started the "Aquatic Species" research project aimed at identifying biological processes capable of producing large amounts of vegetable oils, to be used as an alternative biofuel. One of the conclusions was that microalgae cultivation could provide a technically viable alternative source for transportation fuels, but in order to keep costs low, domestic wastewater, rather than fertilizers, should be used to supply the nutrients necessary for the biomass to grow.

Experiments with High Rate Algal Ponds were initiated in California and Hawaii, and later on in Roswell-NM, showing very promising results (Sheehan et al., 1998). In the following decades, in which very low petroleum prices inhibited economic viability of alternatives, these experiences were almost forgotten, but since the beginning of the 2000s, as a result of increasing oil prices, but also because of the increasing urgency to limit global warming, the cultivation of oil-rich microalgae using wastewater became a hot topic again (Chisti, 2007; Wijffels & Barbosa, 2010). Since those early days, a lot has been done, and because of cost concerns, currently the focus of research on microalgae cultivation in wastewater has shifted from optimizing production of triglycerides and biodiesel to actually treating the wastewater, and the production of interesting products from these algae, which can be much more than only algae oil, has turned into a secondary, albeit very interesting, objective (de Leite & Daniel, 2020).

Following up on the results of the Aquatic Species program, the approach is often to run the algae-based processes close to a facility that produces CO_2, like a fermentor, an anaerobic digester, or even a gas-fired electric power plant: additional carbon is necessary for algal biomass production, as treated sewage has a carbon deficit, when considering its C, N, and P contents and comparing these with the Redfield ratio (C:N:P = 106:16:1), a typical ratio for algal biomass composition often used as a starting point for studies and calculations (Rogers et al., 2014; Dammak et al., 2023). Such CO_2-supply is not essential though; in the absence of a supply of CO_2, the algae will use atmospheric CO_2 introduced by the mixing of the HRAP with a paddlewheel.

The growing importance of the use of microalgae in wastewater treatment can be explained when looking at various factors. In the first place the increasing necessity of nutrient removal. Biological wastewater treatment processes are designed to remove organic matter, in order to avoid receiving waters to become anaerobic and devoid of life. But activated sludge processes, and especially anaerobic digestion, generally do not remove significant amounts of nutrients (Chernicharo, 2007). Nutrient removal is becoming increasingly necessary, not only to avoid eutrophication of receiving water bodies, but even more so to move on to a circular economy, in which materials are not wasted (creating problems elsewhere), but recovered and reused (Manthos et al., 2022). In contrast to the bacteria normally used in wastewater treatment, microalgae need relatively large amounts of nutrients, which will accumulate in the produced biomass, and, when separating the algae, can be recovered, permitting reuse when using algae or derived products as fertilizers (Calicioglu & Demirer, 2015; Yang et al., 2022). Also, microalgae are capable of absorbing heavy metals, and thus much higher removal efficiencies of heavy metals can be achieved then when only using bacterial processes (Muñoz & Guieysse, 2006). And in the third place, microalgae are capable of limiting the amount of pathogens present in the effluent, for various reasons, like the variations in pH and oxygen level they induce, but also because of the release of some antibacterial compounds (Muñoz & Guieysse, 2006).

Actually, there are three main lines of research on the use of microalgae in wastewater treatment, which will be discussed in separate sections below. In the first line of work, an algae-based process is used for treatment of a secondary effluent, often domestic sewage that has been treated in a upflow anaerobic sludge blanket (UASB) reactor (anaerobic digester), but this may also be the secondary effluent of an activated sludge process. This way, the emphasis is on polishing the secondary effluent: removing nutrients, pathogens, and emerging contaminants, and the biogas produced in the UASB reactor can be upgraded by the algae-based process, which benefits from the CO_2 present in the biogas (del Rodero et al., 2019; Scarcelli et al., 2021).

In a second, more recent line of work, an algae reactor, often a HRAP-type reactor, is fed with primary sewage, and the mixed culture present in the reactor contains a significant amount of activated sludge, while algae growth may be limited by the reduced light penetration in the medium. In this case, the emphasis is on removing organic matter, nutrients, and pathogens (Park et al., 2011; Ruas et al., 2020; Farias et al., 2023). Of course, depending on the situation, the general composition of the algae cultivation medium (the [pretreated] wastewater) will vary greatly, as shown in Table 1. As sewage characteristics depend heavily on the economic and cultural situation, but also on, for instance, water availability and the use of kitchen grinders, the actual characteristics of a local domestic wastewater may be outside the ranges presented here (Metcalf & Eddy et al., 2014).

In a third and last line of research, microalgae systems are integrated with phytoremediation, particularly constructed wetlands—or treatment wetlands (Silveira et al., 2020). In this case, removal of the bulk of the pollutants occurs in

Table 1 Characteristics of primary and secondary domestic sewage—upflow anaerobic sludge blanket (UASB) and activated sludge reactors (Von Sperling, 2007; Metcalf &Eddy et al., 2014; Dammak et al., 2023)

Parameter	Primary treatment	Secondary treatment	
		Effluent UASB	Effluent Act. sludge
TSS (mg L^{-1})	120–400	60–100	10–30
COD (mg L^{-1})	250–800	200–280	50–100
TOC (mg L^{-1})	80–260	100–130	20–40
Ammonia (mg L^{-1})	12–45	>15	1–10
TKN (mg L^{-1})	30–70	40–50	10–20
TN (mg L^{-1})	20–70	>20	15–35
TP (mg L^{-1})	4–11	8–12	1–5

Abbreviations: *TSS* Total Suspended Solids, *COD* Chemical Oxygen Demand, *TOC* Total Organic Carbon, *TKN* Total Kjeldahl Nitrogen, *TN* Total Nitrogen, *TP* Total Phosphorus

the microalgae-based system, and the constructed wetland serves to improve the quality of the final effluent.

2 Leading Aspects of Algae-Based Wastewater Treatment Processes

2.1 Types of Photobioreactors

As stated in the introduction, microalgae can be cultivated in two fundamentally different types of systems: open and closed photobioreactors (Barboza-Rodríguez et al., 2024), but nowadays there are also hybrid systems (Li et al., 2019). The choice of the best reactor design for a specific situation is made on the basis of a cost-benefit analysis, taking into consideration the desired efficiency and treatment requirements.

Open photobioreactors are the most common design used for domestic wastewater treatment, because they are simple to install, operate, and maintain, and provide an efficient removal of organic matter and nutrients (Muñoz et al., 2006). Among the open photobioreactor designs available, the High Rate Algal Pond (HRAP) stands out for operating in such a way that autotrophic and heterotrophic metabolisms coexist and support each other. The HRAP, originally developed by Oswald in the 1950s (Oswald & Gotaas, 1957; Oswald & Golueke, 1960; Nurdogan & Oswald, 1995; Sutherland & Ralph, 2020) is an obvious choice because of its simplicity and low operational costs (Barboza-Rodríguez et al., 2024). This system comprises shallow and open ponds to ensure light penetration, and the use of paddlewheels to facilitate thorough mixing of the culture prevent sedimentation of microalgae/bacteria flocs and enhance nutrient diffusion across the cellular boundary layer, without damaging the cells through excess shear forces (Sutherland & Ralph, 2020). Key features of these reactors include high efficiency, operational simplicity,

scalability, and an energy consumption below 10 W/m^3, while there are already patented models with an even lower energy requirement of less than 2 W/m^3, significantly outperforming other reactor technologies. Given the need for a low depth and a high hydraulic retention time to permit sufficient biomass growth and pollutant removal, the large land requirement for implementation is the most significant restriction for this technology (Torres-Franco et al., 2021). However, it is crucial to note that while conventional technologies rely on mechanical and electrical equipment that depreciates over time, the main investment for HRAPs is the acquisition of land, of which the value may actually increase in the long term, while reducing capital costs (Alcántara et al., 2015b).

In contrast, the closed photobioreactors, which eliminate the risk of contamination, are more commonly used for microalgae cultivation when the biomass has a higher valued destination, such as the production of nutraceuticals (supplements, antioxidants, etc., for human or animal nutrition), dyes, phytochemicals (polyphenols, polysaccharides), medicines, and biochars (da Rosa et al., 2023), as the high costs of building, operating, and maintaining closed reactors are only justified for biomass production systems when the sale of the product and its added value will support the viability of the system's operation. However, in these cases often more severe restrictions concerning the quality and cleanliness of the cultivation medium exist (Lorenz & Cysewski, 2000; Severo et al., 2024). As a result of higher costs and the lack of need to avoid contamination, closed photobioreactors are less relevant for wastewater treatment processes. Although the results in removing pollutants are significant given the considerably efficient performance (Scherer et al., 2017).

2.2 Microalgae Species

Many different species of microalgae can be used in photobioreactors, and some species have special characteristics and capabilities for producing certain specific products of interest. However, in the case of wastewater treatment, the cultivation medium is never sterile, and an invasion by another species is always possible. A complete change of the dominant biomass species can occur within a week (Sutherland et al., 2017). Thus, relatively little importance should be paid to the selection of specific microorganisms, as over time natural selection will inevitably cause the biomass composition to evolve to a certain, case-specific optimal composition anyway (Bohutskyi et al., 2015), depending on the type of effluent, cultivation system, and applied conditions (Abdelaziz et al., 2014). What has been gaining momentum is prospecting mixed cultures in natural environments and effluents to arrive at a favorable population more quickly (Pompei et al., 2023, 2024).

The use of native and bioprospected microalgae in the effluents to be treated has been a successful alternative for improving treatment, as it reduces the time needed to adapt the culture and shows better removal of target pollutants (Wilkie et al., 2011; Krustok et al., 2015). The more diverse the community of microalgae present, the more resilient and efficient the system, especially regarding the removal or

inactivation of pathogens (Krustok et al., 2015), since we know that different species of microalgae affect the pathogens in domestic effluents differently (Ruas et al., 2023). In most cases we will see the presence and/or dominance of *Chlorella* spp. and *Scenedesmus* spp. (Msanne et al., 2020; Silambarasan et al., 2023; Al-Hammadi & Güngörmüşler, 2024). The Solids Residence Time (SRT) in the reactor is a critical process parameter, as longer SRTs create less diverse biomass, while shorter SRTs increase selective pressure on the microbial community structure and force a higher biomass diversity, thus affecting stability, efficiency, and reliability of the process as a whole (Bradley et al., 2019).

2.3 Biomass and By-products

Even though microalgae can be used for the production of many different types of compounds, often with a higher aggregated value (Suganya et al., 2016; Hassan et al., 2022; da Rosa et al., 2023), when primary and secondary domestic effluents are used as a growing medium, the recommended applications are production of biofuels, bioplastics, biopolymers, and biochars. Even though these are less valuable products, their production may still contribute to a circular economy and to a more cost-effective operation of the Wastewater Treatment Plant (WWTP). Biomass productivity in HRAPs fed with anaerobically digested effluent can be around 0.30 $g.L^{-1}.d^{-1}$, at a final dry weight concentration of $\approx$ 2.2 $g.L^{-1}$ (Ziganshina et al., 2022). For a city of 100,000 inhabitants producing 100 L of sewage per capita per day, treated in a HRAP with a HRT of 5 days this would come down to 15 tons of biomass per day. This biomass can then be further processed, depending on its composition; a detailed example of biomass composition as a result of different types of feed is given by Villaró-Cos et al. (2024). In general, processes should be prioritized in which the biomass will be processed chemically and/or thermally, in order to minimize the risk of product contamination by pathogens, heavy metals, and inorganic pollutants (Markou et al., 2018). Another prominent use of the produced biomass is in its anaerobic digestion for biogas production. Several studies already combine effluent treatment, algal biomass production, and biogas production and purification (Oswald et al., 1956; Olguín, 2012; Scarcelli et al., 2021; Ruas et al., 2022b). Given the importance of natural gas in the world's energy matrix, and the possibility to upgrade biogas to compatibility with natural gas (Muñoz et al., 2015), this will remain an important ongoing research topic in the foreseeable future.

2.4 Microalgae-Based Systems as Nature-Based Solutions

The concept of nature-based solutions (NbS) appeared in scientific literature in the 2000s and is used to define the sustainable use of nature, or systems mimicking nature, to solve society's problems and challenges (IUCN, 2013; Eggermont et al.,

2015). NbS are different from ecosystem-based approaches in that they must follow three principles: (i) application alone or integrated with other solutions; (ii) application on a landscape scale; (iii) integrating a management policy with the participation of all stakeholders, with the aim of solving a problem/challenge (Cohen-Shacham et al., 2019). The famous HRAP system, detailed in the previous section, as well as other types of microalgae-based systems (Faraloni et al., 2023; Malyan et al., 2024), has already been identified as an NbS (Vassalle et al., 2023).

The use of microalgae-based NbS for effluent treatment is still growing, and the establishment of guidelines and regulations is essential to expand their use. A great example can be seen in South Australia, where the use and application of HRAP systems is already regulated, and where guidelines are drafted to ensure that the characteristics and efficiency of the system are complied with (LGA, 2020). The publication of government documents, standards, and guidelines strengthens the use of NbS with microalgae, since this integration of technology, management, and users is imperative for any ecological solution to be considered a NbS (Cohen-Shacham et al., 2019).

3 Algae-Based Processes for Secondary Wastewater Treatment

Microalgae-bacteria systems, and especially the High Rate Algae Ponds, have traditionally been developed as an option for the tertiary treatment of wastewater, removing remaining nutrients and other pollutants and promoting disinfection. However, it is also possible, and increasingly common, to use HRAPs immediately after primary sedimentation, acting as a secondary treatment of an effluent that contains both soluble organic and inorganic compounds, thus enabling the removal of both nutrients and organic carbon in one step (Acién et al., 2016; Posadas et al., 2017a). Utilizing primary treated wastewater for microalgae cultivation presents benefits such as a higher microalgal biomass concentration and a more efficient nutrient recovery. This approach can yield biomass concentrations of up to 0.65 g L^{-1} and enable the production of up to 1 kg of microalgae biomass per m^3 of processed wastewater (Acién Fernández et al., 2018). Considering that this matrix only goes through the physical process to remove coarse materials, with substantial removal of settleable solids of ≈ 70%, and a slight reduction of organic matter of ~40% COD, there is no effective change in the chemical composition of the effluent (Posadas et al., 2017c).

The proportion of macronutrients carbon, nitrogen, and phosphorus in the effluent stands out as one of the main factors that influence biomass productivity. The C/N ratio impacts the equilibrium among heterotrophic, nitrifying bacteria, and microalgae. A higher ratio tends to promote the predominance of heterotrophic bacteria and a higher lipid content in the biomass, whereas a lower ratio supports nutrient treatment and recovery (Zhu et al., 2019; Torres-Franco et al., 2021). The

composition of primary treated wastewater can be closer to the ideal proportion of traditional cultivation mediums for microalgae production. Nevertheless, carbon limitation is a common problem when using sewage as a cultivation medium, which can be solved with supplementation of inorganic carbon. If no source of CO_2 or carbonates can be found, more intense mixing with a paddlewheel permits increased CO_2 absorption from the air.

The N/P ratio affects the elemental composition of the biomass, leading to varying degrees of accumulation of these nutrients, and influencing the biomass growth (Gonçalves et al., 2016). Wang et al. (2010) noted that, when assessing the growth of the green alga *Chlorella* sp. at four distinct points in a wastewater treatment plant's treatment process flow, the most favorable proportions of inorganic N/P were found at the points before and after primary sedimentation, close to the ideal range of 6.8–10.

Among the microalgae reported in the literature, the most representative genera present in primary sewage are *Chlorella* sp. and *Scenedesmus* sp., whose growth is also favorable in secondary and tertiary effluents (de Cassia Soares Brandão et al., 2023). The application of *Chlorella* in treatment of primary effluent stands out for its ability to recover nutrients combined with the production of biomass, and presents great potential for replacing treatment by means of an activated sludge process, for example (Wang et al., 2010). Likewise, *Scenedesmus*, commonly grouped in colonies, is notable for nitrogen and phosphorus removal efficiencies ranging from 30% to 100%, and for its resilience in adverse environmental conditions (Cai et al., 2013; Sánchez Zurano et al., 2020).

The microalgae-bacteria system employed in HRAPs fosters the removal of nutrients and pollutants through a complex, synergistic interaction between these microorganisms. In order to achieve maximum removal efficiencies of both organic matter and nutrients, it is crucial to understand the removal mechanisms, investigating in detail the effect of environmental and operational parameters such as pH, temperature, light intensities, hydraulic retention time (HRT), and CO_2 supplementation. Pilot studies, conducted under real-world environmental conditions, prove more effective than controlled laboratory experiments, offering valuable insights that can guide large-scale system applications (Forruque et al., 2022). In this section, we will explore studies of parameters applied to HRAPs fed with primary domestic sewage, which are summarized in Table 2. These investigations sought to improve reactor performance, with greater nutrient recovery and biomass productivity, guiding the adoption of full-scale technology.

Hydraulic Retention Time (HRT) is an important operational parameter that affects the proportion and composition of biomass and determines the nutrient loading rate. Controlling this parameter makes it possible to overcome system limitations such as long-term operating stability and biomass production limitations (Rani et al., 2021). It is also an important parameter in relation to costs, as the final energy consumed per cubic meter of treated wastewater is a function of the specific energy consumption in the reactor and the hydraulic retention time of the system (Acién et al., 2016). The determination of the HRT must consider characteristics of wastewater and climatic factors, considering that solar irradiance impacts light

Table 2 Reported operational parameters with percentage nutrient removal and biomass production in primary domestic wastewater treatment used as the influent in High Rate Algal Pond studies on a pilot scale

	Operational parameters						Results				
									Removal efficiency (%)		
Reference	HRT (d)	Temp (° C)	Water depth (m)	CO_2 addition (L min^{-1})	Feeding regime	Shading	Representative species	Biomass productivity (g m^{-2} d^{-1})	TN	TP	COD
García et al. (2000)[a]	4	21.7	0.3	–	Continuous	–	*D. pulchellum*, *Chlorella* sp., *M. pusillum*, *S. armatus*, *S. acutus*	N/R	57	N/R	N/R
	10	11.8							73		
Craggs et al. (2012)[a]	N/R	15.4 ± 3	0.35	Unreported flow	N/R	–	*Micractinium* sp., *Desmodesmus* sp.	11.5	64–67% (only NH_4-N)	14–24% (DRP)	82–91% ($fBOD_5$)
Sutherland et al. (2014a)[a]	4	19.4 ± 4	0.3	–	Continuous	–	*Mucidosphaerium pulchellum*	13.8 ± 2.6	75 ± 21 (NH_4-N)	58 ± 29 (DRP)	N/R
Sutherland et al. (2014b)[a]	4	16 ± 2.4	0.2	1.6	Continuous	–	*M. pulchellum*	150 ± 36	70.3 ± 6.5	23.4 ± 18	N/R
		16.1 ± 2	0.4					201 ± 61 (Chl-α mg m^{-2} d^{-1})	68.5 ± 5.9 (NH_4-N)	19.8 ± 16 (DRP)	
Kim et al. (2014)[a]	2	25.8	0.3	–	Semi-continuous	–	*Chlorella* sp., *Scenedesmus* sp., *Stigeoclonium* sp.	0.5 ± 0.03 (g L^{-1} d^{-1})	92.68	82.65	85.44
Matamoros et al. (2015)[a]	4	25 ± 1	0.3	–	Continuous	–	*Chlorella* sp., *Stigeoclonium* sp., *Monoraphidium* sp., *Stigeoclonium* sp.	316 ± 50	99	N/R	75
	8							346 ± 38	99		84

(continued)

Table 2 (continued)

Reference	Operational parameters						Results				
									Removal efficiency (%)		
	HRT (d)	Temp (° C)	Water depth (m)	CO_2 addition (L min^{-1})	Feeding regime	Shading	Representative species	Biomass productivity (g m^{-2} d^{-1})	TN	TP	COD
Posadas et al. (2015)[a]	2.7 ± 0.1	23 ± 1	0.3	20	Continuous	–	*Scenedesmus* sp.	17 ± 1	73 ± 1	3 ± 0	88 ± 0
Ruas et al. (2018)[a]	5	35 ± 4.5	0.15	0.005 of 30% CO_2	Continuous	–	*Chlorella vulgaris*, *Microspora* sp.	4.1 ± 1.3	27 ± 11	8 ± 1	88 ± 9
Arashiro et al. (2019)[a]	4.5	N/R (cold/ warm seasons)	0.3	–	Continuous	–	*Chlorella* sp.	15 ± 6	49 ± 17	37 ± 52 PO_4^{3-}	62 ± 22
Serejo et al. (2020), Farias et al. (2023)[a]	7	27.4 ± 2	0.16	–	Continuous	–	*Scenedesmus* sp.	1.5	65 ± 8	65 ± 8	74 ± 8
		27.3 ± 2			Semi-continuous: 12 h d^{-1}			3.6	74 ± 6	86 ± 1	73 ± 8
					0.1 h d^{-1}			3.3	76 ± 8	80 ± 7	70 ± 13
Ruas et al. (2020)	5	21.9 ± 3	0.16	0.025 ± 0.4	Continuous	–	*Scenedesmus* sp.	3.2 ± 0.2	78 ± 4	58 ± 9	41 ± 5
	5	22.3						2.0 ± 0.2	80 ± 6	58 ± 5	34 ± 4
	7	22.3 ± 3						2.6 ± 0.2	90 ± 1	87 ± 7	67 ± 6
Pham et al. (2021)[a]	4	18.2 ± 3	0.3	–	Continuous	–	*Chlorella* sp., *Ulothrix* sp., *Scenedesmus* sp.	4.1 ± 0.2	77 ± 11.4	47.2 ± 25	57.7 ± 189
Ruas et al. (2022a)	5	21.9 ± 3	0.16	0.025 ± 0.4	24 h d^{-1}	50%	*Scenedesmus acutus*	3.94 ± 1.00	73 ± 3	29 ± 7	63 ± 8
		21.6 ± 4		–	24 h d^{-1}			2.83 ± 0.51	67 ± 2	26 ± 12	66 ± 13

		21.7 ± 3		–	Semi-continuous: 0.1 h d^{-1}		*Scenedesmus obliquus*	5.93 ± 2.0	60 ± 18	35 ± 14	59 ± 16

Abbreviations: *HRT* Hydraulic Retention Time, *Temp* Temperature, *TN* Total Nitrogen, *TP* Total Phosphorus, *COD* Chemical Oxygen Demand, *N/R* Not Reported

[a]Data from the best conditions tested in relation to biomass productivity, considering the different stages developed

penetration and temperature affecting respiration and decomposition (Posadas et al., 2017a). A shorter HRT can be applied at higher temperatures, intensifying productivity; however, greater dilution can impact the quality of the final effluent (Sutherland et al., 2014b; Sutherland & Ralph, 2021). Long HRTs, on the other hand, are more suitable in winter, considering that microalgae growth is slower during this period.

García et al. (2000) observed that for nitrogen removal from primary effluent with low organic matter, an HRT of 4 days is adequate in the spring and summer seasons, while for autumn/winter the HRT of 10 days was more appropriate. Ruas et al. (2020) obtained similar results of biomass gain with the application of HRT of 5 and 7 days, but the higher HRT improved the removals of COD, TOC, TN, and TP.

Temperature and light are critical environmental factors influencing microalgae growth. Given the diverse environmental conditions in open cultivation systems, microalgae strains must exhibit tolerance to variables like temperature fluctuations and changes in illumination. Temperature plays an essential role in the metabolic development of microalgae, as well as influencing the composition of biomass, cell physiology, and can affect the solubility of CO_2 in the cultivation medium (Dammak et al., 2023). The optimal growth temperatures for most microalgae species, including the genera *Chlorella* sp. and *Scenedesmus* sp., commonly found in primary wastewater, typically range from 15 °C to 35 °C (Posadas et al., 2017a).

While higher temperatures are advantageous for microalgae production, it's crucial to assess whether they surpass the ideal range, as excessive heat can have more detrimental effects than low temperatures, diminishing both photosynthesis and respiration (Ras et al., 2013). Echenique-Subiabre et al. (2023), comparing predicted biomass productivity based on local climatic conditions, revealed significant variability across sites, not solely explicable by these conditions. It emphasizes the need to differentiate and quantify the influence of local climatic conditions versus experimental conditions on productivity to increase confidence in scaling up experimental results.

Linked directly to light, the ratio of illuminated surface to volume (depth) is an important parameter affecting both volumetric productivity and pollutant removal rates (Muñoz & Guieysse, 2006). The depths applied in cultivation with primary sewage reported in the literature vary from 0.1 to 0.4 m (Table 2). Correlated with HRT, shallower depths may enhance productivity; however, microalgae become more vulnerable to temperature fluctuations and the risk of overheating (Béchet et al., 2016). Sutherland et al. (2014b), when comparing HRAPs with depths of 0.2 and 0.4 m, noted that doubling the depth resulted in up to a 200% increase in productivity, in which despite the reduction in the euphotic zone, microalgae proved to be more photosynthetically efficient and suffered less carbon limitation during certain parts of the day.

Low light intensity restricts the metabolic energy of algae, whereas high intensity induces photoinhibition or photooxidation, which occurs when the cellular capacity of algae to utilize energy is exceeded (Assemany et al., 2014). In this context, there are studies reported in the literature that evaluated the effect of light intensity on productivity, using shading screens. Ruas et al. (2022a) conducted an experiment in

a tropical climate using primary wastewater as a cultivation medium, with a 50% shade screen. The productivity results closely resembled values obtained in HRAPs operated with 5–7 days of HRT and light intensities ranging between ~156 μmol m^{-2} s^{-1} and ~725 μmol m^{-2} s^{-1}. Consequently, it was observed that under these conditions, light intensity was not a limiting factor for microalgae growth.

The external addition of CO_2 is an operational arrangement that makes it possible to overcome the common carbon limitation in sewage due to unbalanced nutrient ratios (Torres-Franco et al., 2021). CO_2 addition also contributes to reducing pH, thus mitigating the environmental impact of ammonia volatilization and preventing phosphorus precipitation, which typically occurs at pH levels above 9 (Sutherland & Ralph, 2020). Life Cycle Assessment (LCA) studies identify that one of the potential highest investment cost points in the cultivation of microalgae in sewage is CO_2 supplementation, which demonstrates that it is a gap that is still under constant investigation for better economic viability aimed at applications on a large scale (Magalhães et al., 2021). A cost-effective and environmentally sustainable solution for CO_2 supplementation involves utilizing biogas produced during anaerobic wastewater treatment. This process requires scrubbing to eliminate H_2S and harness the CO_2, presenting the algal-bacterial process as a promising option for upgrading this biogas (Chaudhary et al., 2017).

CO_2 supplementation in primary wastewater in HRAPs, reported in the literature, proves the efficiency in the growth of microalgae, but depending on the flow rate applied and other factors, it may not significantly influence the removal of nutrients and other pollutants. Ruas et al. (2022a) observed that the addition of CO_2 did not impact the removal of pathogens, but improved the productivity and sedimentability of the microalgal biomass. Similar findings were observed in a study of Sutherland et al. (2015), where the addition of CO_2 enhanced the photophysiology of microalgae, resulting in increased microalgal biomass.

An additional critical factor that can enhance productivity and lower costs is the feeding regimen. This cultivation method has the potential to surpass the efficiency constraints of batch regimes and overcome operational control and cost limitations of continuous systems, making it a highly suitable option for large-scale cultivation (Yadav et al., 2020). The comparative performance of removal of pollutants such as surfactants, heavy metals, and pathogens and microalgae growth of semi-continuous and continuous regimes in HRAPs treating primary sewage was explored (Serejo et al., 2020; Ruas et al., 2022a; Farias et al., 2023). Ruas et al. (2022a, b) observed that the semi-continuous feed regime improved the biomass productivity and TSS concentration while reducing *Escherichia coli* removal. Serejo et al. (2020) also considered the condition with continuous feeding at 0.1 h d^{-1} to be more advantageous, with respect to both the microalgal biomass productivity and the nutrient removal efficiencies. However, under these conditions, the removal of *E. coli* was lower, likely attributed to the high dilution over a short timeframe, which might have compromised the treatment efficiency (Farias et al., 2023).

4 Combining Anaerobic Digestion and Microalgae-Based Processes for Tertiary Wastewater Treatment

When combining microalgae with activated sludge processes, probably a secondary treatment process is more efficient (*vide ante*), but microalgae processes can also be very well combined with anaerobic digestion (AD). Anaerobic digestion is a biological process without oxygen, resulting in the production of biogas (~60% methane, ~35% carbon dioxide, ~5% nitrogen, and trace gasses, like NH_3, H_2S, and VOCs), a source of renewable energy, and a digestate rich in nutrients (Guan et al., 2024). The use of anaerobic processes to treat domestic wastewater is more attractive in tropical and subtropical regions, due to the necessity of temperatures above 20 °C for the microbial metabolism (Chernicharo, 2007), and also because at lower temperatures increased methane solubility will cause significant loss of methane, a potent greenhouse gas, to the environment with the treated effluent (Stazi & Tomei, 2021). In spite of advantages like easy operation, and low installation and operating costs, there are also drawbacks, as AD requires post-treatment, since removal of organic carbon is limited to around 80%, and AD does not remove nutrients (nitrogen and phosphorus) and pathogens (Bressani-Ribeiro et al., 2019; Guan et al., 2024). In addition, when not used in situ, the biogas produced needs to be purified ("upgraded") in order to meet standards for distribution and use (Vasan et al., 2024), removing especially the large amount of CO_2 present, as well as the traces of H_2S, which is corrosive.

The use of microalgae-based processes to treat secondary domestic wastewater (digestates) is a good option for recovering macro- (nitrogen (N), phosphorus (P), and potassium (K)) and micronutrients (sulfur (S), magnesium (Mg), cobalt (Co), iron (Fe), molybdenum (Mo), and nickel (Ni)) and thus promoting the circular economy (Chojnacka & Chojnacki, 2024; Guan et al., 2024). Depending on the predominant species of microalgae produced, these can be rich in proteins, sugars, and/or fats (Villaró-Cos et al., 2024), and thus can be used as a source of animal feed, or as a feedstock for fuels, chemicals, cosmetics, and/or fertilizer production (Stiles et al., 2018). In addition, microalgae-based systems can be used for biogas upgrading, as their growth is often carbon limited, and biogas upgrading requires exactly removing the excess of CO_2 present in it (*vide ante)* (Serejo et al., 2015; Posadas et al., 2017b). Biogas upgrading was seen to be more efficient when cultivating microalgae in secondary wastewater than in primary wastewater, as secondary wastewater has a lower C/N ratio than primary wastewater and thus a higher demand for CO_2 (del Rodero et al., 2019).

4.1 Design

When operating a full-scale HRAP for treatment of secondary effluent, a number of parameters need to be taken into account. The first is the HRT to be applied. The

process depends on both bacteria and microalgae, of which the latter depend on sunlight for photosynthesis, producing the oxygen the bacteria will need for degradation of organic matter and nitrification, among others. In order to guarantee exposure to sunlight for all wastewater that enters the process, the HRT needs to be at least 24 hours, but generally HRAPs tend to be operated at longer HRTs of between 3 and 8 days (Arbib et al., 2013; Posadas et al., 2015), with a recommended minimum of 4 days (Craggs et al., 2014; Ortiz et al., 2022). Second, attention should be paid to light penetration. Illumination matters, and as a result, the reactor depth is limited by the need to permit light penetration. The darker or more turbid the suspension is, the shallower the reactor should be, with a proportionally larger footprint. Typical depth of a HRAP for tertiary treatment is between 30 and 70 cm (Sutherland & Ralph, 2020; Ortiz et al., 2022). A shallower pond does not always result in better performance though. In a shallower pond, biomass concentration will increase more, and this may cause light shielding, and the effect may be that a deeper pond, in spite of lower biomass concentration, offers a higher volumetric and especially areal rate of nutrient removal (Sutherland & Ralph, 2020). HRT and depth being defined, the necessary surface area can be calculated, defining reactor length and width. CFD calculations show that HRAP performance tends to be optimized at length to width ratios higher than 10, as this gives a better velocity uniformity and reduces shear stress (Hadiyanto et al., 2013). A paddlewheel is used for mixing the liquid. Mixing is required for introducing CO_2, for keeping the cells in suspension and avoiding precipitation, and also for maintaining a flow of nutrients to the biomass, and maintaining liquid homogeneity. Paddlewheel speed should be kept low to avoid shear stress on the cells and reduce turbulence, and the number of paddles in the paddlewheel should be high, in order to avoid backflow and improve paddlewheel efficiency (Ortiz et al., 2022).

As in other suspended cell processes, without intervention, the Solids Retention Time (SRT) in the reactor would be the same as the HRT, and the amount of solids in the effluent would be the same as in the reactor. To avoid this situation, solids (the macroalgae/bacteria suspension) need to be separated and partially recycled, just as is done in the activated sludge process. In order to do so, microalgae/bacterial flocs can be separated in a settler, kept in the system using membrane filtration, or dissolved air flotation can be used. Generally, a SRT of around 7 days is applied, but a higher SRT, and the resulting higher biomass concentration, results in a higher process efficiency (Xu et al., 2015). Settleable biomass recycling also exerts selective pressure on the biomass, leading to a higher proportion of better settleable biomass, facilitating process operation (Sutherland & Ralph, 2020). The biomass that is not going to be recycled in the process can be separated from the effluent using coagulants. In this case, the possibilities depend on the pretended use of the biomass. The best results are usually obtained with $FeCl_3$ or alum ($Al_2(SO_4)_3 \cdot 12H_2O$), although at $130 and $65 per megaton of algae harvested these are also the most expensive options (Udom et al., 2013). However, when the microalgae will be used as fertilizer, for instance, the use of such metal salt flocculants should be avoided, as these salts will accumulate in the fertilized soil, and a cationic polymer, also cheaper with $50 per Mton, might be considered. Given the problems arising from the use of

traditional coagulants (Scherer et al., 2018), a shift toward natural (and biodegradable) coagulants, like those derived from *Moringa oleifera* seeds (Cassini et al., 2017), but more recently also those based on vegetable tannins, like the commercial Tanfloc SG, can now be observed (Dammak et al., 2023).

4.2 Operation

Operation of a large-scale HRAP is straightforward and does not require much interference. The reactor contents are to be kept mixed using the paddlewheel, which should maintain the reactor contents at a horizontal speed of 15–30 cm/s (Craggs et al., 2014). During operation, the pH in the reactor will vary, as during the day bicarbonate is consumed through photosynthesis, while during the night aerobic respiration produces bicarbonate. Usually, the pH will not drop below 6.5. If the pH tends to rise to values above 10.0, this indicates carbon limitation, and nutrient removal will be affected. To make matters worse, at pH $>$ 9.0, ammonia may be lost to the environment by volatilization, as a result of the shift of the ammonia—ammonium ion equilibrium. In such cases, the pH can be controlled (lowered) by admitting CO_2 gas; in this case the pH will vary between 6.5 and the set upper value. Good results were obtained limiting the pH at a value of 8.0 using CO_2 sparging (Park & Craggs, 2010). Biomass concentration can be optimized controlling the sludge recirculation rate, as is the case in activated sludge plants; a low biomass concentration will result in reduced removal efficiency, but a too high biomass concentration will result in internal shading, reduced photosynthesis, reduced algal activity, and an increase of the bacteria/algae ratio in the biomass.

4.3 Results

Given the operational parameters (HRT, SRT, pH, feed) as stated before, the use of microalgae/bacterial combined biomass for tertiary treatment tends to present good results. Removal efficiencies of N and P of >90% or even close to 100% are frequently observed (Alcántara et al., 2015a; Romero Villegas et al., 2017; Yu et al., 2023), and pathogen removal, depending on the species tested, can be of 1–3 units log (Ruas et al., 2021; Bhatt et al., 2023). Factors that may be responsible for pathogen removal in algae-based processes may be pH, dissolved oxygen, and sunlight-mediated processes; microalgae-based processes tend to result in fluctuating values for all these parameters, with the bacteria lacking capabilities to respond to rapidly changing environmental conditions. Entrapment of the pathogens in the microalgal/bacterial flocs may also play a role (Bhatt et al., 2023). Pathogen removal is not always complete though, and pending better results, chlorine disinfection after this process may still be necessary, depending on discharge requirements. Data on removal of heavy metals, of which especially lead, mercury, and cadmium are

reasons for concern (Wilde & Benemann, 1993), show that heavy metal removal by microalgae can reach from tens to a few hundred mg per gram of dry biomass (Kumar et al., 2015), but heavy metal fixation is favored at lower pH, around 5, which is below typical operating range. Metal removal may be desired, but especially when the intended final destination of the biomass is agriculture, avoiding metal removal by the microalgae, for instance by maintaining a certain pH, may be preferred.

Biomass productivity in laboratory and small-scale experiments has been good, but biomass productivity on treated wastewater when using a full-scale system is significantly less, with productivities of around 6 $g.m^{-2}$ d^{-1} being recorded for such cases. Considering 40 cm reactor depth and a 5-day HRT, this corresponds to 15 $g.m^{-3}.d^{-1}$.

5 Integration of Microalgae-Systems with Constructed Wetlands

5.1 Potential of Microalgae Systems Integrated with Constructed Wetlands

The development of microalgae cultivation at high densities in wastewaters is a technology that, besides providing efficiency in the treatment and attend discharge standards, can offer several possibilities, from nutritional supplements, treatment of diseases, obtainment of pigments, and biotechnological applications such as production of biodiesel and biofertilizers, among others (Ramos & Pizarro, 2018; Ramírez Mérida & Rodríguez Padrón, 2023).

The fundamental objective of applying microalgae for wastewater treatment is the use and biotransformation of the nutrients present into the biomass. Furthermore, the removal of ammonia, nitrites, nitrates, and phosphates, in addition to the increase in pH that favors the precipitation of phosphates, the oxygenation of water, the oxidation of organic matter, the reduction of pathogenic organisms, and the recovery of O_2 released in the process photosynthetic are other benefits arising from the use of microalgae (Ramos & Pizarro, 2018; Amenorfenyo et al., 2019; Abdelfattah & El-Shamy, 2024). However, post-treatment and/or a more sustainable alternative for separating algae from the liquid fraction may still be necessary, that is, economically viable and which mitigates the environmental impacts of conventional processes.

Constructed wetlands (CW) or treatment wetlands (TW) are a wastewater treatment technology based on natural floodplain ecosystems, and as such included in the category of Nature-based Solutions (NbS) (Costa et al., 2018; Ji et al., 2022). CW can be used for secondary or tertiary treatment of domestic and industrial effluents, and are composed of substrate, plants, and microorganisms. The type of vegetation used has a direct impact on the microbial community present, the disinfection potential of the system, and the ability to remove emerging contaminants

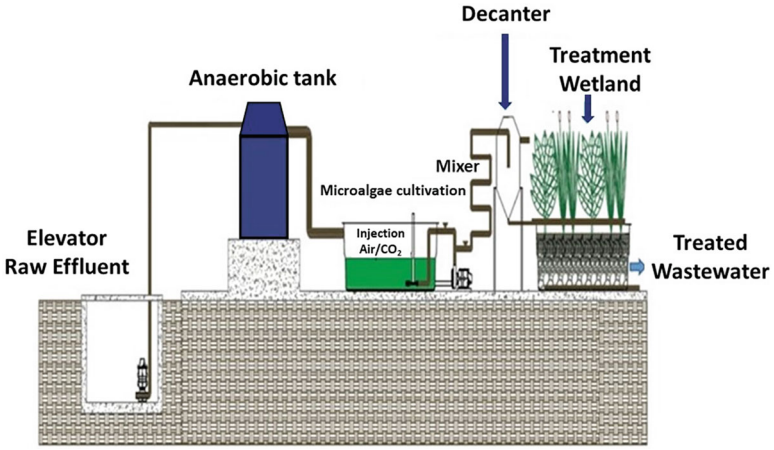


Fig. 1 Schematic representation of the experimental unit recommended for studies involving the combination of microalgae and vertical flow treatment wetlands (TW)

(pharmaceuticals, personal care products, plasticizers, surfactants, and pesticides) (Fadanelli et al., 2019; Mumtaj et al., 2024).

In integrated Microalgae-Wetlands systems, different metabolic conditions allow nitrification and denitrification processes to occur, which increase nitrogen removal and offer low costs and a high aesthetic value, while generating biomass and creating habitats for different animals (Caselles-Osorio et al., 2018; Zhao et al., 2023).

Subtropical climatic conditions may be exceptionally well-suited for such systems, with amounts of precipitation and temperature and humidity permitting year-round good performance of anaerobic processes, microalgae cultivation, and the constructed wetland. Such a climate (Köppen and Geiger classification: Cfa) can be found, for instance, in Santa Cruz do Sul, in the south of Brazil (xxx W, yyy S). The average temperature is 18.9 °C, and with an annual average rainfall of 1790 mm, even the driest month still has mm of precipitation (Kottek et al., 2006). There is the potential to combine anaerobiosis with a microalgae production system and vertical flow treatment wetlands (TW), which have a similar configuration to the CW of the French system (Von Sperling & Sezerino, 2018).

A reference experimental unit is presented in this chapter (Fig. 1), containing an upward flow anaerobic tank, with an ascent speed of 1 m h^{-1}, Hydraulic Detention Time (HDT) of up to 27 hours, a nominal volume of 1.1 m^3, and a load factor of 0.26 kg COD.m^{-3} day^{-1}. The microalgae unit can consider an oval-shaped raceway of 1 m^3; useful volume of 300 L; and load factors of 26.7 g COD.m^{-1} day^{-1}, 1.7 g N-NH^{4+}.m^{-1} day^{-1} of total, and 0.6 g Ptotal m^{-1} day^{-1}. The microalgae production system normally presents a raceway feature, that is, a centrifugal pump is connected to a Venturi-type air injector, that can both add carbon dioxide to the effluent and at the same time be responsible for circulating water in the tank. A tank



Fig. 2 Pilot-scale system, implemented at the Wastewater Treatment Plant (WWTP) of the University of Santa Cruz do Sul (UNISC), considering the stages of anaerobic reactor, microalgae production, decantation, and treatment wetlands

with a 100 L conical bottom, with a diameter of 0.54 m, a height of 1.19 m, and a cone height of 0.31 m, can also be considered as a decanter compartment.

The TW post-microalgae unit must assemble an integrated vertical flow system (French first-stage system) aiming to separate the coagulated/flocculated fraction from microalgae. The unit must be designed with a top drainage layer of at least 30 cm (30–80 cm) of zero gravel (with 4.8–9.5 mm), an intermediate transition layer of 10–20 cm of gravel number two (19–25 mm), and a lower layer of number 3 crushed stone of 20–30 cm (25–50 mm), as indicated by Von Sperling and Sezerino (2018). Considering the need for microalgae separation, a 5 cm layer of medium sand (with a grain size of 0.2–4 mm) can be added. The hydraulic application rate can be up to 0.4 m day^{-1}. The treatment wetland system under discussion was vegetated with: *Chrysopogon zizanioides* (six units), *Typha domingensis* (four units), and *Dracaena trifaciata* (two units). Von Sperling and Sezerino (2018) also recommend the use of Tifton 85 grass (Fig. 2).

The use of microalgae makes it necessary for the registration in the National System for Management of Genetic Heritage and Associated Traditional Knowledge. So, the microalgae *Chlorella vulgaris* and *Scenedesmus subspicatus* (A256CC3) as well as the macrophytes *Chrysopogon zizanioides*, *Typha domingensis*, *Dracaena trifaciata* (AA24A11) were registered.

5.2 Combined Use of Microalgae and Vertical Flow Constructed Wetland: Performance Details

The application of phyto- and phycoremediation systems has performance characteristics that depend on the climatic conditions where they are located. For ambient temperatures found in wastewater, raw and treated, with a minimum of 9 °C (recorded in July in the subtropical region) and a maximum of 26 °C recorded in December, the performance of microalgae development changes a lot. As we know, temperature, light, and nutrient availability are relevant factors for the production of microalgae. Even in areas with a large amount of shade, good growth of microalgae can be observed, reaching absorbance measurements of up to 0.349 A (690 nm), obtaining an average of 0.152 A throughout the year. It is important to note that during cold periods, the measured absorbance decreased to 0.049 A. It is important to highlight that during cold periods absorbance measurements in the range of 0.049 A can be observed (Oliveira et al., 2020).

It can also be observed for the Chemical Oxygen Demand (COD) very characteristic stages of reduction in the integrated system, with a reduction already occurring in the first stage of the process, that is, in the anaerobic reactor (Fig. 3). Subsequently, in the microalgae stage, the COD decrease reaches 52%. Finally, after the CW unit, an increase of approx. 5% in COD (not significant) in relation to the MA stage was observed. It is important to highlight that the values obtained for the treated wastewaters fully comply with standards required by local legislation (330 mg L^{-1}), for flows of up to 100 m^3 day^{-1}.

Regarding ammonia nitrogen (N-NH_4^+) and total phosphorus (total P), the system presented opposite results. While for N-NH_4^+ the system may be considered efficient, reaching an average removal of 71%, for total P, surprisingly, the concentrations increased in the system, mainly in the microalgae unit. A plausible explanation would be that when inoculating microalgae into the system, quantities of phosphorus from their cultivation medium were also added, but with N-NH_4^+ values that would still be below the 20 mg L^{-1} referenced by Beuckels et al. (2015) and thus there is an increase in this nutrient in the system. Comparing only the MA and TW stages, it becomes evident that the TW was efficient with a mean removal of 55%, which shows that this stage performed satisfactorily in removing this pollutant. Moreover,

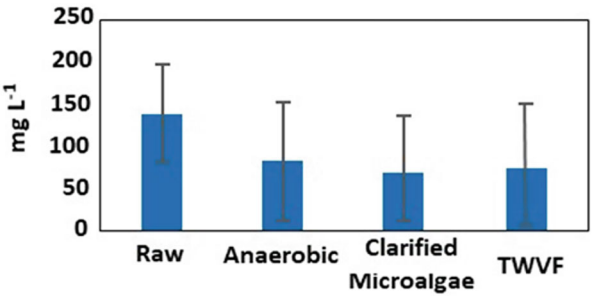


Fig. 3 Mean values and standard deviation obtained in the COD analyses

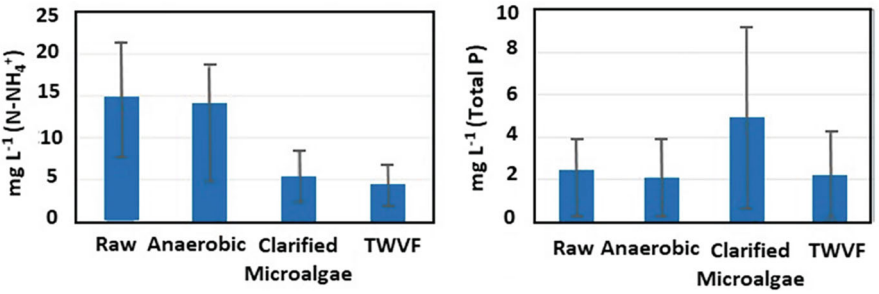


Fig. 4 Mean values obtained for ammonia nitrogen (N-NH_4^+) and total phosphorus (total P) with the MA + WC system in the treatment of urban effluents

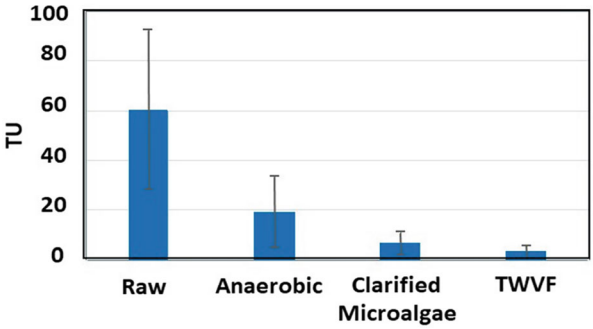


Fig. 5 Mean values and standard deviation obtained in turbidity analysis

it must be pointed out that both N-NH_4^+ and total P present values under the maximum discharge limits established by the Rio Grande do Sul state (South Brazil) legislation (CONSEMA, 2017), that for flows of up to 100 m^3 day^{-1} are 20 mg L^{-1} and 4 mg L^{-1}, respectively (Fig. 4).

In their study, Lima et al. (2020) evaluated the performance of the microalgae *Chlorella* sp. isolated and in consortium with bacteria from activated sludge, in urban wastewater. Satisfactory results were verified, especially when *Chlorella* sp. was inoculated with activated sludge in proportions of 1:2 and 2:1, attaining reductions of 81.39%, 86.12%, 82.89% and 82.5%, 72.66%, 97.15% of COD, total nitrogen, and total phosphorus, respectively. A parameter that needs to be highlighted for the combined MA + WC system is turbidity. As can be seen in Fig. 5, satisfactory results were achieved in both, microalgae production tank and the TW unit, with a mean reduction of 95%. Turbidity is a physical parameter which indicates the scattering of light in suspended particles and is commonly used to estimate water quality under ambient conditions. High turbidity is responsible for the increased oxygen consumption normally associated with high sediment loads (Liu et al., 2023).

Another important factor, when it comes to the management of urban effluents, is associated with the treatment of sludge from the process of phase separation with microalgae by flocculation coagulation. The accumulation of sludge on the surface



Fig. 6 Sludge (biomass) retained in the first layer of filter media with coarse sand

of vertical flow TW systems (Fig. 6), the first stage of the French model, can have positive effects on the results to be obtained.

However, due to reduced permeability, the mud layer can restrict hydraulic capacity in treatment wetlands (TW), requiring careful consideration of feeding and resting strategy (Khomenko et al., 2019).

The type of the plant used in TW also affects the efficiency in removing pollutants from organic sludge treatment stage coming from WWTP. In a study by Gagnon et al. (2012), 16 TWs with different macrophytes compositions were used to treat sludge from fish farming, four replications with *Phragmites australis*, *Typha angustifolia*, and *Scirpus fluviatilis* plants, in addition to unplanted controls. The results showed a high efficiency of the treatment wetlands, with removal rates between 94% and 99% for most pollutants. The planted systems outperformed the unplanted control, with wetlands vegetated with *Phragmites australis* being the most efficient, followed by *Typha* and then *Scirpus*. The distinct influence of plant species on pollution removal is explained by the nitrogen and phosphorus sequestration capacity in plant tissues and the effect of the rhizosphere.

6 Current Limitations in Advancing Large-Scale Application of Microalgae-Based Wastewater Treatment

Despite the high potential of the studies, results presented here in this chapter, and the advancement in this scientific area, the large-scale algal biomass production technologies are not popular—are not part of the daily operation units of conventional Wastewater Treatment Plants (WWTP), not even in decision-making, particularly in Latin America. In other countries, Europe and the United States, they already have larger plants, coming from financing from large network and collaboration scientific and innovation projects.

Most of the studies presented in the literature have been carried out on a small laboratory scale. There are limits about the possibility of obtaining reliable data for a comprehensive assessment of the efficiency of such solutions. Therefore, there is a need to verify the results in pilot-scale and the full technical-scale studies (Dębowski et al., 2020). This chapter summarizes information of microalgal technologies for wastewater treatment applications.

Limitation exists because the systems for algal biomass production, separation, and conversion into energy carriers and other benefits still are difficult to bring economic feasibility in the face of usual simplified processes. Where the sector view is only to (wastewater)treat and meet environmental conditions, without incorporating the context of circular economy, water reuse, and the nexus approach (water, energy, and food) as part of a holistic, systemic, and sustainable management sight of sanitation based on the concepts of resource-oriented sanitation.

The road to large-scale implementation—production and use of microalgal biomass—is not only fraught with economic difficulties, but also with technological, normative, and legal challenges. Unsuitable climate conditions are also an impediment; proper thermal and light conditions are crucial factors in microalgal growth and improved performance. Sites of anaerobic reactor exploitation can serve as technologically and commercially viable locations. However, it is reasonable to assume that microalgae, with the multitude of commercial applications, possess properties that grant them a competitive advantage over conventional WWTP in terms of commercial applicability (Leu & Boussiba, 2014; Dębowski et al., 2020). This will require local public policies and state strategies (climate change, nature-based solutions, urban resilience) to encourage the local productive sector in the implementation of a WWTP-biorefinery approach—including economic benefits—and facilitate the bureaucratic legal and regulatory process. The concept of recovering resources affords an opportunity for the development of technologies based on the use of microalgal biomass.

Some important limitations were pointed out by Yap et al. (2021), based on a review carried out with several studies in the area. Therefore, this chapter was based on bringing information to full-scale projects, based on specificities that must be considered on a real scale. Some relevant and highlighted points are as follows: (a) high cost in cultivation and harvesting process; and (b) criteria to design based on studies that are not comparable because data on yields and costs relate to different cultural systems, different stresses, and different environmental and social conditions.

For large-scale application open raceway ponds appear to be more favorable—with the potential for CO_2 sequestration. With biomass harvesting being one of the major challenges at full-scale application, some of the recovery methods being considered include filtration, centrifugation, gravity sedimentation, chemical flocculation, and dissolved air flotation. However, the method of choice has to be selected based on a positive techno-economic and environmental evaluation, also taking the energy balance into consideration. Open pond systems and photobioreactors for large-scale commercial production are limited to culturing microalgae for high value

products and not biodiesel. Some criteria that need to be optimized for large-scale application include (Rawat et al., 2013):

- strain selection and seed culture preparation;
- biomass and lipid yield optimization;
- bioreactor configuration;
- physic-chemical parameters; and most importantly
- harvesting and extraction of the lipid from the biomass.

Major breakthroughs are still needed toward design and development of technologies (Amaro et al., 2011) at full scale that can reduce costs while increasing yields. Integrated studies, following coherent and long-run, well-funded R&D program, with social, governance, and environmental management goals, are necessary. The processes should be integrated in the existing biofuels industry to accelerate full-scale implementation, especially in Third World countries. Developing this sector within an integrated social and economic policy agenda and not just through disjointed environmental actions will speed things up: not only meant for profit making and benefiting the environment, but also to help local communities in terms of food and energy security (Lam & Lee, 2012).

The scale-up process is often expensive and frequently carries high risks. It will be important that projects adapting technologies to local contexts, as they are not the same criteria and operational practices for all cases, clearly indicate: (i) practical recommendations and discuss common challenges of large-scale systems associated with; (ii) cost-effective design; (iii) operation and maintenance; (iv) shortage of experienced staff (especially phycologists); and (v) social barriers, such as wider acceptance of algae as a food source and regulatory gaps (Novoveská et al., 2023).

In addition to the issues inherent to the specific innovation process itself and the challenges to disseminate the technology on a real scale, it is important to highlight that transforming urban society from a linear to a circular metabolism is a trend in many developed countries. In low-income countries, green values and opportunities are less developed and have a generally lower priority among many key stakeholders, and public budgets are too constrained to make the right investments. With the development of microalgae-based wastewater treatment, many innovative components should be developed to meet the requirement of wastewater treatment and result in the appearance of the new related technologies. The cost of some components (e.g., solid-liquid separators) may decrease with manufacturing development and mass production, which could improve the high cost dilemma. Actions are needed that go beyond process optimization, but public policies that integrate social and economic-environmental dimensions (Magalhães Filho et al., 2022).

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Large-Scale Harvesting of Microalgae



Emma Muir, Benoit Guieysse, and Maxence Plouviez

Abstract While microalgae biotechnology already supports the production of food and pharmaceuticals, its commercial applications remain limited by costs and difficulty to scale. Among various challenges, harvesting can account for up to 30% of the total production costs because microalgae cultures typically contain less than 0.5 g of dry biomass per liter of cultivation broth, meaning that significant volumes of water must be removed relative to the amount of biomass recovered. Consequently, microalgae harvesting typically requires the use of several processes in series to deliver cost-efficiency, and each step must be selected and/or developed depending on the biomass characteristics and other key process parameters. This chapter presents and discusses the suitability of established and emerging microalgae biomass harvesting technologies in light of their technical feasibility, economics, and sustainability.

Keywords Microalgae biotechnology · Mass scale production · Microalgae harvesting · Commercial harvesting technologies · Emerging harvesting technologies

E. Muir
School of Food Technology and Natural Sciences, Massey University, Palmerston North, New Zealand

Cawthron Institute, Nelson, New Zealand

B. Guieysse
BG Bioprocess Consulting Limited, Palmerston North, New Zealand

M. Plouviez (✉)
Cawthron Institute, Nelson, New Zealand
e-mail: Maxence.Plouviez@Cawthron.org.nz

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1 Introduction

The industrial production of microalgae began over 60 years ago when species of *Chlorella*, *Arthrospira* (Spirulina), and *Dunaliella* became cultivated to produce health supplements and aquaculture feed. The commercial production of microalgae is today dominated by: (1) *Arthrospira*, a pigment-rich cyanobacteria expected to support a US $2 billion market by 2026; (2) *Chlorella*, which contains compounds such as beta-1,3-glucan (an immunostimulatory, free radical scavenger, reducer of blood lipids), vitamins, and carotenoids that are valued in food and feed applications; (3) *D. salina*, which has a dry cell mass of up to 14% b-carotene and includes valuable lipids, proteins, and glycerol; and (4) *Aphanizomenon flos-aquae,* which is rich in pigments such as chlorophyll and is utilized in food (Spolaore et al., 2006). Aside from these "Big 4," *Haematococcus pluvialis* is commercially cultivated for astaxanthin production, and *Crypthecodinium cohnii* and *Schizochytrium* are grown as sources of docosahexaenoic acid (DHA). Table 1 lists the primary uses and estimated global annual production of these algae as reported in 2017. Driven by the food, nutraceuticals, and pharmaceuticals industries, the microalgae market is projected to reach US $6.3 billion by 2028 (https://www.credenceresearch.com/report/algae-products-market).

To date, countless publications state that "harvesting accounts for 20–30% of total costs of microalgae biomass production", often indirectly (and possibly unknowingly) referring to a single and nearly four-decade-old original work (Gudin and Thépenier, 1986). In contrast, a more recent techno-economic analysis of a theoretical 100 ha plant puts this value at 3–15%, with this relative contribution decreasing with scale (Fasaei et al., 2018). As the largest microalgae farms currently operating are 36–44 ha in size (Guieysse & Plouviez, 2024; Plouviez et al., 2022), the relative impact of harvesting of total production costs remains unclear. This

Table 1 Current commercially relevant species, their uses, and global production

Species or genus	Primary uses	Global production (ton dry weight per year) (García et al., 2017)
Arthrospira platensis (Spirulina)	Food supplement, feed, cosmetics, phycocyanin, γ-linoleic acid, oleic acid	12,000
Chlorella	Food, feed, phenolic compounds	5000
Dunaliella salina	Feed, β-carotene, linoleic acid, oleic acid, sterols	3000
Aphanizomenon flos-aquae	Food, chlorophyll A	1500
Haematococcus pluvialis	Astaxanthin, vitamins, and carotenoids	700
Chrypthecodinium cohnii	DHA	500 ton DHA per year
Chizochytrium	DHA	20 ton DHA per year

chapter reviews the traditional and emerging harvesting technologies and discusses their suitability in different applications.

2 General Considerations

Microalgae can be cultivated in open ponds (unstirred, circular, or raceway) and closed systems (photobioreactors). Open raceway ponds are generally preferred for large-scale microalgae cultivation (e.g., nearly all the commercially produced *Arthrospira* is cultivated in open raceway ponds) because these cultivation systems incur lower capital and energy expenditures than closed systems. Open systems are, however, more prone to water evaporative losses and contamination that can limit biomass productivity. Regardless of the cultivation system, the cell concentration achieved during commercial phototrophic cultivation is typically small, ranging from 0.5 to 0.75 g L^{-1} in open ponds and only reaching up to 2 g L^{-1} (0.2%) in closed systems (Davis et al., 2011; Fasaei et al., 2018). Harvesting microalgae biomass, therefore, involves separating large volumes of water from relatively small amounts of biomass. This requires significant labor, maintenance, energy, chemicals (e.g., flocculants), and capital expenditures. The performance of microalgae harvesting can be assessed by the recovery efficiency (RE), which quantifies how effective the separation is in recovering all the biomass, and the concentration factor (CF), which quantifies how much the biomass is concentrated.

The low cell concentration achieved during phototrophic microalgae cultivation also means that biomass recovery must often be staged via a series of technologies to improve cost efficiency (Fig. 1). Pre-concentration and thickening are indeed needed to reduce the volumes treated and, therefore, reduce the energy required at the dewatering and drying steps. Soomro et al. (2016) computed the costs and carbon footprint incurred by microalgae harvesting using various technology chains. Using various assumptions for energy demand and removal efficiency, these authors

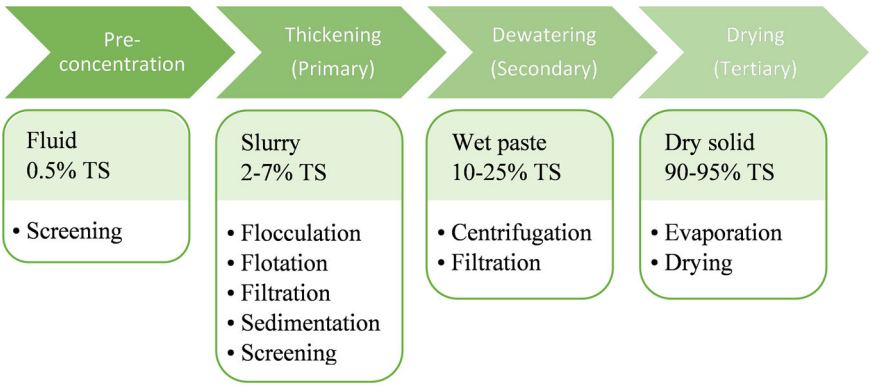


Fig. 1 Biomass recovery stages (*TS*: total solids)

Table 2 Technologies commonly used for microalgae harvesting

Technology	Advantages	Limitation	Key performance indicators
Screening	Simple, low capital and operational costs	Only suitable for large microalgae (>100 μm)	0.4 kWh m^{-3} RE 89% (Soomro et al., 2016)
Settling	Simple, low operational costs	Slow, low recovery, and low biomass quality	
Chemical flocculation	Cost-effective and relatively simple	Biomass contamination with chemicals Variable RE	0.15 kWh m^{-3} SS 3–8% RE 80–98% (Fasaei et al., 2018)
Dissolved air flotation	Suitable in freshwater, >10,000 m^3 d^{-1}	High energy demand	1.5–7.6 kWh m^{-3} RE >99% SS 5% (Soomro et al., 2016)
Electrocoagulation	Reliable Cost-effective	High energy demand Electrode replacement Biomass contamination with chemicals	0.15–1 kWh m^{-3} RE 80–95%
Centrifugation	High recovery Reliable	High costs Best for dewatering	0.7–1.30 kWh m^{-3} (Fasaei et al., 2018) SS 10–20% RE 95–99%
Membrane filtration	High recovery Reliable	High operational costs Limited by biomass characteristics	0.8–2.51 kWh m^{-3} permeate SS 1.5–10% RE 99% (Fasaei et al., 2018)

reported a harvesting cost and carbon footprint of \$0.37 kg^{-1} dry biomass recovered and 3.91 kg CO_2 equivalent per kg biomass recovered, respectively, when using centrifugation only (1 kWh m^{-3}, 95% RE), against \$0.92 kg^{-1} and 5.15 kg CO_2 kg^{-1} when combining electrocoagulation with centrifugation (0.6 kW m^{-3}, 88% RE); \$0.11 kg^{-1} and 1.12 kg CO_2 kg^{-1} when combining alum-flocculation (0.1 kWh m^{-3}, 99% RE) with centrifugation, and \$0.004 kg^{-1} and 0.05 kg CO_2 kg^{-1} when combining bioflocculation (negligible energy demand, 99% RE) with tangential flow filtration (0.2 kWh m^{-3}, 99% RE). While these numbers should be taken cautiously considering the variability in reported RE and energy demands (Table 2), they evidence that staging and technology selection are paramount to reducing cost and impacts of harvesting. Process selection also depends on the operational mode as biomass can be harvested in batches (entire or near entire volume at once), semi-continuously (i.e., 20–40% of the entire cultivation volume at periodic intervals), or continuously. Batch operation is only technically feasible for small volumes, such as during cultivation in photobioreactors, whereas continuous or semi-continuous harvesting is generally required for large ponds (i.e., hectare scale) to reduce storage demands and/or minimize the costs of harvesting

and processing equipment. For example, pilot-scale raceways (80 m^2) cultivating *Scenedesmus* spp. in Spain are operated semi-continuously, with 20% of the culture being harvested daily by centrifugation and replaced with fresh culture medium to maintain the biomass concentration around 1.0 g L^{-1} (Cardias et al., 2023). Critically, the process must handle the physical (e.g., viscosity and temperature), biological (e.g., cell size, shape, and sensitivity), and chemical (e.g., pH and alkalinity) characteristics of the culture, which are all influenced by the process operation and environmental parameters. The following section describes these interactions in greater detail.

3 Commercial Versus Emerging Harvesting Technologies

Microalgae can be harvested using "traditional" technologies that are already used at commercial scale, and "emerging" technologies that have mainly been tested experimentally. The costs of traditional technologies range from US$28 ton for coagulation/flocculation with alum to US$500 ton for centrifugation. By contrast, costs of emerging technologies sit at around US$1350 ton (see further details in de Morais et al., 2023). Consequently, harvesting is still mainly performed by traditional technologies, such as centrifugation. Another reason is that these techniques still offer the best performance. Tables 3 and 4 present examples of performance data for the different microalgae harvesting technologies at laboratory and pilot scales, respectively. As can be seen, the high efficiencies of emerging technologies achieved in the laboratory (>90%) are often not reproduced at scale (50–80%) because scale influences biomass characteristics and water chemistry and, therefore, harvesting efficiency.

Interestingly, the technologies currently used for commercial microalgae harvesting have often been developed for other applications, such as water treatment or food production. While direct technology transfer to the microalgae industry can be challenging due to differences in scale and cell characteristics (e.g., fragile biomass), well-established manufacturers are now actively collaborating with microalgae growers and researchers to develop more bespoke systems (Fig. 2). SANI membranes that developed Vibro® technology, a microfiltration and ultrafiltration process, is one example (Harvest and concentration of algae (sanimembranes.com)). Alfa Laval and GEA are examples of large manufacturers of food, beverage, and pharmaceutical equipment that now market specific microalgae harvesting solutions (see: Algae processing | Alfa Laval; GEA solutions for algae processing spans 50 years; GEA credentials for microalgae processing). Technology transfer is also happening in the other direction as Evodos is a company that began in 2007 by developing dynamic settlers for microalgae and is now reaching out to other industries (Evodos Dynamic Settlers| Algae). As an example of very targeted process, Bernhard Rampelt has developed a specific rotative spirulina harvester that is now commonly used by small and medium size farms in France (Harvester – Spirutech).

Table 3 Examples of performance achieved with traditional and emerging technologies at the laboratory scale

Method	Microalgae species	Initial culture volume and biomass concentration	Output	Recovery efficiency or concentration factor	Energy demand	Reference
Autoflocculation + Sedimentation	*Chlorella*	100 mL	–	>95%[a]	–	Vandamme et al. (2012)
Flocculation + Sedimentation	*Chlorella* (freshwater)	0.9 L	–	90%[a]	–	Bilanovic et al. (1988)
	Chlorella (marine)	0.9 L	–	10–15%[a]	–	Bilanovic et al. (1988)
Flocculation +45 μm sieve	*Tetraselmis suecica*	200 mL 0.42 g L^{-1}	0.56 mL 150 g L^{-1}	100% 357-fold	14.81 kWh m^{-3} (impeller)	Danquah et al. (2009)
Sedimentation with recycling	*Pediastrum boryanum*	100 mL	–	~85%	–	Park et al. (2015)
Inclined thickener	*Chlorella*	733 mL 0.7 g L^{-1}	8.3 mL 59 ± 1 g L^{-1}	96 ± 4% 84-fold	–	Smith and Davis (2013)
Bioflocculation	*Desmodesmus brasiliensis*	200 L 0.5 g L^{-1}	–	99%	–	Ndikubwimana et al. (2016)
Dissolved-air flotation	*Dunaliella salina*	1.8 L 0.4–0.6 g L^{-1}	8–12 g L^{-1}	80% 20-fold	–	Besson and Guiraud (2013)
	Pond mix, *Chlorella* and *Scenedesmus*	1.2 L 0.11 g L^{-1}	2.5 mL 45 g L^{-1}	84.9% 409-fold	7.6 kWh m^{-3}	Wiley et al. (2009)
Micro-flotation	*D. salina*	1 L	–	98.2%	–	Hanotu et al. (2012)
Suspended air flotation	Pond mix, *Chlorella* and *Scenedesmus*	1.2 L 0.114 g L^{-1}	2.2 mL 48 g L^{-1}	76.6% 421-fold	0.003 kWh m^{-3}	Wiley et al. (2009)
Dispersed-air/foam flotation	*Chlorella* *Scenedesmus*	200 mL	–	CTAB: >93.7% Saponin: 22.5% Chitosan + saponin: ~98%	–	Kurniawati et al. (2014)

Electrocoagulation-flotation	*Microcystis aeruginosa*	1 L Up to $1.55x10^6$ cells mL^{-1}	–	100%	0.4 kWh m^{-3}	Gao et al. (2010)
Foam fractionation	*Chlorella*	9.5 L 0.11 g L^{-1}	14–24 g L^{-1}	– 127–218-fold	0.015 kWh m^{-3}	Coward et al. (2013)
Submerged microfiltration	*Chlorella*	25 L 0.41 g L^{-1}	1.67 L 3.5 g L^{-1}	57% 8.5-fold	0.48 kWh m^{-3}	Bilad et al. (2012)
Electrofiltration	*Arthrospira*	1 L tank 0.5 g L^{-1}	208 g L^{-1}	–	0.105 kWh kg^{-1}	Aoude et al. (2023)
Centrifugation	*Chlorella*	35 mL	–	98%	–	Japar et al. (2017)
Autoflocculation	*Scenedesmus*	160 mL 2×10^6 cells mL^{-1}	–	77 ± 17%[a]	–	Roccuzzo et al. (2020)
Ultrasound	*Monodus subterraneus*	10 L day^{-1} 20.8 mL 0.49×10^7 cells mL^{-1}	$9.7x10^7$ cells mL^{-1}	81.4% 19.8-fold	14.4 kWh m^{-3}	Bosma et al. (2003)
Magnetic nanoparticles	*Chlorella*	1 g L^{-1} 20–500 mL	–	99%	–	Lee et al. (2013)
Phototaxis	*Euglena*	250 mL day^{-1} $4.8x10^4$ cells mL^{-1}	$1.19x10^5$ cells mL^{-1}	91%	–	Nakajima and Takahashi (1991)

[a]Flocculation efficiency, by sampling clarified zone. Biomass was sedimented but not actually removed

Table 4 Examples of performance achieved with traditional and emerging technologies at pilot to large scales

Method	Microalgae species	Initial culture volume and biomass concentration	Output	Recovery efficiency or concentration factor	Energy demand	Reference
Vibro-screening	*Spirulina*		80–100 g L^{-1}	Up to 95%	–	Habib and Hasan (2008)
Vibro-screening + hydraulic press	*Spirulina*	75 m^3 0.6–1.2 g L^{-1}	Filtration: 50–100 g L^{-1} Press: 200–250 g L^{-1}	Filtration: 41–167-fold Press: 167–417-fold 70% RE	–	Kurpan et al. (2024)
Chemical flocculation	*Scenedesmus*	1000 L 0.5 g L^{-1}	4.8 L 100 g L^{-1}	96% 200-fold	–	Selesu et al. (2016)
pH flocculation	*Scenedesmus, Chlorella*	1000 L		75%, 60% at 1 h	0.2 kWh kg^{-1}	Koley et al. (2017)
Dissolved-air flotation with alum	Mixed—*Chlorella* and *Oocystis* etc.	9750 L 1.055 g L^{-1}	142 L 43.3 g L^{-1}	60%	0.43 kWh m^{-3}	Sim et al. (1988)
Foam fractionation	*Chaetoceros* spp.	220 L	–	90% 7.5-fold	–	Csordas and Wang (2004)
Electrocoagulation-flotation	Mix: Blue-green, green, and diatoms	100 L 2.7–36.1 mg L^{-1}	–	96.3%	0.331 kWh m^{-3}	Poelman et al. (1997)
Electrocoagulation-flotation	*Scenedesmus, Chlorella*	1000 L	–	48–50% at 1 h >90% at 24 h	60–65 kWh kg^{-1}	Koley et al. (2017)
Electrocoagulation with settler	*Chlorella*	111 L 0.1 g L^{-1}	~0.45 L 22 g L^{-1}	85–93% 220-fold	Electrolysis only: 0.04–0.22 kWh kg^{-1} Total: 0.61–1.56 kWh kg^{-1}	Lucakova et al. (2022)

Tangential flow filtration	*Tetraselmis suecica*	80 L 0.6 g L^{-1}	0.093 L 88.8 g L^{-1}	17.2% 148-fold	2.06 kWh m-3	Danquah et al. (2009)
Belt filtration	Mixed species in pond water	0.5 g L^{-1}	30 g L^{-1}	80% 60-fold	0.3–0.5 kWh m^{-3}	Sim et al. (1988)
Autoflocculation sedimentation + disk-stack centrifuge	*Staurosira, Desmodesmus*	60 m^3	Sedimentation: 10 g L^{-1} Disk-stack: 200–300 g L^{-1}	–	–	Huntley et al. (2015)
Disk-stack centrifuge + bowl-decanter	Mixed—*Chlorella* and *Oocystis* etc.	31–300 m^3 0.4–0.7 g L^{-1}	Disc-stack: 40–50 g L-1 Bowl decanter: 150 g L^{-1}	>90%	1.3 kWh m^{-3}	Sim et al. (1988)
Continuous-flow centrifuge	*Nannochloris* sp.	55-L from 979 L tank, 0.1 g L^{-1}	–	94%, 0.94 L min^{-1} 17%, 23 L min^{-1}	20 kWh m^{-3} 0.8 kWh m^{-3}	Dassey and Theegala (2013)
Flocculation + sedimentation	Mixed	2500 m^3 ~0.15 g L^{-1}	–	70%	–	Park et al. (2019)
Phototaxis	*Dunaliella salina*	Pond 5×10^5 to 5×10^6 cells mL^{-1}	2.5×10^6 to 3×10^7 cells mL^{-1}	66–68% 5–6-fold	–	Borowitzka and Borowitzka (1990)

[a]Flocculation efficiency, by sampling clarified zone. Biomass was sedimented but not actually removed



Fig. 2 SANI Membranes—Vibro-Lab3500 system running a concentration step with microalgae (top left). SANI Membranes—Vibro-I connected to a feed system (top right). SANI Membranes—Vibro-I and feed system in operation at a microalgae farm (bottom). (Photographs re-used with permission from SANI Membranes)

3.1 Filtration

Screens/sieves, vacuum/pressure filters, and membranes are all sub-categories of filtration. The following sub-sections focus on screening and membrane filtration which are widely used in microalgae biotechnology.

3.1.1 Screening

Screens with pore size larger than 25 μm can be used to pre-concentrate and even dewater large microalgae (>100 μm) such as *Arthrospira* and *Aphanizomenon* (Roselet et al., 2019). Screening is simple, requires little initial capital, and typically does not require added chemicals. However, it requires frequent backwashing to unclog pores and poorly handles fluctuations in particle size. Unfortunately, since smaller cells can pass through screens while larger cells are retained, screening can lead to the culture being dominated by smaller trichomes (filaments) over time (Kurpan et al., 2024; Vonshak & Richmond, 1988). Cheng et al. (2018) also found that light intensity and temperature influenced *Spirulina* helix pitch and trichome length, which in turn influenced biomass harvesting: for example, when the average helix pitch was below the filter pore size and trichome length was <300 μm, harvesting efficiency decreased by 28%.

Various screen designs can be used, either alone or in combination, such as inclined or vibrating screens and micro-strainers (rotary drums with polyester or straining fiber). Vibrating screens can filter microalgae culture at the same rate as inclined screens for one-third of the filter area. However, vibrating screens are more likely to cause a gradual decrease in cell size in the culture (Vonshak & Richmond, 1988) as small cells that are not harvested are recycled back into the culture. The harvesting flow and cell size largely determine the economics of screening, including the energy input and harvesting efficiency. Large algal strains can be separated with a larger screen mesh and, therefore, at a higher flow rate (for the same screen area) and/or at reduced operational costs (Barros et al., 2015). The slurry obtained from screening is typically further concentrated by filtration or pressing.

3.1.2 Membrane Filtration

Membrane filtration is typically driven by pressure or vacuum and generally incurs lower capital and energy expenditures than centrifugation for the equivalent performance (Greenwell et al., 2010). Filtration can achieve a high biomass recovery at a low energy demand and is scalable. However, it is limited by the characteristics of the biomass, and membrane fouling can be a significant issue. For example, *Arthrospira* produces extracellular polymers (EPS) that cause fouling and reduce filterability (Depraetere et al., 2015).

The most common membranes for filtration are microfiltration and ultrafiltration membranes. Crossflow filtration is preferred over dead-end filtration as this mode reduces fouling and produces a more concentrated sludge (Roselet et al., 2019). Membrane filtration is more cost-efficient at lower biomass concentrations and is, therefore, best used to concentrate biomass to a slurry prior to other dewatering methods (Bilad et al., 2014; Roselet et al., 2019).

3.2 Settling

Gravity settling (sedimentation) is simple and requires little energy input. However, this process is only suitable for harvesting large nonmotile species as small cells do not settle fast enough, especially in dilute cultures (Roselet et al., 2019). Long retention times can still be required, which increases capital costs (large settling volume) and can impact biomass quality.

Gravity settling can be implemented in settling ponds, clarifiers, or inclined thickeners. Conventional clarifiers have a cylindrical shape with a funnel-shaped bottom. The culture is gently pumped to minimize turbulence and allow biomass to settle to the bottom while clarified medium flows out of the top. Inclined thickeners are similar but use inclined parallel plates to increase the settling surface and, thereby, the settling efficiency. Regardless of the technology used, the final biomass concentrations remain low (2–3% TSS) and gravity settling must be followed by additional dewatering.

3.3 Coagulation and Flocculation

Microalgae cells are usually 2–20 μm in size and have negative surface charges and a low specific gravity of 1.05–1.1. These characteristics prevent them from being effectively harvested by screening and settling. To circumvent this limitation, coagulation and flocculation are complementary processes that allow small and charged cells to combine into larger particles that can be economically settled or screened (Fig. 3). Coagulation describes the process of neutralizing the cell surface to reduce repulsion between cells and allow small cell aggregates to form. Flocculation enables further aggregation into large settling flocs via charge neutralization, bridging, patching, or sweeping (Li et al., 2022) (Fig. 3).

Chemically induced settling generally requires the addition of metal salts or polymeric flocculants. Aluminum and ferric salts are widely used coagulants because they form positively charged hydroxides that neutralize negatively charged cells. Polymeric flocculants are long-chain molecules that act by bridging, charge-neutralization, and electrostatic patching. A key disadvantage of chemically enhanced settling is that the chemicals contaminate the biomass and/or water recovered. Safe biochemicals such as chitosan effectively remove cells, but their

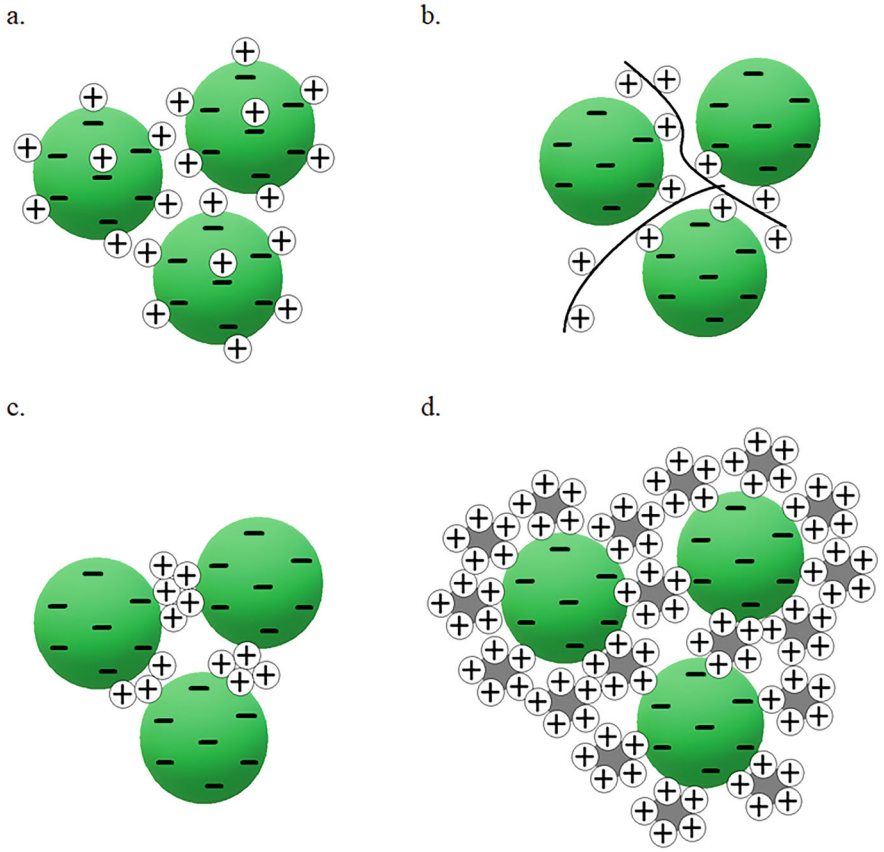


Fig. 3 Mechanisms of flocculation: (**a**) charge neutralization: neutralization of surface charges by positive ions leading to coagulation, (**b**) bridging and (**c**) electrostatic patch: during bridging or patching, polymers with positive charged functional groups neutralize charges across cells, thus connecting cells via the polymer, (**d**) sweeping: the trapping of cells in a large structure of inorganic precipitate

cost remains too high for economic use during microalgae production in most applications (Laamanen et al., 2016).

Careful selection of chemical, dosage, and mixing conditions is required as the formation, structure, and recovery of flocs are influenced by the microalgae species, the loading rate, and the water chemistry. For further information on chemically enhanced gravity settling, please consult Barros et al. (2015).

3.4 Flotation

Flotation can be used to concentrate cells into a slurry containing 2–7% solids (Laamanen et al., 2016). This process is based on the ability of small air bubbles to adhere to microalgae cells to increase cell buoyancy. This lifts microalgae cells to the culture surface, where they can easily be mechanically skimmed. Surfactants, polymers, and other additives can assist this adhesion by neutralizing electrostatic repulsion between cells (Laamanen et al., 2016; Roselet et al., 2019). Flotation is reliable, inexpensive, and feasible at large scale (Sim et al., 1988).

Dissolved-air flotation (DAF): Dissolved-air flotation can remove up to 99.7% of cells when used with flocculants (Laamanen et al., 2016). In this process, atmospheric air is pressurized into a water stream that is injected into a flotation tank. Microbubbles (<100 μm) are formed upon decompression of the supersaturated water. DAF involving microbubble clouds with a bubble size of about 50 μm can be referred to as microflotation (Hanotu et al., 2012). DAF can handle large volumes (>10,000 m^3 d^{-1}) but has a high energy demand due to the need for pressurization (Roselet et al., 2019). DAF may not be suitable for marine species because salinity negatively influences cell-bubble adhesion (Barros et al., 2015).

Dispersed-air flotation (DiAF): Dispersed-air flotation only differs from DAF in the way that air is directly diffused into the flotation tank (Laamanen et al., 2016). This creates larger bubbles (700–1500 μm) that are less prone to adhere to microalgae cells and, therefore, DiAF requires flocculants or surfactants to increase efficiency (Laamanen et al., 2016; Roselet et al., 2019). Surfactant-aided DiAF is also called foam fractionation.

Electrocoagulation-flotation (ECF): Electrocoagulation-flotation, or electrolytic flotation, involves converting water into hydrogen and oxygen bubbles using electricity. These bubbles are much smaller (15–45 μm) than the bubbles generated during DAF or DiAF and are therefore more efficient at adhering and lifting the microalgae cells (Roselet et al., 2019). Sacrificial electrodes made of aluminum or iron also release metal ions that directly neutralize the microalgae surface or react with hydroxide ions to form the flocculants $Al(OH)_3$ or $Fe(OH)_3$ (Barros et al., 2015; Roselet et al., 2019). As these metallic hydroxides contaminate the biomass harvested, non-sacrificial carbon electrodes that do not form a flocculant can be used; these electrodes alter the pH which, following the addition of an electrolyte, such as sodium chloride, aggregates the biomass (Roselet et al., 2019). Alternatively, cells move toward the anode and lose their charge, forming aggregates (Barros et al., 2015). ECF is more energy-efficient for marine cultures than freshwater cultures, and its recovery efficiency is influenced by the type of anode used and the pH, among other parameters (Vandamme et al., 2011). Careful process control is required because water chemistry affects the current density achievable and thereby the flocculation speed and the formation of undesirable compounds. The state of the art of electrocoagulation has been reviewed by Boinpally et al. (2023).

Foam flotation/fractionation: Foam flotation involves the formation of a stable foam to float the biomass. Foam flotation typically involves the addition of surfactants before air injection, creating a foam to lift particles to the surface for removal.

3.5 Centrifugation

Centrifugation utilizes a higher gravitational force through high-speed rotation to increase the settling velocity of microalgae. Both the recovery efficiency and the final solids concentration achieved are high, and the process requires no chemicals. However, fragile species such as *Dunaliella salina* can be damaged due to the high force (Xu et al., 2015). Energy demand is high (up to 20 kWh m^{-3}) but depends on process design and operation (Dassey & Theegala, 2013). Consequently, centrifugation is best used for harvesting high-value biomass from photobioreactors and/or concentrating cells following primary concentration. Depending on the application, several centrifuge designs can be used (e.g., conical plate/disk stacked centrifuge, perforated basket, imperforated basket, decanters, and hydro cyclones).

4 Emerging Harvesting Technologies

Innovative harvesting approaches are under investigation to increase the cost-efficiency of microalgae harvesting. Some of these approaches are discussed below.

4.1 Bioflocculation

Bioflocculation utilizes extracellular polymeric substances (EPS) produced by microalgae and other microorganisms to induce floc formation. EPS include polysaccharides with functional groups capable of bridging cells. EPS-producing organisms can be co-cultured with the microalgae or added prior to harvesting; however, this contaminates the microalgal biomass. The use of bioflocculation for microalgae harvesting was reviewed by Ummalyma et al. (2017).

4.2 Autoflocculation

Autoflocculation, sometimes referred to as self-flocculation, can occur spontaneously due to abiotic and biotic stressors. Although sometimes used to specifically describe pH-induced flocculation, autoflocculation is most generally used to describe any flocculation mechanism that occurs spontaneously without the addition

of a flocculant (excluding chemicals used to alter the pH). While autoflocculation occurs through charge neutralization; high alkalinity and other stressful conditions can also increase the production of cell-bridging EPS. The principle of autoflocculation for microalgae harvesting was reviewed by Li et al. (2021) and Muir et al. (2024).

Another example of autoflocculation is the spontaneous flocculation of cells in response to predators. Microalgae such as *Chlamydomonas, Scenedesmus, Chlorella,* and *Microcystis* indeed flocculate when these sense predators such as Cladocera (e.g., *Daphnia* spp.), rotifers (e.g., *Brachionus calyciflorus*), phagotrophic unicelled euglenoids (e.g., *Peranema trichophorum*), phagotrophic unicelled motile microalgae (e.g., *Ochromonas* sp.), and protozoa (e.g., *Tetrahymena thermophila*) (Becker, 2010; Berger, 2017; Kapsetaki et al., 2016; Lurling & Beekman, 2006; Sathe & Durand, 2015). The mechanisms by which microalgae sense predators (or their products) and initiate flocculation are largely unknown. However, predator-mediated autoflocculation may involve the production of extracellular polysaccharides or incomplete cell division (Muir et al., 2024).

4.3 Ultrasound/Hydrodynamic Cavitation

Ultrasound and hydrodynamic cavitation are techniques that generate and collapse gas bubbles. Collapsing the bubbles physically deteriorates the structural characteristics of the cells attached to them and causes the release of biopolymers (e.g., EPS) triggering flocculation (Zhu et al., 2024). The use of ultrasound and hydrodynamic cavitation at scale may be hampered by high energy inputs (>1 kWh m^{-3}) and physical constraints (Zhu et al., 2024). In addition, these techniques may be better suited for cell disruption and are therefore not discussed further.

4.4 Magnetic Nanoparticle (MNP) Flocculation

Magnetic nanoparticles have applications in many fields, including the removal of pollutants from wastewater. In brief, the application of an external magnetic field magnetizes nanoparticles and allows the "manipulation" of these nanoparticles (and any other particles that are complexed to the nanoparticles).

Several types of materials have been tested for microalgae harvesting with a predominance of iron oxide materials such as magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3). Importantly, the nanoparticle surface needs to be functionalized with compounds such as silica or chitosan to enhance the "binding" between the microalgae and improve the stability of the flocs. Small-scale trials showed that MNPs can remove up to 99% of microalgae cells from solution (Table 3), although performance still needs to be demonstrated under a broad range of conditions.

While MNP harvesting includes several steps (i.e., nanoparticle production/functionalization, auxiliary chemical input for particle recovery, and magnetic separation system), this technique could be more economical than conventional harvesting ($0.07–0.16 kg^{-1} vs. $0.15–0.30 kg^{-1} microalgal biomass, respectively) (Zhu et al., 2024). Unfortunately, the potential introduction of nanoparticles in the biomass means MNP harvesting should be best suited for non-food/feed applications (Malhotra et al., 2020). The separation and recovery of nanoparticles from biomass is possible, although this requires extra steps, such as acid digestion and Fe^{3+} reduction by polyphenols, that would affect the economics and sustainability of the process. Other methods could potentially be used: the company Manta Biofuel has, for example, developed a harvesting system where microalgae form a complex with magnetic particles and are harvested with magnetic discs. The microalgae can be separated from the particles using a three-step process involving the reduction of calcium concentration in the solution, hydrodynamic shears to separate the algae from the particles, and harvesting of the particles with a magnetic separator (see US 11904326 B2 for further details). A full techno-economic analysis is, however, still required.

4.5 Phototaxis

Phototaxis describes the modulation of flagellar beating (i.e., motility) as a response to light sensing, a phenomenon well characterized in *Chlamydomonas reinhardtii* (Martinez Carvajal et al., 2024). This movement of motile microalgae toward or away from light (referred to as positive or negative phototaxis) has been investigated as a low-energy harvesting solution.

Photobioreactors have been designed to take advantage of phototaxis and concentrate the microalgae in specific locations within the reactor. The addition of porous components such as mesh or beads can improve efficiency, as shown by Prakash and Croze (2021) for *C. reinhardtii*. Alternatively, a settler enabling cell exposure to high light intensity at its top was developed to concentrate microalgae at its bottom via negative phototaxis (Nakajima & Takahashi, 1991).

Phototaxis-based harvesting is only applicable for motile species. While providing a low-energy option, the recovery efficiencies currently reported are low (Table 3). In addition, microalgal biology and parameters such as wavelength, intensity, and directionality of light stimuli all influence the photo-response. Consequently, a better knowledge of species-specific phototactic behaviors is needed to optimize the design of reliable and versatile phototaxis-based harvesting systems. Low light penetration in dense cultures and the influence of environmental factors may also limit the implementation of this technology.

5 Key Considerations for Selecting Harvesting Technologies

The selection of an appropriate harvesting method depends on numerous considerations, such as the characteristics of the microalgae cells, water chemistry, environmental conditions, such as light intensity and temperature (which largely depend on design, operation, and location), and the final application considered (which dictates product specification, how much can be afforded, and subsequent downstream processing). The following sections discuss some of the most important parameters.

5.1 Microalgae Characteristics

The characteristics of microalgae cells that influence harvesting efficiency include the cell size, shape, the surface charge, EPS production, motility, and cell features such as the presence of a cell wall. For example, large filamentous algae such as *Arthrospira* and *Aphanizomenon* can be screened easily, but small unicellular algae such as *Chlorella* and *Scenedesmus* must be either floated, centrifuged, or filtrated unless they are first flocculated. Some species are motile and do not readily sediment, such as *Dunaliella* which will move upward toward light (Borowitzka & Borowitzka, 1990), but may then be suitable for phototaxis-based harvesting. EPS-producing cells such as *Arthrospira* may not be suitable for filtration as the EPS may cause excessive membrane fouling (Kurpan et al., 2024), but could be prone to autoflocculation prior to settling. *Dunaliella salina* has no cell wall and is an example of cell that deforms and loses valuable pigments during centrifugation or membrane filtration (Borowitzka & Borowitzka, 1990; Xu et al., 2015). *Chlorella minutissima* cells are lysed by aluminum salts (Papazi et al., 2010) and, therefore, cannot be chemically flocculated using alum. The high ionic strength and salinity of media used to cultivate marine microalgae such as *Chlorella stimatophora* severely limits the use of cationic flocculants but may be highly suitable for electrocoagulation-flotation harvesting.

5.2 Downstream Processing and End-Use

Commercially relevant microalgae species are used for food, animal feed, and cosmetics, with significant research also exploring the potential of utilizing microalgae biomass as a biofuel feedstock. These uses must be considered when selecting a harvesting method as some technologies can damage or contaminate the biomass with chemicals. As a general guideline, the use of chemical flocculants, sacrificial electrodes, and magnetic nanoparticles should be avoided when the biomass harvested is used to produce food and feed. Food-grade chitosan can be

used as a flocculant but is relatively expensive so the use of food-grade sodium hydroxide to induce autoflocculation at high pH could be preferable. In certain cases, damaging cells during harvesting could be beneficial to further processing (e.g., by facilitating subsequent extraction) or the end-use (e.g., by increasing digestibility). This may, however, be detrimental if this causes losses (e.g., if the biomass needs to be washed after harvesting) or reduces product quality (e.g., by causing the oxidation of certain molecules and the generation of bad smells and taste). Low-cost harvesting is paramount for the production of low-value commodities such as biofuel feedstock, but more feasible when biomass quality (and contamination) is less critical.

6 Conclusions

Harvesting microalgae has always been a challenge. This critical processing step requires significant labor, maintenance, energy, chemicals (e.g., flocculants), and capital expenditures. At scale, the cost of harvesting is however expected to be lower, i.e., up to 15%, than the 30% of production costs usually referred to. Currently, "traditional" technologies are still cheaper than "emerging" technologies and they offer better performance. Consequently, harvesting is still mainly performed by traditional technologies such as centrifugation. Staging and technology selection are paramount to reducing cost and impacts of harvesting. This selection also depends on the operational mode of the culture, the biomass characteristics, and the volume of culture to be harvested.

Finally, significant efforts are focusing on the development of materials or techniques to reduce the cost and improve the sustainability of harvesting. The collaboration between algae growers and industries specialized in harvesting is one of the steps needed to improve microalgae harvesting.

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Large-Scale Microalgae Drying



Crisóstomo Barajas-Ferreira, Jefferson E. Contreras-Ropero, Janet B. García-Martínez, Andrés F. Barajas-Solano, and Antonio Zuorro

Abstract Drying harvested algal biomass on a large scale is a critical challenge in the commercial production of algal biomass, as it plays a fundamental role in preserving product quality and the economic efficiency of the process. The unique characteristics of algal biomass, such as its high moisture content, cellular fragility, and susceptibility to the thermal degradation of valuable bioactive compounds, significantly influence the drying process. These particularities require careful selection and optimization of drying technologies to maintain the integrity and quality of the final product. Among direct thermal methods, spray drying is efficient for producing fine powders and is ideal for food and nutraceutical applications, although it involves high costs and potential thermal degradation. Drum drying, which is energy-efficient and has high capacity, can degrade nutrients via direct heat. Solar drying is sustainable and low cost but weather-dependent and time-consuming for indirect thermal methods, making it suitable for sunny regions. Fluidized bed drying provides uniform drying through hot air, preserves bioactive compounds, and is effective for granular materials. Freeze drying, which uses sublimation under vacuum, preserves the structure and bioactivity of microalgae, producing a high-quality product, although it is expensive, slow, and suitable for high-value products. Among emerging technologies, microwave drying offers rapid and uniform drying through microwave energy absorption, although its large-scale implementation is costly and requires specialized equipment. Supercritical drying, which uses supercritical fluids such as carbon dioxide to extract water at low temperatures, preserves heat-sensitive

C. Barajas-Ferreira
Research Center for Sustainable Development in Industry and Energy, Program of Chemical Engineering, Universidad Industrial de Santander, Bucaramanga, Colombia

J. E. Contreras-Ropero · J. B. García-Martínez · A. F. Barajas-Solano (✉)
Department of Environmental Sciences, Universidad Francisco de Paula Santander, Cúcuta, Colombia
e-mail: andresfernandobs@ufps.edu.co

A. Zuorro (✉)
Department of Chemical Engineering, Materials, and Environment, Sapienza University, Rome, Italy
e-mail: antonio.zuorro@uniroma1.it

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compounds and shows excellent potential for the future. This chapter evaluates each method in terms of energy efficiency, final product quality, and environmental impact, allowing the identification of critical characteristics of the methodologies. Additionally, case studies in the food, cosmetic, pharmaceutical, and biofuel industries demonstrate the successful implementation of microalgae drying, enhancing operational efficiency and sustainability. These studies provide valuable insights into best practices and lessons learned.

Keywords Biomass · Microalgae drying technologies · Bioactive compound preservation · Energy efficiency · Sustainable drying methods · Industrial applications.

1 Introduction

Microalgae are unicellular photosynthetic organisms found in both marine and freshwater aquatic environments. These organisms are notable for their taxonomic diversity and ability to accumulate valuable compounds such as lipids, proteins, carbohydrates, and pigments, making them attractive to various industries (Barajas-Solano et al., 2024). The most well-known groups of microalgae include chlorophytes (green algae), diatoms, and red algae, each with unique characteristics in terms of biochemical composition and growth requirements. For example, green microalgae contain chlorophyll a and b, allowing them to perform photosynthesis efficiently (Cancela et al., 2019; Schmid et al., 2022). Diatoms, conversely, contain chlorophyll a and c and fucoxanthin, pigments that give them their characteristic color and allow them to absorb different wavelengths of light, which is crucial for their survival under various light conditions (Zhang et al., 2021). Finally, red algae contain chlorophyll a and phycobiliproteins such as phycoerythrin and phycocyanin, which enable them to absorb light at greater depths where blue and green light penetrate more deeply (Markou, 2020). Several critical factors within the upstream process chain must be optimized for large-scale algal production to be technically and economically viable. Among these factors is the selection of suitable species, as different algal species have varying capacities to accumulate valuable compounds such as lipids, proteins, and pigments. For example, biomass from *Spirulina* sp. and *Chlorella* sp. is widely used in the food industry because of its high protein content and essential nutrients (Contreras-Ropero et al., 2024).

The selection of species also depends on their adaptability to the specific environmental conditions of the cultivation site, which can affect the overall efficiency of the process. Additionally, the optimization of cultivation conditions is another crucial factor. This includes controlling variables such as light, temperature, pH, and nutrient concentration in the culture medium. Research has shown that the intensity and quality of light can significantly influence the growth rate and biochemical composition of microalgae (Barajas-Solano et al., 2024; Guarin-Villegas et al., 2020; Contreras-Ropero et al., 2022). For example, diatoms require high-quality light to maximize their fucoxanthin content, whereas red algae need specific

light conditions to optimize the production of phycoerythrin and phycocyanin (Markou, 2020). In the downstream process chain, harvesting and drying methods absorb the most significant amount of available economic resources, representing up to 70% of the total production cost because of the high energy and technology demand (Gifuni et al., 2019; Mat Aron et al., 2021; Sanchez-Galvis et al., 2020). Standard harvesting processes include centrifugation, a technique widely used for its high efficiency in biomass concentration. However, it is costly and energy intensive (Muhammad et al., 2021). Filtration is another technique that can be more economical but is less efficient for very small microalgae biomass. Flocculation involves the addition of chemical agents that aggregate microalgae cells, facilitating their subsequent separation. This technique can be cost-effective and less energy intensive, but choosing the appropriate flocculant is crucial to avoid contaminating the final product (Sanchez-Galvis et al., 2020).

Drying harvested algal biomass is crucial because of its role in preserving and stabilizing the biomass, helping to prevent degradation during storage. Factors such as moisture and the presence of microorganisms can negatively affect the stability of microalgae, making it essential to implement effective methods to maintain their integrity (Muhammad et al., 2021). Moisture and microbial activity are critical factors that can degrade the quality of microalgal biomass. Effective drying prevents this degradation by inhibiting microbial growth and reducing enzymatic activity, which could break down valuable compounds. Methods such as lyophilization and microwave drying have proven to be particularly effective in preserving the integrity of bioactive compounds (de Oliveira et al., 2020; Chen et al., 2019). Additionally, drying significantly reduces the biomass volume and weight, providing logistical and economic benefits. By removing water, the transport and storage of microalgae are facilitated, reducing the costs associated with these processes (May-Cua et al., 2018).

From an economic perspective, drying represents a significant portion of the total cost of microalgae production. Studies have shown that the operational cost of drying can vary considerably depending on the method used. For example, spray drying and lyophilization have high operational costs due to high energy consumption and the need for specialized equipment, with costs reaching up to $210 per kilogram of dry biomass (Valdovinos-García et al., 2020). In contrast, solar drying, while less costly in terms of operation, may not be viable in regions with low solar radiation, affecting the efficiency and quality of the final product (Schmid et al., 2022). However, the implementation of each drying method depends on various factors. Solar drying is sustainable and low cost and is ideal for regions with high solar radiation, but its efficiency is affected by climatic conditions. Spray drying is quick and efficient, maintaining high quality but expensive in terms of energy and equipment. Lyophilization preserves the structure and bioactivity of compounds, making it ideal for high-value products, although its cost is high and time-consuming. Roller drying is efficient and high-capacity and suitable for large-scale production, although it can degrade nutrients at high temperatures. Finally, microwave drying is fast and allows precise control, maintaining the quality of bioactive compounds, although its initial cost is high (de Oliveira et al., 2020; Chen et al., 2019).

2 Dehydration

Dehydration is crucial in preserving and stabilizing microalgal biomass, which prevents degradation during storage. This process inhibits microbial growth and reduces enzymatic activity, thereby maintaining the integrity of valuable compounds within the algal biomass and facilitating subsequent material handling (Ryckebosch et al., 2011). Dehydration transfers thermal energy to the microalgal biomass, increasing its temperature and allowing water evaporation (Widjaja et al., 2009). This process is critical because the application of sufficient heat causes the conversion of water to vapor, which is then removed from the biomass. Research has demonstrated that heat transfer efficiency depends on the air temperature and relative humidity (Hosseinizand et al., 2018). The optimal temperature range to avoid the degradation of bioactive compounds and ensure drying efficiency is between 60 and 80 °C (Widjaja et al., 2009).

Additionally, the generated water vapor is removed during dehydration, displacing it from the dehydrated material to the surrounding environment. This mass transfer is achieved by creating a vapor pressure gradient, where water moves from areas of high concentration in the biomass to low-concentration regions in the surrounding air (Aziz et al., 2014). A constant and adequate airflow is required to optimize mass transfer and maintain the vapor pressure gradient, thus facilitating efficient dehydration. The airflow rate and temperature are critical factors affecting this transfer, as shown in studies evaluating different drying methods (Widjaja et al., 2009). Constant and controlled airflow ensures that water vapor is effectively removed, maintaining the drying rate and preventing rehydration of the biomass (Aziz et al., 2014). Combining these principles ensures that the dehydration process is both time- and energy-efficient and effective in preserving the quality of microalgal biomass (Silva et al., 2020).

2.1 Factors Affecting Dehydration

Several factors can influence the efficiency and effectiveness of the microalgae dehydration process. The following describes these factors:

- **Air temperature**. Higher temperatures increase the capacity of air to absorb moisture, accelerating dehydration. However, excessively high temperatures can cause the degradation of heat-sensitive compounds, such as certain pigments and proteins. One study reported that dehydrating *Chlorella vulgaris* at temperatures between 60 and 80 °C better preserves bioactive compounds, whereas temperatures above 100 °C can cause significant damage (Hosseinizand et al., 2018).
- **Relative humidity of the air**. Air with lower relative humidity can absorb more water vapor, improving drying efficiency. In environments with high relative humidity, the capacity of air to absorb additional moisture is reduced, slowing the drying process. A previous study indicated that maintaining low relative humidity during microalgae drying significantly improved the evaporation rate (Widjaja et al., 2009).

- **Air velocity**. Faster airflow helps remove water vapor from the surface of the microalgae, maintaining constant evaporation. However, excessively high air velocities can cause material losses due to turbulence. A study on drying *Spirulina platensis* in a rotary dryer revealed that moderate air velocity is optimal for balancing drying efficiency and retention of bioactive compounds (Silva et al., 2020).
- **Characteristics of the algal biomass**. The particle size, cell structure, and initial moisture content of the microalgae also influence the dehydration rate. Microalgae with denser cell structures and higher initial moisture contents require more time and energy to dry completely. A comparative study of different microalgal species revealed that species with more minor, less dense cells dry more quickly than those with more complex cell structures do (Chen et al., 2015).

2.2 Stages of the Dehydration of the Algal Biomass

The dehydration process of microalgae can be divided into several key stages, each with objectives and challenges.

- **Preliminary Heating Phase**: In this initial stage, the microalgal biomass and the surrounding air are heated to reach a stable temperature. This initial heating is crucial for efficient water evaporation in the subsequent phases. Hosseinizand et al. (2018) reported that preliminary heating at moderate temperatures helps maintain the cellular structure of the microalga *Chlorella vulgaris*, which is vital for preserving the quality of the bioactive compounds present in the biomass.
- **Constant Rate Drying Phase**: During this phase, a steady flow of heat is maintained toward the microalgal biomass, facilitating the continuous evaporation of free water. Most free water evaporates at this stage, keeping the temperature and airflow constant to maximize the drying efficiency. The generated water vapor is continuously removed by adequate airflow, maintaining the efficiency of the process. Schmid et al. (2022) demonstrated that maintaining a constant and proper airflow is crucial for the drying efficiency and quality of the final product (Schmid et al., 2022).
- **Falling Rate Drying Phase**: The drying rate decreases as bound water is removed. Bound water is more firmly attached to the cellular structures of microalgae, requiring more energy for evaporation. In this phase, precise control of the temperature and airflow is needed to continue the dehydration process without damaging the quality of the final product. Silva et al. (2020) reported that proper control of drying conditions, such as temperature and air velocity, is essential to avoid the loss of bioactive compounds during the removal of bound water in *Spirulina platensis*.

2.3 Applications of Microalgal Dehydration

- The pharmaceutical industry has found that microalgal dehydration is an effective technique for preserving bioactive compounds with therapeutic and nutritional applications. Microalgae such as *Spirulina platensis* contain high levels of proteins, vitamins, and antioxidants that are valuable for dietary supplements and pharmaceutical products. A study by Silva et al. (2023) demonstrated that the use of refractance window (RW) dryers to dehydrate *Spirulina platensis* maintains high levels of phenolic compounds and flavonoids, which are essential for the antioxidant properties of the final product (Silva et al., 2023). Silva et al. (2020) also evaluated a nonconventional rotary dryer and reported that this method significantly preserves bioactive compounds, which is crucial for pharmaceutical applications where product stability is paramount (Silva et al., 2020).
- Dehydrated microalgae are widely used in the cosmetic industry because of their antioxidant and rejuvenating properties. Microalgae such as *Chlorella vulgaris* are rich in chlorophyll, carotenoids, and vitamins, which are critical ingredients in antiaging and skin-regenerating products. A study by Stramarkou et al. (2017) revealed that freeze-drying *Chlorella vulgaris* preserves high levels of carotenoids and chlorophyll, making it beneficial for cosmetic formulations (Stramarkou et al., 2017). Additionally, García-Vaquero et al. (2020) reported that conventional extraction methods, such as maceration and steam distillation, combined with dehydration, are effective for obtaining high-purity bioactive compounds in premium cosmetics (Garcia-Vaquero et al., 2020).
- Microalgae dehydration is particularly relevant in the food industry, where they are used as nutritional supplements and functional additives. *Spirulina* and *Chlorella* are popular because of their high protein and antioxidant contents. A study by Dehnad et al. (2016) reviewed various dehydration techniques and reported that spray drying and fluidized bed drying are effective methods for preserving the functional properties of microalgae, making these methods ideal for use in food products (Dehnad et al., 2016).
- The biofuel industry also benefits from the microalgae dehydration process. Microalgae can accumulate large amounts of lipids, which can be converted into biodiesel. To maximize lipid extraction efficiency, reducing the moisture content of the microalgal biomass is crucial. A study by Liang et al. (2012) highlighted that proper dehydration is essential for optimizing lipid extraction from microalgae, thus improving biodiesel production (Liang et al., 2012). Additionally, Zainith et al. (2021) reported that dehydrated microalgae can be effectively used for biofuel production, thereby reducing reliance on fossil fuels.

3 Solar Drying

The solar drying of microalgae is a process that uses the sun's radiant energy to remove water from microalgae on the basis of the principles of solar radiation and natural convection. Solar radiation is the electromagnetic energy that directly heats the microalgae and the surrounding air, facilitating the evaporation of the water in them (Schmid et al., 2022). Simultaneously, natural convection, which involves the movement of heated air, transports the evaporated moisture and maintains a constant flow of dry air around the microalgae (Hosseinizand et al., 2018). This process involves a complex interaction between heat and mass transfer. Solar energy heats the microalgae, increasing the vapor pressure of the water inside them. Once this pressure exceeds that of the surrounding air, evaporation occurs, and the moving air transports the moisture away from the microalgae, continuing the drying process (Valdovinos-García et al., 2020). Innovations in the design and optimization of microalgae solar dryers have improved the efficiency and economic viability of these processes in various industries. In India, an intelligent solar dryer integrating IoT and machine learning was developed, improving the energy efficiency by 25% and reducing the drying time by 15% through automatic parameter adjustment, optimizing the process, and ensuring the quality of the microalgal biomass (Valdovinos-García et al., 2020). Similarly, in Brazil, a hybrid solar dryer was used to dry *Spirulina*, combining solar energy and waste heat recovery, providing an efficient and sustainable solution for large-scale microalgae drying (May-Cua et al., 2018).

3.1 Types of Solar Dryers

Several types of solar dryers are suitable for microalgae, each with specific characteristics that affect their performance and critical variables that must be considered when optimizing their operation. In direct solar dryers, microalgae are exposed directly to solar radiation. Examples of these dryers include open trays and cabinet dryers. The main advantages of this type of dryer are its simple design and low cost. However, they present disadvantages, such as potential degradation by UV light and the risk of contamination. UV light degradation can reduce the quality of bioactive compounds in microalgae, whereas exposure to an open environment increases the risk of contamination by dust and other external agents (Schmid et al., 2022). The most essential variable for direct solar dryers is the intensity of solar radiation, which affects the amount of energy available for drying. For example, open trays in regions with high solar radiation have significantly reduced drying times (Schmid et al., 2022).

- Indirect solar dryers use solar radiation to heat the air, which then passes through the microalgae. Examples of these dryers are tunnel dryers and greenhouse dryers. The advantages of these systems include better quality control and a

lower risk of contamination, as the microalgae are not directly exposed to the external environment. However, these dryers are more complex and costly to build and operate (Caceres et al., 2021). The most critical variable for these dryers is the wind speed, which affects the renewal capacity of hot air and drying efficiency. A practical example is tunnel dryers in windy regions, where high wind speeds significantly reduce the drying time (May-Cua et al., 2018).

- Mixed solar dryers combine the elements of direct and indirect systems. These dryers offer greater operational flexibility and improved efficiency, although their design is more complex and has higher initial costs. The combination of direct and indirect exposure allows better temperature and humidity control, optimizing the drying process. The most essential variables for mixed dryers are the intensity of solar radiation and wind speed, which influence drying efficiency (Agbede et al., 2020). An example is mixed solar dryers in tropical climates, where efficient drying has been achieved even under high humidity conditions (Al-Saadi et al., 2023).

3.2 Factors Influencing Microalgal Solar Drying

Various factors affect the efficiency of the solar drying process for microalgae, such as the intensity of solar radiation, ambient temperature, relative humidity of the air, wind speed, and characteristics of the microalgae.

- **The intensity of solar radiation** varies according to geographical location, season, and atmospheric conditions. A higher solar radiation intensity provides more energy for water evaporation, improving the drying efficiency. In regions with high solar radiation, such as the western desert in Egypt, indirect solar dryers significantly reduce the drying time (Schmid et al., 2022). However, in geographical areas with low solar radiation, it may be necessary to use hybrid solar dryers or assist with additional energy sources to maintain efficiency (Valdovinos-García et al., 2020).
- **The ambient temperature** influences the capacity of air to absorb moisture; higher temperatures increase the drying capacity of air. For example, direct solar drying in warm climates such as Australia has shown excellent cost efficiency because high temperatures favor rapid evaporation (Al-Ismaili, 2021).
- **The relative humidity** of the air directly affects the evaporation rate; drier air can absorb more moisture from the microalgae, accelerating the drying process. In places with high relative humidity, such as Malaysia, mixed solar dryers are needed to compensate for the low drying capacity of humid air, resulting in better performance (Valdovinos-García et al., 2020).
- **The wind speed** impacts the rate of renewal of moist air around the microalgae. A higher airflow helps to remove evaporated moisture, quickly improving drying efficiency. Studies on tunnel solar dryers in windy regions have shown that higher wind speeds can significantly reduce drying times (Hosseinizand et al., 2018).

- **Characteristics of the algal biomass.** The particle size, cell structure, and initial moisture content of the microalgae also affect the drying rate. Microalgae with more compact cell structures and higher initial moisture contents require more drying time. For example, the drying time of *Chlorella vulgaris*, which has a high initial moisture content, significantly decreased when nanoparticles were used to improve mass transfer (Chen et al., 2019).

3.3 Design Considerations for Solar Dryers for Microalgal Biomass

When designing a solar dryer for microalgae, it is crucial to consider several factors to optimize performance and adaptability. The loading capacity determines the volume of microalgae that can be dried per batch. Properly sizing the loading capacity is essential to maximize the system's efficiency and productivity. A higher loading capacity allows more microalgae to be processed in a single drying cycle, increasing overall production. However, an excessive loading capacity can lead to uneven heat and airflow distributions, reducing drying efficiency (Agbede et al., 2020).

The thermal efficiency maximizes the capture and retention of solar heat. Using materials with high thermal conductivity and good thermal insulation can significantly improve the efficiency of a solar dryer. The thermal efficiency ensures that the heat captured from the sun is effectively used to evaporate the water from the microalgae. A well-insulated system minimizes heat losses, which can reduce drying time and operational costs (Chen et al., 2019). Additionally, optimized airflow is crucial for the circulation of hot air and the efficient removal of moisture. The design of the drying chamber and the use of solar fans can improve air circulation and drying efficiency. Adequate airflow ensures that the evaporated moisture is transported out of the drying chamber, maintaining a dry environment for the microalgae. Insufficient airflow can lead to uneven drying and the formation of wet spots, whereas excessive airflow can increase energy consumption (Schmid et al., 2022).

Furthermore, temperature control is essential to avoid overheating, which can damage microalgae. Automated control systems and continuous temperature monitoring are fundamental to maintaining optimal drying conditions. Precise temperature control is crucial to prevent the degradation of sensitive compounds in microalgae. An effective temperature control system can dynamically adjust the drying conditions to maximize efficiency and product quality (Al-Saadi et al., 2023). On the other hand, protection against UV radiation is vital to minimize the degradation of light-sensitive components. Protective materials and coatings can safeguard microalgae from UV light degradation. UV exposure can degrade the bioactive compounds in microalgae, reducing their quality and value. Implementing practical UV barriers can help maintain the integrity of bioactive compounds during drying (Valdovinos-García et al., 2020).

Moreover, ease of loading and unloading simplifies operations and reduces downtime. This design should allow easy and quick handling of microalgae to improve their operational efficiency. Easy loading and unloading enable more efficient handling of large biomass volumes, reducing preparation time and increasing overall productivity (Chen et al., 2019). Finally, scalability allows for future expansion according to production needs. The modular and scalable design of the solar dryer can facilitate its adaptation to different production volumes and operating conditions. Scalability ensures that the system can grow alongside production demands, providing a flexible and efficient solution for large-scale drying operations (Valdovinos-García et al., 2020).

3.4 Industrial Applications of Solar-Dried Microalgal Biomass

- **Food Industry:** Solar drying of microalgae has been successfully implemented to preserve biomass rich in proteins, lipids, and other essential bioactive compounds. A recent study evaluated the indirect and hybrid solar drying of *Tetraselmis chui* and *Nannochloropsis oceanica*. The results revealed no significant differences in the biochemical profiles between the drying technologies applied, with solar drying being the most economical (Schmid et al., 2022). Additionally, an autonomous parabolic solar collector was designed for drying *Spirulina* sp. and *Nostoc sphaericum*, achieving a processing capacity of 5 kg of wet microalgae while preserving their high protein concentration, which is crucial for use in food products (Caceres et al., 2021).
- **Cosmetic Industry:** Microalgae are valued for their bioactive compounds, such as antioxidants, vitamins, and essential fatty acids. Solar drying preserves these compounds, improving the stability and quality of the final products. A solar dryer was designed for *Spirulina platensis*, allowing the biomass to be dehydrated to a moisture content below 10% while preserving bioactive compounds and maintaining the integrity of antioxidants and other essential compounds (Valdovinos-García et al., 2020). Additionally, the solar drying of *Chlorella vulgaris* significantly reduces drying time and improves energy efficiency, which is crucial for maintaining the quality of cosmetic products (Hosseinizand et al., 2018).
- **Energy Industry:** Microalgae are used as raw materials for biofuel production. In Mexico, a technoeconomic evaluation was conducted on the use of *Chlorella vulgaris* for CO_2 capture in a thermal power plant, with operational costs ranging between $4.75 and $6.55/kg dry biomass, indicating that solar drying is a more economical and energy-efficient option (Valdovinos-García et al., 2020). In another study, the hydrothermal processing of microalgae for bio-oil production was investigated, and the results demonstrated that solar drying before this treatment is crucial for reducing moisture content and improving process efficiency (Vlaskin et al., 2020).

- **Pharmaceutical Industry:** Solar drying of microalgae is fundamental because it preserves bioactive compounds with therapeutic and nutritional properties. A recent study evaluated indirect solar drying for *Spirulina platensis*, allowing the biomass to be dehydrated to a moisture content below 10% while preserving bioactive compounds, making it an exciting methodology for the pharmaceutical industry (Hosseinizand et al., 2018). Additionally, a hybrid solar dryer was developed for drying *Spirulina*, combining solar energy and waste heat recovery, which allowed for continuous operation and reduced energy costs by 40% (May-Cua et al., 2018).

4 Spray Drying

Spray drying is a dehydration method that transforms a liquid mixture into a dry powder by atomizing the mixture in a stream of hot air. This highly efficient process allows control over the final product's characteristics, making it widely used in the food and pharmaceutical industries (Chen et al., 2015). The fundamental principles of spray drying involve atomizing the microalgae suspension into tiny droplets through an atomizer, which immediately encounters a hot air stream. This contact causes rapid evaporation of the water and the formation of dry particles (Park et al., 2020). Atomization can be carried out via different atomizers, such as centrifugal and nozzle atomizers, each with advantages and specific applications. Centrifugal atomizers use centrifugal force to create fine droplets from the liquid suspension, whereas nozzle atomizers work by forcing the liquid through a narrow opening, creating a dispersion of droplets (Hosseinizand et al., 2018). The choice of atomizer type depends on the properties of the liquid to be dried and the desired final product. For example, the use of centrifugal atomizers has proven effective in producing microalgae powders with a uniform particle size distribution, improving the rehydration and stability of the final product (Vilatte et al., 2023). Additionally, advancements in atomization technology, such as the development of high-precision nozzles, have allowed greater control over droplet size, further optimizing the drying process (Shi et al., 2020).

4.1 Types of Equipment and System Configurations

Several types of spray drying equipment are designed for specific applications and have different system configurations. The most common spray dryers are single-stage and two-stage spray dryers, each with advantages and applications.

- **Single-stage dryers**: The atomized droplets are entirely dried in a single pass through the hot air stream. This type of dryer is suitable for products that are not heat sensitive and require a rapid drying process. The main advantage of single-

stage dryers is their simplicity and efficiency, as the entire process is completed in one step. However, they may not be suitable for products that require more precise control over the final moisture content and granulation. These dryers are also energy efficient and have lower operating costs (Chen et al., 2015).

- **Two-stage dryers**: A combination of rapid initial drying and slower final drying is used to achieve a product with specific moisture and granulation characteristics. In the first stage, most of the water evaporates quickly, whereas in the second stage, drying is completed at a lower temperature in a more controlled manner. This is useful for heat-sensitive products, such as certain microalgae, where it is vital for maintaining the integrity of bioactive compounds. Using two-stage dryers in *Chlorella* production has improved nutrient retention and the quality of the final powder, making it more suitable for use in dietary supplements (Nespi et al., 2021). These dryers can also significantly reduce operating costs and improve energy efficiency (Ryckebosch et al., 2011).
- **Continuous flow and batch dryers:** Another necessary configuration is the distinction between constant flow and batch dryers. Continuous flow dryers are ideal for large-scale industrial processes requiring constant, high-volume production. In contrast, batch dryers are more suitable for small productions or when process flexibility is needed. A study comparing both systems revealed that while continuous-flow dryers have a relatively high production capacity, batch dryers offer better control over the final product characteristics, such as moisture and granulation (Biz et al., 2019). Additionally, batch dryers allow easier adjustments in operating conditions, which is beneficial for researching and developing new products (Bonilla-Ahumada et al., 2018).

4.2 Critical Operating Parameters

Precise control of the operating parameters is essential to ensure the efficiency and quality of spray drying. As shown in Table 1, the main parameters to consider are temperature, airflow, and the size of the atomized droplets.

- **Temperature control**: The hot air temperature must be high enough to allow rapid evaporation of water but not high enough to degrade the bioactive compounds of the microalgae. The optimal temperature range depends on the type of

Table 1 Critical operating parameters for spray drying of microalgae

Parameter	Optimal range	Determining factors	References
Temperature	Inlet: 140–220 °C Outlet: 40–65 °C	Microalgae type, solids content, thermal stability	Zhang et al. (2021)
Airflow	2–5 m^3/min	Nozzle position, chamber geometry, humidity	Biz et al. (2019)
Droplet size	10–50 μm	Atomizer type, liquid viscosity, differential pressure	Konar et al. (2023)

microalgae and the desired final product. For *Chlorella vulgaris*, an inlet air temperature of approximately 140 °C and an outlet air temperature of 40–60 °C are recommended (Hosseinizand et al., 2018). Researchers have improved this parameter via advanced temperature control systems that allow precise and real-time adjustments, ensuring the quality and stability of the final product (Shao et al., 2018).

- **Airflow**: Airflow must be controlled to ensure a constant and efficient evaporation rate. Adequate airflow ensures that the atomized droplets remain suspended long enough to dry completely before reaching the drying chamber wall. Too little airflow can result in partially dried droplets, whereas excessive airflow can lead to particle aggregation (Viswanathan et al., 2011). Researchers have developed computational models to optimize airflow in spray dryers, improving process efficiency and reducing variability in the final particle size (Valdovinos-García et al., 2020).
- **Droplet size**: The size of the atomized droplets influences the drying rate and the final product characteristics. Smaller droplets have a larger surface area in contact with hot air, allowing faster evaporation. However, too few droplets can lead to more significant product loss as a fine powder. Droplet size control is achieved by adjusting the pressure and type of atomizer used (Hosseinizand et al., 2018). Optimizing droplet size through adjustable nozzle technology has significantly improved the uniformity and quality of the produced powder (Homayoonfal et al., 2024).

4.3 Applications of Spray-Dried Microalgal Biomass

Spray drying has become a versatile and efficient technique for dehydrating microalgae and has been applied in various industries because of its ability to produce fine and uniform powders with specific characteristics. Below are some of the main applications of spray drying in the cosmetic, pharmaceutical, and food industries, supported by case studies and recent research.

- In the cosmetic industry, spray-dried microalgae are primarily used for their antioxidant and nutritional properties, which benefit the skin. *Spirulina* and *Chlorella* are two commonly used microalgae in cosmetic products because of their high contents of vitamins, minerals, and bioactive compounds. A study conducted in France evaluated the effectiveness of spray-dried Spirulina as an ingredient in antiaging creams. The results showed that Spirulina retained its antioxidant and essential nutrient contents after drying, contributing to improved skin hydration and elasticity in the study participants (Couteau & Coiffard, 2018). Moreover, spray drying produced a fine powder that was easy to incorporate into cosmetic formulations, enhancing the final product's homogeneity and stability.

  Similarly, a study in South Korea reported positive results when the inclusion of spray-dried Chlorella in cosmetic products was investigated. Research has

shown that *Chlorella* sp. retained its antioxidant properties and significantly improved skin moisture retention (Martínez-Ruiz et al., 2022). Another study in Brazil focused on the stability of bioactive compounds in Spirulina after spray drying and their application in cosmetic products. The results indicated that spray drying effectively preserved phycocyanin, a critical antioxidant pigment, thereby maintaining the antiaging properties of the resulting products (De Luca et al., 2021).

- In the pharmaceutical industry, spray-dried microalgae produce nutritional supplements and medications. The bioactive properties of microalgae, such as the presence of omega-3 fatty acids, proteins, and antioxidants, make them valuable ingredients in pharmaceutical product formulations. A study in Japan evaluated the efficacy of spray drying in the production of omega-3 supplements from *Nannochloropsis* sp. biomass. The results indicated that, compared with other drying methods, spray drying preserved omega-3 fatty acid levels and improved the bioavailability of these compounds (Vilatte et al., 2023). Additionally, the process yielded a fine and homogeneous powder, facilitating its encapsulation and oral administration. Similarly, a study in the United States explored the use of spray drying to stabilize antioxidants in Spirulina-based supplements. The results revealed that antioxidant compounds remained stable throughout the process, increasing the quality and efficacy of the resulting supplements (Bonilla-Ahumada et al., 2018).
- In the food industry, spray-dried microalgae are used as functional ingredients in various products, including beverages, energy bars, and dietary supplements. The high protein, vitamin, and mineral contents of these products make them ideal superfoods for diet enrichment. A study in Spain investigated the incorporation of spray-dried Chlorella in functional beverages. The results revealed that spray drying preserved the bioactive compounds of Chlorella, such as chlorophyll and B vitamins, and improved the nutritional profile of the beverages without negatively affecting their taste or texture (Biz et al., 2019). Additionally, the homogeneity of the Chlorella powder facilitated its dissolution in the liquid matrix of the beverages, ensuring a uniform distribution of nutrients. Similarly, a study in the Netherlands evaluated the incorporation of Spirulina in energy bars. These findings indicated that spray-dried Spirulina significantly improved the nutritional value and stability of the product, making it more appealing to health-conscious consumers (Park et al., 2020). Furthermore, a study in Canada explored the use of spray-dried microalgae as an ingredient in dietary supplements. The results showed that the drying process preserved essential nutrients and improved supplement bioavailability, offering consumers a healthy and efficient alternative (Vieira et al., 2020).
- The animal feed and aquaculture industry also benefits from the use of spray-dried microalgae. Microalgae are rich in essential nutrients for feeding fish and other aquatic organisms, increasing their growth and health. A study in Norway investigated the supplementation of salmon diets with spray-dried *Nannochloropsis* biomass. The results revealed improvements in the development and quality of salmon fillets, highlighting the effectiveness of microalgae as

a dietary supplement (Yang et al., 2022). Additionally, a study in China evaluated the use of spray-dried *Chlorella* in shrimp diets. The results indicated a significant improvement in shrimp survival rates and growth, underscoring the viability of spray-dried microalgae in aquaculture (Santiesteban-Romero et al., 2022). Therefore, spray-dried microalgae in the animal feed and aquaculture industry enhance aquatic organism growth and health and offer a sustainable and nutritious feeding solution.

- In the agricultural industry, spray-dried microalgae are used as biofertilizers and biostimulants. High nutrient and bioactive compound contents promote plant growth and improve soil health. A study in Italy evaluated the effectiveness of spray-dried Chlorella as a biofertilizer in tomato crops. A study in Mexico investigated the use of spray-dried Spirulina as a biostimulant in maize crops. The results indicated an increase in plant height and grain yield, highlighting the effectiveness of *Spirulina* as a biostimulant (Vieira et al., 2020).

5 Freeze Drying

Lyophilization, also known as freeze drying, is a drying method that involves the removal of water from a frozen material through sublimation (Stirk et al., 2020). This process is based on three fundamental principles: freezing, primary sublimation, and secondary desorption. During freezing, the material is rapidly frozen to form ice crystals. In the primary sublimation phase, the ice is converted directly to vapor without passing through the liquid phase. Finally, secondary desorption removes the remaining bound water through gentle heating (Hosseinizand et al., 2018). For microalgae, the freeze drying process involves several critical phases, each crucial in preserving the biomass. The preparation phase consists of concentrating the biomass through centrifugation or filtration and, in some cases, adding cryoprotectants to protect the cells during freezing. Rapid freezing is essential for the formation of tiny ice crystals between −40 and −80 °C, which are crucial for maintaining cellular integrity (Stirk et al., 2020). During the primary sublimation phase, pressure is subsequently reduced, and gentle heat is applied to convert the ice to vapor, creating a porous structure in the microalgae matrix. Controlling the temperature and pressure during this phase prevents the collapse of the cellular structure. Then, in the secondary desorption phase, the remaining bound water is removed by gradually increasing the temperature under vacuum, optimizing the preservation of thermosensitive compounds (Ryckebosch et al., 2011). Finally, during the storage phase, the freeze-dried product is sealed under vacuum or in an inert atmosphere to prevent rehydration and maintain low water activity, which is essential for long-term stability (Hosseinizand et al., 2018). In this context, the freeze drying of microalgae offers unique advantages because of its ability to preserve the cellular structure and heat-sensitive bioactive components (Liu et al., 2022). This method is useful for preserving bioactive compounds such as pigments and antioxidants, which are highly susceptible to thermal degradation (Hosseinizand et al., 2018).

5.1 Types of Equipment and System Configurations

In microalgae freeze-drying (lyophilization) processing, various types of equipment are employed, each designed to meet specific requirements. The most commonly used methods include:

- **Tray lyophilizers**: These are the most common and are used for processing medium to large batches. Tray lyophilizers can handle multiple trays simultaneously, making them ideal for microalgae in the form of paste or thin sheets. This design ensures uniform heat distribution, ensuring that the entire biomass is adequately treated. Liu et al. (2022) validated the efficiency of these lyophilizers in drying food products, highlighting their ability to maintain cellular integrity and preserve bioactive compounds (Liu et al., 2022).
- **Manifold lyophilizers**: These are used for smaller volumes or samples in vials, making them suitable for research and developing new microalgal strains. These systems allow precise control of drying conditions in small quantities, which is crucial for scientific research. Shivkumar et al. (2019) emphasized the versatility of these lyophilizers in experimental studies, underscoring their ability to maintain stable and reproducible conditions in small volumes (Shivkumar et al., 2019).
- **Continuous lyophilizers**: These are designed for large-scale production and utilize conveyor belts or rotating drums. However, they are less common in the microalgae industry because of scalability challenges and costs. Nevertheless, their application in other industrial sectors has proven effective in continuously producing dehydrated products. Sickert et al. (2023) noted that while these lyophilizers are highly productive, their implementation in the microalgae industry requires significant adaptations to manage biomass variability and volume (Sickert et al., 2023).

5.2 Critical Operating Parameters

The critical operating parameters of microalgae freeze drying (lyophilization) are fundamental to ensuring process efficiency and the quality of the final product. As shown in Table 2, the critical parameters for freeze drying include the freezing temperature, chamber pressure, condenser temperature, heating rate, and total process time.

- **Freezing temperature**: Rapid freezing between −40 and −80 °C is essential for the formation of tiny ice crystals that preserve cellular integrity. In contrast, slow freezing can result in large crystals that damage cells, negatively impacting the final product's quality (Stirk et al., 2020).
- **Chamber pressure**: The chamber pressure must be maintained below the triple point of water (6.1 mbar) to allow for ice sublimation. This adequate pressure

Table 2 Critical operating parameters for microalgae freeze drying

Parameter	Optimal range	Influencing variables	References
Freezing temperature	−40 °C to −80 °C	Freezing rate, ice crystal size, cell integrity	de Oliveira et al. (2020)
Chamber pressure	<6.1 mbar	Balance between sublimation and structural collapse of cells	Vilas et al. (2020)
Condenser temperature	<−50 °C	Efficient water vapor capture, prevention of recondensation	Schmid et al. (2022)
Heating rate	<1 °C	Prevention of structural collapse, protein stability	Stramarkou et al. (2017)

facilitates the direct conversion of ice to vapor, preventing structural collapse of the cells and ensuring complete dehydration (Ryckebosch et al., 2011).

- **Condenser temperature**: The condenser temperature, which must be significantly lower than that of the product, is generally below −50 °C, ensures efficient water vapor capture during sublimation, and prevents recondensation of the product (Stirk et al., 2020).
- **Heating rate**: Keeping the heating rate below 1 °C/min during primary sublimation is crucial to avoid the structural collapse of cells and protein denaturation. Rapid heating can cause irreversible damage to biomass (Stirk et al., 2020).
- **Total process time**: The total process time varies depending on the microalgal species and sample volume; insufficient time can result in incomplete dehydration, whereas excessive time can increase costs and contamination risks (Hosseinizand et al., 2018).

5.3 Applications of Freeze-Dried Microalgal Biomass in Industry

Freeze drying has become a versatile and efficient technique for dehydrating microalgae, with applications across various industries due to its ability to produce fine, uniform powders with specific characteristics. This technique has significant applications in the food, cosmetic, pharmaceutical, and nutraceutical industries, each of which is supported by recent research.

- Freeze drying of microalgae is widely utilized in the food industry because of its ability to preserve essential nutrients and bioactive compounds. Microalgae such as *Spirulina* and *Chlorella* are dietary supplements rich in proteins, vitamins, and antioxidants. For example, research by Liu et al. (2022) demonstrated that freeze-dried microalgae such as *Tetraselmis chui* and *Nannochloropsis oceanica* maintain high levels of proteins, lipids, and pigments, making these products suitable for food and feed applications (Liu et al., 2022). Additionally, Raja et al. (2018) highlighted that freeze-dried microalgae, such as *Spirulina* and *Haematococcus*, are used in the food industry to enrich products with β-carotene and other

antioxidants, providing significant nutritional benefits (Raja et al., 2018). Similarly, Amorim et al. (2020) investigated the impact of different drying processes on the antioxidant properties and chemical constituents of tropical macroalgae and reported that freeze drying better preserves functional compounds than other drying methods do (Amorim et al., 2020).

- The cosmetic industry benefits from the freeze drying of microalgae because of their high content of bioactive compounds, such as proteins, lipids, and vitamins. Microalgae such as *Chlorella* and *Dunaliella* are used in cosmetic formulations because of their moisturizing, antioxidant, and rejuvenating properties. Couteau and Coiffard (2018) reported the use of microalgae in cosmetics to increase skin hydration and firmness (Couteau & Coiffard, 2018). De Luca et al. (2021) also explored the use of microalgal lipids in the cosmetic industry, highlighting their role in formulating products such as moisturizers, emollients, and fragrance carriers (De Luca et al., 2021). Furthermore, Vieira et al. (2020) reviewed microalgae encapsulation systems for cosmetic applications, emphasizing how these techniques improve the stability and bioavailability of bioactive compounds in microalgae (Vieira et al., 2020).
- In the pharmaceutical industry, freeze-dried microalgae are utilized to develop new drugs and supplements because of their anti-inflammatory, anticancer, and antiviral properties. Udayan et al. (2021) highlighted the use of microalgae in the production of bioactive compounds for use in medications and nutritional supplements (Udayan et al., 2021). Moreover, Mehariya et al. (2021) reviewed the applications of microalgae in the pharmaceutical industry, noting their potential to replace conventional chemical compounds and enhance the sustainability of drug production (Mehariya et al., 2021). Finally, Pardeshi et al. (2022) explored thin-film freezing (TFF) technology for dry powder inhalable formulations, demonstrating its effectiveness in preserving therapeutic compounds in freeze-dried microalgae (Pardeshi et al., 2022).

6 Current Challenges and Future Perspectives

Microalgae drying methods present various technical and economic challenges. Solar drying faces limitations in regions with low solar radiation and adverse climatic conditions, restricting its universal application. While it is a low-cost option, its efficiency can be compromised by environmental factors, requiring optimized design and management to maximize performance (Schmid et al., 2022). Additionally, implementing hybrid systems can mitigate some of these challenges by combining solar energy with other renewable sources (Caceres et al., 2021). Spray drying, though efficient in terms of time, faces challenges because of its high energy consumption and associated costs. Optimizing atomization and temperature control is crucial for improving efficiency and reducing energy consumption. Comparative studies have shown that heat recovery techniques can reduce energy consumption by 15–20% (Valdovinos-García et al., 2020).

Moreover, the integration of advanced technologies can increase the economic viability of this method (Vilatte et al., 2023). On the other hand, while freeze drying is the most effective method for preserving product quality, it is also among the most expensive and energy-intensive methods. The need for specialized equipment and high energy consumption during the freezing and sublimation stages represent significant economic barriers. However, studies suggest the use of more efficient refrigerants and advanced control technologies that optimize the sublimation process. These innovations are crucial for addressing current challenges and ensuring the sustainable development of these drying methods (Bhatta et al., 2020). Therefore, market trends and technological innovations are driving the growth of more efficient and sustainable microalgae drying methods capable of meeting the growing demand for high-quality products. These advances improve the economic viability of microalgae production and contribute to environmental sustainability and the diversification of industrial, food, and pharmaceutical applications (Soares et al., 2019; Herrera et al., 2021).

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Building-Integrated Microalgae Photobioreactor: An Adaptive Solution to Urban Heat Island Mitigation



Maryam Talaei, Mohammadjavad Mahdavinejad, and Hadi Motevali Haghighi

Abstract Building envelopes and their materials can play a considerable role in mitigating the urban heat island (UHI) phenomenon as one of the crucial challenges of human life. Acting as an interface between inside and outside, building envelopes have not only an essential role in enhancing the sustainability-oriented performance of buildings but also in UHI mitigation (UHIM) by providing energy efficiency for building and controlling heat/light absorption. Accordingly, adopting suitable facade technologies can noticeably contribute to improving buildings' environmental condition and alleviating UHI. Meanwhile, integrating microalgae photobioreactor (PBR) with the building envelope, known as microalgae bioadaptive façade (MBF), is regarded as a newly emerged biofaçade technology with great potential to enhance the capacity of buildings to meet UHI and its adverse environmental impacts. However, this issue has not been addressed by previous research, comprehensively.

This chapter is to examine and categorize different potential aspects of MBF as an adaptive strategy to reduce the UHI effect and analyze its performance in comparison to common green wall systems. In light of the conclusions achieved during the course of the research, it was discussed that MBF prospects for UHI alleviation can be considered as providing energy efficiency through biomass production, (adaptable) shading and thermal insulation, as well as air purification. Besides, the role of MBF as the building's second skin in providing natural ventilation and controlling wind flow can be considered potentially in regulating the building's thermal load.

Keywords Microalgae photobioreactor · Microalgae bioadaptive façade · Urban heat island (UHI) · Energy-efficient building · Building envelope

M. Talaei (✉) · H. M. Haghighi
Faculty of Architecture and Urbanism, Ferdowsi University of Mashhad, Mashhad, Iran
e-mail: m.talaei@ferdowsi.um.ac.ir

M. Mahdavinejad
College of Engineering and Architecture, University of Nizwa, Nizwa, Oman

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1 Introduction

Urban heat island (UHI) as an augmentation of the urban temperature deviating from the rural areas is regarded as one of the main crucial challenges of the twenty-first century as the consequence of growing urbanization and industrialization (Rahman et al., 2022; Rizwan et al., 2008). Climate change and UHI are critical factors affecting biodiversity, human health, well-being, and urban ecosystems (Rizwan et al., 2008; Jain et al., 2020; Kandya & Mohan, 2018). UHI can be investigated as surface or atmospheric UHI based on the surface or air temperature, respectively (Manni et al., 2022). The detrimental effects of UHI on the environment also include the deterioration of the living environment (Sadik-Zada & Gatto, 2022), an increase of ground-level ozone and smog (Fallmann et al., 2016), concentration of air pollutants (Santamouris & Osmond, 2020), and increased carbon emissions due to higher cooling energy demand of buildings (Kandya & Mohan, 2018). Meanwhile, the interaction between UHI and buildings, as one of the significant parts of the built environment, is a critical issue to be addressed in mitigating the UHI phenomenon. Different research methods examined the role of several UHI mitigation (UHIM) strategies in building energy efficiency (Sedaghat & Sharif, 2022a; Morales-Inzunza et al., 2023; Romano et al., 2021), greenhouse (GHG) emissions (Chen & You, 2020), and thermal discomfort (da Silva Espinoza et al., 2023). Furthermore, miscellaneous studies highlighted the role of building envelope/materials (Tabatabaei & Fayaz, 2023; Garshasbi et al., 2019; Santamouris & Yun, 2020) and energy consumption on UHI alleviation. Accordingly, building envelopes and adopting suitable materials are regarded as one of the main solutions to meet UHI challenges (Kandya & Mohan, 2018; Feng et al., 2022). Building envelope, as an interface between indoor and outdoor, plays a key role in meeting the sustainability targets set for the building sector (Loonen et al., 2017). Hence, there is a need to develop new building concepts, technologies, and materials that can further improve the energy efficiency of buildings and simultaneously enhance the occupant's comfort. Recent studies developed advanced mitigation strategies to counterbalance the UHI phenomenon and buildings' energy consumption by adopting state-of-the-art façade technologies and materials. Phase change materials (PCMs) (Yang et al., 2017, 2019), thermochromic coatings like quantum dots, plasmonic, photonic crystals (Fabiani et al., 2019; Berardi et al., 2020; Garshasbi & Santamouris, 2019), and fluorescent materials (Levinson et al., 2017) are among recent developments of UHI-mitigating materials that can be integrated with building envelope to help regulate building's cooling energy demand. Meanwhile, integrating microalgae culture system with building envelope as microalgae bioadaptive façade (MBF) is regarded as one of the recently emerged façade technologies showing great potential to address sustainability-oriented performances of building (Talaei & Prieto, 2024).

MBF as one type of biofaçades has great potential in providing energy efficiency (Trombadore et al., 2022), daylight control (Lo Verso et al., 2019), reducing air pollution (Oncel et al., 2020), and wastewater treatment (Foix-Cablé et al., 2018) for the building. The great potential of green walls in reducing building cooling energy

demand and climate change (Yang et al., 2018), UHIM (Bahrami et al., 2022), and reduced CO_2 emission (Charoenkit & Yiemwattana, 2016) has been approved by previous research. Green walls, categorized as living walls and green façade, contributes to UHIM up to 5 °C through decreasing building heating energy demand by 16.5% and cooling energy demand by about 51% considering almost all Köppen climate classifications (Susca et al., 2022). However, the role of integrating microalgae photobioreactor (PBR) with building façade as a smart green biofaçade technology (Habibi et al., 2022) and an adaptive strategy in UHIM has not been studied, comprehensively.

This chapter seeks to investigate the potential of symbiosis between microalgae culture systems and building envelope in terms of contribution to UHIM. To achieve this objective, at first, the interaction between the building envelope and city overheating is studied and their effective parameters are investigated, then the interrelation between MBF performance and the studied parameters is examined. Besides, this chapter makes a comparison between MBF as types of biofaçade and common vertical gardens.

2 Building Envelope and UHI

Urban spaces suffer from an imbalanced microclimate due to high-density building areas, low green surfaces, and excessive heat generated by different anthropogenic sources. These parameters noticeably can affect surface heat equilibrium, exchanged convective heat among building and the ground, wind flow, and evapotranspirative cooling (Kandya & Mohan, 2018), which influence urban microclimate. Parameters affecting UHI can be classified as controllable and uncontrollable (Rizwan et al., 2008). Controllable parameters refer to those related to design and planning in building and urban scales including sky view factor (Bernard et al., 2018), surface materials (Rizwan et al., 2008), urban size/form/density (Wang et al., 2021; Santamouris, 2015), population (Santamouris, 2015), anthropogenic heat (e.g., vehicles) (Kalogirou, 2022; Sailor, 2013), and GHG emission (Wang et al., 2021). Uncontrollable ones address environmental and climatic factors including anticyclone conditions (Santamouris, 2015; Pongracz et al., 2006), seasonal solar radiation, wind speed, sky condition (Akbari et al., 2016), geographical location (Wang et al., 2021), and diurnal conditions (Rizwan et al., 2008). UHI can affect surface and the air temperature (Taha, 2004). Surface urban heat island (SUHI) is characterized by temperature differences between the surfaces in the urban, which is commonly examined by the satellite land surface temperature data (Zhou et al., 2019). This can be calculated though measuring the temperature differences between urban and rural areas at the same time as follows (Oke, 1987):

$$SUHI = TST_{urban} - TST_{rural} \tag{1}$$

Meanwhile, land surface temperature (LST) related to the emissivity level of the earth surface is measured by the following formulas (Weng et al., 2004):

$$LST = \frac{T}{\left(1 + \left(\lambda \frac{T}{C_2}\right) \times log(\varepsilon)\right)} \tag{2}$$

where λ, T, and ε indicate the wavelength of the emitted radiation, temperature of Landsat brightness, and the emissivity of the ground, respectively. Also, C_2 can be calculated as:

$$C_2 = \frac{h \times c}{s} \tag{3}$$

where h indicates Planck's constant (6.626×10^{-34} Js), s shows the Boltzmann constant (1.38×10^{-23} J/K), and c is the speed of light (2.998×108 m/s). Besides, air temperature is categorized as the urban canopy (UCL) and boundary layer (UBL). While, UCL indicates urban atmosphere, measured from ground level up to the nearly mean building height and affect people's lives both indoors and outdoors (World Meteorological Organization, 2023), UBL refers to the air layer extended just above the UCL (Tabatabaei & Fayaz, 2023). Meanwhile, different research explored the interrelation between building, its envelope, and UHI phenomenon. These studies can be categorized in terms of the role of building in UHIM in two building and urban scales, while they have bilateral interactions. Figure 1 presents the role of building and related parameters in contributing to the UHI effect considering urban and building scales.

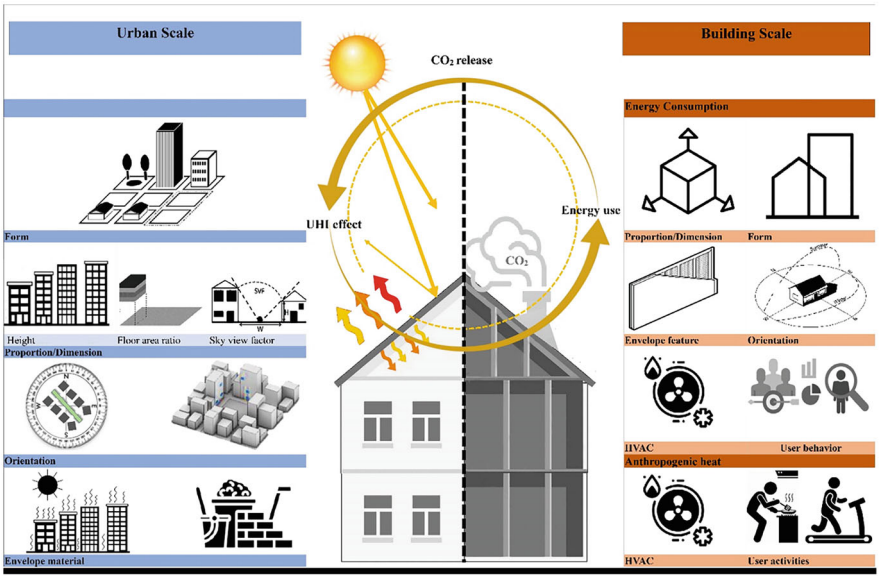


Fig. 1 Building parameters affecting UHI considering building and urban scales

Urban scale Urban morphology has a considerable role in the thermal performance of the cities by facilitating wind velocity and reducing air temperature (Golany, 1996). It also contributes to energy saving for cooling urban areas (Javanroodi et al., 2018). Parameters of urban morphology include urban density (Taleghani et al., 2013), building's form/geometry (Chen & Hong, 2018; Gracik et al., 2015), orientation (Chatzidimitriou & Yannas, 2017), urban canopy/street geometry (Allegrini et al., 2012; Niachou et al., 2008), the ratio of building height to street width (H/W) (Evins et al., 2014), and shading obstacles (Ichinose et al., 2017; Wang et al., 2016) (e.g., trees, buildings, vehicles). Building H/W can be used to calculate the sky view factor (SVF). SVF, as a key factor for urban climatology, is defined as a ratio of visible sky hemisphere from the ground and shows street geometry and building density. From a street canyon point considering a 2D and 3D perspective, SVF can be calculated based on the following formula (Dirksen et al., 2019):

$$SVF_{2D} = \cos\left(arctan\left[\frac{H}{0.5W}\right]\right) \tag{4}$$

$$SVF_{3D} = \int_{\theta=0}^{2\pi} \cos^2\left(\beta\left(R,\theta\right)\right) d\theta \tag{5}$$

where H and W are building/obstacle height and distance between the building/obstacles, respectively. Also, β indicates the angle from the center point to the maximum height of the building/obstacle at a maximum distance equivalent to R as the constant search radius.

On the other hand, one of the most significant factors affecting UHI is urban surfaces, which can be categorized as ground and building surfaces (Croce & Vettorato, 2021). Building surfaces defined as building roofs and facades have a significant impact on counterbalancing the UHI effect (Fig. 2). Different research methods have focused on the role of building envelope in UHIM based on its potential to reduce ambient air temperature like using (high) reflective (Kandya &

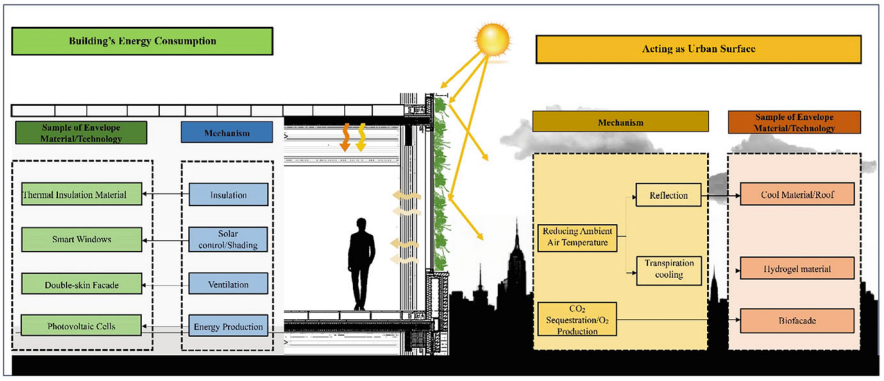


Fig. 2 The mechanism by which the building facade affects UHIM

Mohan, 2018), cool materials (Santamouris & Yun, 2020; Santamouris et al., 2011), and green envelope systems providing evapotranspiration and CO_2 sequestration for the urban and built environment (Susca et al., 2022; Koch et al., 2020).

Building scale UHI and climate change are two crucial parameters influencing building energy demand (Kamal et al., 2023), while energy use in buildings contributes to these phenomena by enhancing anthropogenic heat via using HVAC systems (Sailor, 2013) and GHG emission through fossil fuel consumption and gas flaring (Balaras et al., 2024). Different research methods have estimated that UHI exacerbates the building energy demand by about 20–100% (Kandya & Mohan, 2018; Hassid et al., 2000; Santamouris et al., 2001; Papadopoulos, 2001; Li et al., 2019; Akbari & Konopacki, 2004). It has also been shown that improving building energy efficiency contributes to UHI mitigation (M López-Guerrero et al., 2022). According to Sedaghat and Sharif (2022b), using high-albedo material in combination with vegetation can result in reducing 3–6 °C UHI by 2.1 °C and cooling load by 29%, in Tehran, Iran. The other study (Santamouris et al., 2018) demonstrated that enhancing global high-albedo materials in cities leads to reduced peak ambient temperature and cooling demand by up to 3 °C and 20% respectively, in Sydney, Australia. Research also reported a 46% cooling energy decrease and 20% power saving by enhancing roof reflectance (Akbari et al., 1997) as well as 31–39 Wh/m^2/day energy saving by using 0.70 reflectivity white elastomer on the roof (Akbari, 2003).

Factors affecting building energy consumption include location and climate (Santamouris et al., 2001), orientation, form (Mangkuto et al., 2016), energy systems (Alsharif et al., 2021), occupant behavior (Delzendeh et al., 2017), and socio-economic characteristics (Suleimany, 2023). Also, the building envelope and its materials affect energy consumption (Chandel et al., 2016; Chen et al., 2018) and waste heat generation by regulating conductive, convective, and radiant heat flow through the envelope (Outline, 2018). Building skin materials can also indirectly affect urban overheating through their effects on building energy consumption (Mangkuto et al., 2016). Accordingly, the idea of using advanced façade technologies like adaptive envelopes (Loonen et al., 2013; Sadegh et al., 2022), building-integrated smart materials such as PV (Azami & Sevinç, 2021), PV-PCM cells (Elarga et al., 2016), shape memory alloy (Li et al., 2023), thermoelectric skins (Martín-Gómez et al., 2021), and smart windows (Aburas et al., 2021) have been developed to regulate building thermal load, and reduce energy consumption and the consequence anthropogenic heat. Furthermore, façade technologies have focused on reducing air pollution with state-of-the-art technologies such as artificial leaf-based facade cladding (Azari & Asadi, 2019), TiO_2-integrated double-skin façade (Li et al., 2020), MBF technology (Rezazadeh et al., 2021), and providing natural ventilation with advanced systems like Smart Thermobimetal Self-Ventilating Skin (Sung, 2011) and sweating skin (Cui et al., 2016), which highlights the role of building envelope in UHIM.

3 Microalgae Bioadaptive Façade (MBF) and UHI Mitigation

The symbiosis between the microalgae culture system and the building envelope has increasingly drawn scientists' interest due to its potential to enhance the sustainability-oriented performance of the host building (Talaei & Prieto, 2024) and the PBR energy efficiency (Pruvost et al., 2016). Research also showed the superiority of tubular MBF to PV panels from an environmental point of view, including water efficiency, reducing air pollution, and CO_2 emission (Biloria & Thakkar, 2020). However, the potential of MBF in contributing to UHI alleviation should be considered to certify the potential performance of this technology to address climate change in a broader sense. The potential of MBF in UHIM can be investigated considering the role of building envelope as an urban surface, directly addresses UHI alleviation by reducing CO_2 emission and air temperature, and indirectly by regulating energy consumption in the following sections. Figure 3 indicates the contribution of MBF to UHI alleviation.

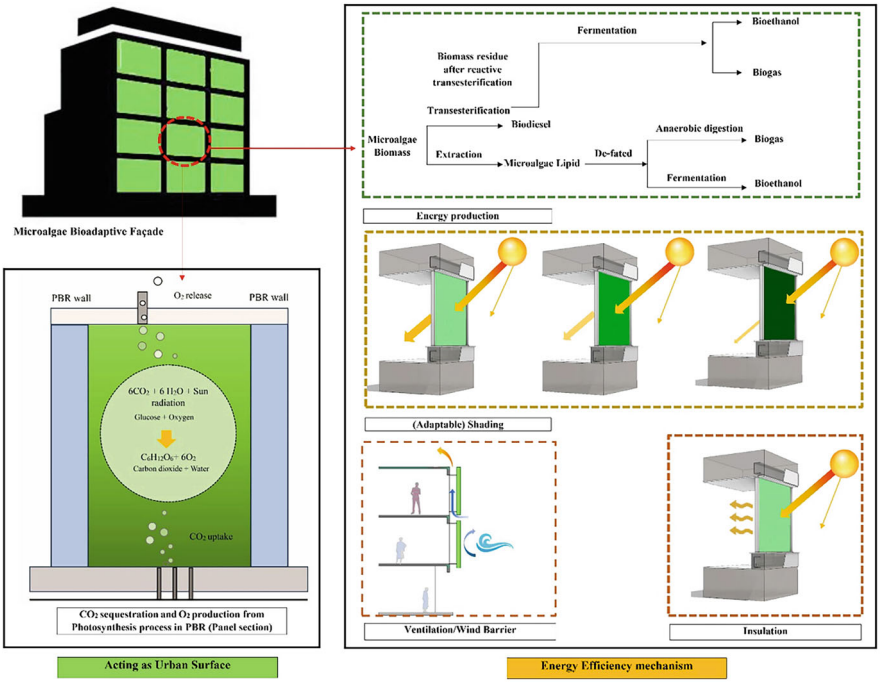


Fig. 3 Contribution of microalgae façade to UHI mitigation

3.1 MBF as an Urban Surface for UHI Mitigation

Air temperature control Directly controlling ambient air temperature can be realized through integrating reflective and cool materials (Morales-Inzunza et al., 2023), latent-heat storage (LHS) materials like PCMs (Yang et al., 2019), hydrogel (Cui et al., 2016), as well as providing evapotranspiration through living walls (Cortês et al., 2022). Although microalgae have been known as a valuable source of smart materials for photonics, electronics, and biomedicine applications (Ragni et al., 2017), and as a kind of building biomaterial (Sandak et al., 2019), they are not recognized as a high-reflective envelope material, which can meet UHI challenges. Since the integration of microalgae with the building façade is feasible through PBR systems, evaluating different PBR materials with high reflectivity can have the potential to reduce ambient air temperature. However, the potential and possibility of using reflective materials should be studied regarding light penetration, photosynthesis process, and biomass productivity of microalgae medium. Moreover, since PBR acts as a solar receiver, its temperature increases by absorbing sun radiation through the walls and the medium culture when the solar energy is not fully used for cell growth (Wasanasathian & Peng, 2006; Tredici & Materassi, 1992). This can result in increased ambient air temperature and UHI, consequently. Hence, to address this challenge, heat sensors, heat exchangers (Pruvost et al., 2016), dark sheets as sun shading (Ugwu & Aoyagi, 2008), and water spraying (Wasanasathian & Peng, 2006) can be used to regulate and convert the produced heat within the system.

Living walls and green facades known as green walls (Cuce, 2017) can regulate urban microclimate by evapotranspiration (Vox et al., 2018; Galagoda et al., 2018), shading/insulation for less energy use (Bakhshoodeh et al., 2022), and CO_2 sequestration (Galagoda et al., 2018). Through transpiration, water is released due to opening the stomata for CO_2 absorption needed for photosynthesis process (Koch et al., 2020). Based on Bakhshoodeh et al. (2022), the evapotranspirative cooling by green façade contributed 25–35% to the overall gap cooling. Evapotranspiration created by a combination of evaporation from the surface of soil, green infrastructures, other surrounding surfaces, and transpiration through plants (Bakhshoodeh et al., 2022) can bestow lower air temperature, enhanced heat loss from outer building skin, and reduced building cooling load (Vox et al., 2018; Schettini et al., 2016). Considering microalgae as photosynthetic organisms, water evaporation from microalgae *Chlorella vulgaris* can be similar to plant transpiration by designing and simulating microalgae as a plant system through increasing the transpiration of reverse osmosis concentrated water and converting pollutant to the biomass (Yu et al., 2021). However, water evaporation and consequent evapotranspiration from microalgae can be regarded neglectable due to closed culture system in PBRs (Martins et al., 2018), which is the common form of microalgae presence in the MBF. Accordingly, the passive cooling of PBR-integrated building envelope through evapotranspiration can also be considered as insignificant compared to that of the plants. Nevertheless, more research needs to evaluate cooling potential of this technology though evapotranspiration.

Although various research has focused on the CO_2 sequestration potential of MBF (Oncel et al., 2020; Vajdi & Aslani, 2023), no study examined the O_2 production potential of this system on UHIM and air temperature reduction. Moreover, since O_2 is not regarded as a GHG, it is often excluded from climate change studies. This is mainly due to the fact that O_2 is not active in the infrared band and radiative forcing contrary to GHGs (Stocker et al., 2013). However, thanks to O_2 impact on atmospheric density, it directly affects solar optical depth (τ_α), which can be simply expressed as:

$$\tau(z) = K\rho\Delta z \tag{6}$$

where k (m^2/kg), ρ (kg/m^3), and Δz (m) are mass absorption coefficient, absorber density, and atmosphere thickness, in order. Reduced pressure of O_2 results in augmented global surface temperatures. The more the earth receives sunlight through a low level of O_2, the more water vapor/moisture is generated trapping more heat and causing higher temperature. Accordingly, considering the potential of MBF in O_2 production, future research needs to investigate the O_2-produced cooling potential of this innovative system to address global warming and UHI.

GHG emission control GHG reduction, especially reducing CO_2 level, as one of the main solutions to reduce global warming and UHI is a critical concern for different environmental/economic scientists and policymakers. Urban areas cover 2% of the earth, while they account for more than 80% of GHG emissions. Research shows 0–100 million kg of CO_2 emission per year in Arizona, Oklahoma, and Missouri associated with 2 °C UHI (Roxon et al., 2020). Meanwhile, using urban green surfaces (e.g., green roofs and vertical gardens) is a noticeable solution to address city overheating not only for the potential of vegetation in insulation, shading, and evapotranspiration but also for their great CO_2 biofixation capacity. Plants have great potential to contribute to UHIM, by up to 2% decreasing CO_2 concentration in the ambient environment (Hong et al., 2012). At the same time, with 15–50 times more CO_2 biosequestration than other vegetation type (Kumar et al., 2024) due to higher chlorophyll per unit area (Raeesossadati et al., 2014), microalgae have superiority in addressing UHIM. They are also primarily photoautotrophs and considerably provide a balanced atmosphere by CO_2 sequestration and O_2 production with the fastest photosynthetic process compared to the other microorganisms. In the early earth, the atmospheric O_2 was built up by microalgae (Bloch & Vardi, 2009). The efficiency range of capture and biosequestration of CO_2 by microalgae is between 40% and 93.7% (Ighalo et al., 2022). *Scenedesmus, Spirulina platensis, and Chlorella* as freshwater microalgae species are commonly used for CO_2 capture (Alami et al., 2020; Mondal et al., 2016). Though using solar energy, microalgae convert CO_2 and other nutrients into biomass leading to biofuel/bio-products production as well as reduced CO_2 levels in urban space (Umdu & Univ, 2020), which contributes to global warming mitigation (Ighalo et al., 2022). Besides, for 1 kg biomass, about 1.83 kg CO_2 fixation occurs in the autotrophic growth process (Chisti, 2007), and some species use SO_x and NO_x along with CO_2 to remove

contamination from flue gas (Yen et al., 2015). PBRs also beat green walls due to providing the chance to be cultivated during a year, short-time growth, and homogeneous culture density (Oncel et al., 2016). Accordingly, the idea of integrating PBR with building skin as a biofaçade has great potential to address global warming and UHI by reducing CO_2 level.

As the first building façade hosting 129 PBR modules, BIQ house (BIQH) in Hamburg, Germany, can potentially reduce CO_2 emission by 6 tons per year. Vajdi and Aslani (2023) analyzed techno-economic aspects of MBF in Tehran, Iran, by designing 2 × 1 × 0.05 m^3 flat-panel PBR containing *C. vulgaris*, which leads to yearly 2.2 and 3.9 ton microalgae production and CO_2 reduction, respectively. Also, the microalgae cultivation cost was estimated at 10$/kg. Another study (Inam & Oncel, 2022) examined *C. vulgaris*-contained MBF to evaluate the effect of microalga culture dilution (10, 20, 40, 80%), recycling of the cell-separated medium, and different levels of CO_2-enriched air (5–10%) on biomass production and photosynthesis process to enhance the sustainability impact of the building. Based on results, up to three medium cycles, 80% dilution, and 5% CO_2-enriched air level bestow high activity level to the microalgae biofaçade. Based on Pruvost et al. (2016), a *C. vulgaris*-contained airlift PBR can produce 25–30 tons of biomass per ha and fix 40–50 tons of CO_2 per year per ha. To predict *C. vulgaris* biomass growth, the following stoichiometric formula can be used (Pruvost et al., 2016):

$$HCO_3^- + 0.495H_2O + 0.159NH_4^+ + 0.006SO_4^{2-} + 0.007PO_4^{3-} \rightarrow CH_{1.750}O_{0.413}N_{0.159}S_{0.006}P_{0.007} + 1.129O_2 + 0.874OH^- \quad (7)$$

Accordingly, the formula shows the necessity of minerals phosphate (*P*source) and nitrate (*N*source) for microalgae culture that can be supplied from the building wastewater. More specifically, it highlights the highest demand for CO_2 sources during the photosynthesis process, which approximately fixes 1.4–1.8 kg CO_2 per kg of dry biomass. CO_2 biofixation and microalgae biomass strongly depend on microalgae features, culture systems, and physicochemical impacts (Ighalo et al., 2022). Also, CO_2 level has a significant impact on biomass production (Jiang et al., 2013). Accordingly, each microalgae species is tolerant to the specific ranges of CO_2, PH level, and light/temperature, which all have a great impact on CO_2 biofixation efficiency among other environmental parameters (Ighalo et al., 2022). If the gas from the industrial/building sectors is injected into algae medium without any pre-process for cost-effectiveness consideration, the high temperature of the gas can result in excessive high temperature in the cultivation which requires specific prerequisites for providing cooling measurements. Hence, using thermophilic microalgae species can address this challenge (Koller et al., 2012). However, their adaptation to the environmental and building's needs parameters is a critical issue, and selecting the proper species for MBF needs specific investigation considering each climate zone.

3.2 MBF as an Energy-Saving Solution for UHI Mitigation

City-related CO_2 emission, attributed to energy consumption, is about 70% of total global CO_2 emission (Seto et al., 2015). Rising urban temperature due to CO_2 emission and UHI results in less indoor thermal comfort and more building cooling energy demand. Meanwhile, green walls perform a considerable role in UHIM by providing building energy efficiency and reducing CO_2 emission through shading, insulation, and ventilation as well as cooling the ambient air through evapotranspiration (Koch et al., 2020). However, research showed that cooling effects are predominantly related to shading compared to transpiration (Hoelscher et al., 2016). MBF also provides energy efficiency for buildings through shading, insulation, heat/biomass production, and ventilation.

3.2.1 Shading

Shading, as a significant cooling performance of green walls, is often conceived as the major capacity of cooling through plants (Pérez et al., 2011), which can provide up to 61% cooling cost savings in warm climates (McPherson et al., 1988). The shading effect of common green walls does not change rapidly as time passes and greatly depends on plant species, their features, and environmental factors (Koch et al., 2020). At the same time, MBF can provide adaptable shading depending on changing microalgae culture density and environmental factors. Having the ability to absorb red sun radiation, green microalgae are considered solar shading systems controlling daylight penetration (Pagliolico et al., 2017).

Microalgae culture density as one of the essential parameters in the light-control performance of the MBF greatly depends on mixing, light, temperature, CO_2, PH level, and nutrients (Mazzelli et al., 2020; Borowitzka, 1998). The more culture concentration, the less light penetration is provided by the PBR-integrated facade (Elnokaly & Keeling, 2016). However, high intensity beyond the light saturation point necessary for the photosynthesis process can lead to cell death (Oncel et al., 2016). Sivakaminathan et al. (2018) reported the relation between the microalgae growth rate μ and photosynthetic efficiency (PE_μ) related to efficient light used by microalgae with the following formulas:

$$\mu = (In\ OD_{750}(t_2) - In\ OD_{750}(t_1))/(t_2 - t_1) \tag{8}$$

where t_1 and t_2 indicate the time at which related optical density (OD_{750}) is measured. By this formula growth rate μ (h − 1) was measured as the change rate of (OD_{750}).

(PE_μ) was supposed to be indicative of efficient light use by microalgae, where μ normalized to the averaged received photosynthetically active radiation (PAR) required for the photosynthesis process,

$$PE = \mu / I_{avg} \tag{9}$$

The I_{avg} is:

$$I_{avg} = \int_0^t I(t)dt * 3.6 \times 10^{-9} \tag{10}$$

where, t_c, I(t), and 3.6×10^{-9} indicate cycle time (mixing rate), irradiance (μmol photonsm^2s^{-1}) at time t_c, and factor converting from μmol photons m^2s^{-1} to mol photons m^2h^{-1}, respectively.

Research (Pagliolico et al., 2019) also highlights the symbiosis between building and PBR as an advanced technology for daylight control, shading effect, and reducing lighting energy demand. Besides, based on Pagliolico et al. (2017) algae shading system enhances daylight level and reduces energy use for daylighting compared to the traditional Venetian blind. Ahmadi et al. (2022) compared microalgae-contained double-glazed windows with Irans' traditional colorful glass-made Orosi windows. They reported the superiority of algae window with 40% and 50% density to the Orosi regarding daylight metric, useful daylight illuminance ($UDI_{100\text{-}2000Lux}$). Besides, results indicated that algae density between 20% and 30% provided less electric lighting consumption in hot and dry climates. However, as microalgae concentration increased, energy use intensity (EUI) enhanced in both cold and warm months owing to incremental lighting energy consumption. In general, microalgae can be regarded as a shading device to regulate light penetration and solar heat gain, which can contribute to energy-efficient building and consequent UHI alleviation.

3.2.2 Insulation and Ventilation

The insulation effect in common plant-based biofaçade is attributed to the still air within the leaves' cavity, which results in regulating surface air temperature in heating and cooling seasons (Koch et al., 2020). According to Poddar et al. (2017), a complete-sealed and full-plant-covered building envelope improves heating and cooling energy performance by 60% and 17%, respectively. Moreover, research expounded on wind flow caused by different morphologies of plant species within green walls (Koch et al., 2019). This contributes to the energy-saving potential of these systems along with their insulation capacity. Vertical greenery systems directly protect the host building/structure from the wind flow and indirectly provide air renovation within the plant layer affecting the insulating feature of the system (Pérez et al., 2014). The green facade also contributes to the energy-efficient building through the wind barrier effect. They provide a 25% heating energy demand reduction by protecting the building from cold wind as a noticeable factor in temperature reduction of the building's inner space (Dinsdale et al., 2006).

Controlling buildings' energy needs by using green walls depends on the substrate characteristics, vegetation features, their density/orientation, plants' albedo/biological activities, irrigation, climate type, seasonal/diary features, and time, as well as building dimensions (Susca et al., 2022; Pérez et al., 2014). Widiastuti et al. (2022) demonstrated that the green façade with more leaf coverage bestows greater thermal insulation potential on the host building's wall. Heat transfer examination showed that bare wall, 50%-, and 90%-covered green façades gained 2.10 W/m^2, 1.81 W/m^2, and 1.46 W/m^2 heat from the ambient environment, respectively. Moreover, the bare wall received radiation heat transfer 4% more than the 50%-covered green façade and 7% more than the 90%-covered one.

MBF also has insulation and ventilation effects for balancing the inner thermal load. However, the ventilation properties of this system depend on the way the PBR system is deployed on the building façade. Used as the building's second skin in a double-skin façade, PBRs can contribute to air movement within the existent cavity. Nevertheless, research has not addressed the ventilation potential of MBF, comprehensively, which needs further investigations.

When it comes to insulation effect, Umdue et al. (2018) showed that one panel of MBF can have thermal transmittance (U-value) between 3.84 and 53.19 W/m^2K, depending on the microalgae reservoir (5–30 cm range) containing *Nannocholoropsis oculata* sp., its thickness (0.6–2 cm range), the air layer (0–5 cm range), and its wall thickness (0.5 cm range). Accordingly, it is also important to examine the thermal transmittance properties of MBF with different layout characteristics like PBR width/wall thickness/material, window frame thickness, and material to investigate its capacity to regulate buildings' thermal load. In another study, Negev et al. (2019) estimated a 4.9 W/m^2 K U-factor of a *Chlamydomonas reinhardtii* and *C. vulgaris* algae window with 2 cm-depth microalgae reservoir between two 0.6 cm-thick clear glass. Although they achieved different U-factor as 4.3 and 4.9 W/m^2 K for summer and winter, in order, due to external alteration in air temperature and heat transfer, they used 4.9 W/m^2 K for the whole year. It was owing to the negligible difference between the values and small error for the evaluated energy consumption. They also achieved up to 20%, 8%, and 14% KWh/m^2 year energy saving for 100%-density algae windows in the South, East, and West, respectively. Also, 18% more energy consumption was calculated compared to the single-glazed window in the Mediterranean climate.

Kerner et al. (2019) investigated the role of adding 1 cm argon-filled glass plates as insulation to the PBR system in BIQH in enhancing heat management efficiency to meet the building's heat demand. They reported that one of the major features that provides sufficient produced heat within the PBR is both-side insulations resembling a double-glazed window regarding thermal transmission coefficient and low heat loss. The other research (Talaei et al., 2022) also investigated the thermal insulation property of microalgae-based windows by altering the culture medium height within the PBR. It was indicated that when the height of the culture medium is elevated within the PBR integrated with a fully insulated chamber, the temperature difference between the inner and outer space becomes greater, where the inner temperature is higher than the outer one. Furthermore, as the process is reversed and the height

decreases, the temperature difference reduces. This experiment showed the potential of the algae window as a barrier for trapping heated air to flow outside and keep the inner space warmer; however, the full-height panel provides less light transmittance within the space.

Todisco et al. (2022) developed a thermal dynamic model for MBF consisting of flat-panel PBRs, an air-cooling channel, and a protection window to anticipate meteorologically dependent dynamic temperature changes of the *C. vulgaris* culture medium. Thermal exchange with the host building was considered as well. They assumed that radiative parameters are constant since they change based on culture density, which depends on different parameters and conditions whose estimation is difficult during the microalgae culture process. Besides, evaporation and consequent heat loss were negligible due to the closed PBR system. Since the air-cooling channel is narrow, in most cases it does not conform to the wind direction and the entered wind speed may also differ from the outer one. Hence, this study was not to precisely estimate wind flow by a detailed fluid dynamic model within the air channel, and it was considered a semi-empirical modification of wind speed within the air channel. The results showed that the opening/closing condition of the air-cooling channel with an automated shutter, which adjusted thermal exchanges with the building, resulted in the energy efficiency of the system to regulate the temperature of the culture medium, particularly in combination with a wide preset temperature range.

Future research also can evaluate the thermal exchange between the MBF and the ambient air to examine the role of PBR biofaçade in the UHI phenomenon. It is based on the fact that PBR presents thermal exchange with the surrounding environment (Pruvost et al., 2016), which indicates the high necessity to study its thermal effect on city scale and adopt suitable PBR design in different climate zones.

3.2.3 Energy Producing/Exchanging

In most research, green walls have not been conceived as energy-generating systems in regulating building thermal performance. However, depending on the cultivated vegetation, this system has the potential to produce renewable energy. Institute for Advanced Architecture of Catalonia developed a moss-based green wall known as Moss Voltaics (Moss Voltaics—The Institute for Advanced Architecture of Catalonia, n.d.). This system produces renewable electricity using bacteria in symbiosis with moss. In this process, some organic compounds are produced due to the moss photosynthesis process and are given to the beneath soil through the moss roots (Fig. 4). As they are fed by the bacteria, the compounds are broken down into some byproducts including electrons. The electrons are donated to an electrode; thus, electricity is generated. Moreover, other plants and algae species can run the system. Moss was chosen in this project based on its adaptation to urban areas, low weight and maintenance, no need for fertilizer, and high resistance to drought. Table 1 provides a comparison between MBF and green walls regarding UHIM.

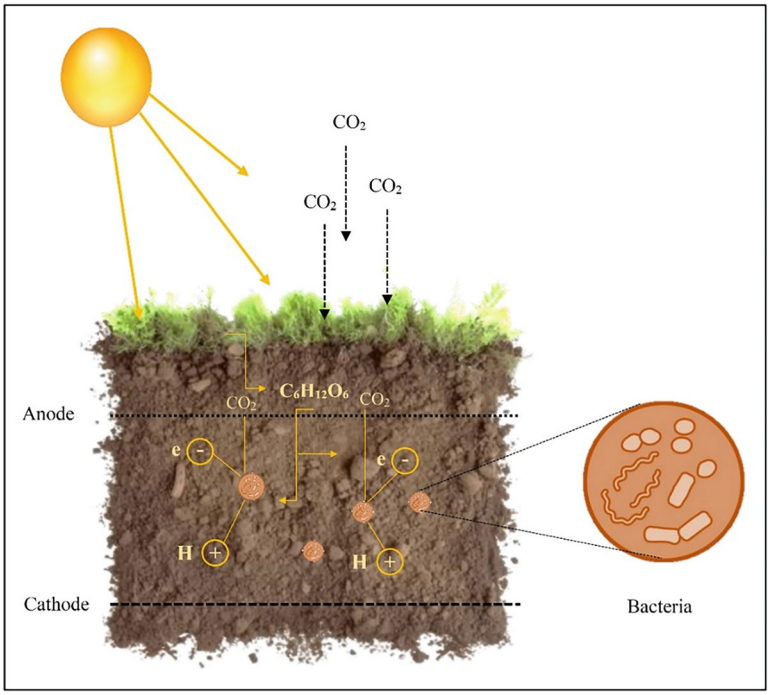


Fig. 4 Energy harvesting by plants in a microbial fuel cell

MBF as a solar-thermal bioenergy façade contributes to UHIM by providing energy-efficient building through biomass/biofuel production and heat generation from received solar radiation and photosynthesis process. In BIQH, along with biomass production, solar radiation is converted to heat by 0.38 energy conversion coefficient, defined as the ratio between received solar radiation by MBF surface and the produced heat throughout the year. This advanced façade technology provides 59% of 63 MWh/total heat consumption of the building for heating and hot water. Moreover, 80% of the extracted heat is directly used for the building's thermal needs, while the extra heat is stored in seven borehole heat exchangers, each with a depth of 80 meters, which can potentially transfer maximal 38 kw thermal power (Kerner et al., 2019). In BIQH, up to 80% of produced biomass is harvested and converted to methane in an external biogas plant and not on site due to deficiency of the technology in symbiosis with residential buildings and legal perspective (IBA_Hamburg, 2013).

The advent of MBF, as one type of urban green surface, converts cities from an absolute resource consumer to a vital place with resource production. Accordingly, one of the most considerable superiorities of MBF compared to the common solar energy systems is its potential to produce biomass via microalgae cultivation (Kerner et al., 2019). Biomass derived from plants, algae, matter, and animal life are organic material that can be converted to biofuel. High petroleum costs, concerns about

Table 1 Microalgae façade vs. green walls for UHI mitigation

Building envelope effect on UHI	Mechanism	Green walls	MBF
Energy efficiency	Shading	Decreasing solar radiation entry into the building through vegetation (Koch et al., 2020)	(Adaptive) shading provided by changing microalgae culture density through absorbing solar radiation for the photosynthesis process (Pagliolico et al., 2017)
	Insulation	Reduced heat transfer via air cavity between plant layers and building as well as foliage density (Pérez et al., 2011) Reduced heat transfer due to the substrate layer acting as a thermal insulation in living walls (Pérez et al., 2014)	Reduced heat transfer through the PBR reservoir by its different layers (wall thickness, air cavity, etc.) and microalgae medium (Umdu et al., 2018; Negev et al., 2019)
	Natural ventilation	Convective air movement in the air cavity (Koch et al., 2020) or within the vegetation (Koch et al., 2020)	NA[a]
	Energy production	Energy production depends on the vegetation type[b]	Acting as a solar-thermal cell: heat production through photosynthetic process/heat absorption, which can be transferred to the host building (Pruvost et al., 2016; Kerner et al., 2019) Biomass and biofuel production (Pruvost et al., 2016)
	Wind barrier effect	Protecting a building from the cold wind via plant leaves (Azkorra et al., 2015)	NA[c]
Acting as urban surface	Reducing ambient air temperature	Evapotranspiration through vegetation/substrate (Bakhshoodeh et al., 2022; Blanco et al., 2020)	NA[d]
	CO_2 sequestration/O_2 production in urban area	Air purification by CO_2 absorption /O_2 production (Koch et al., 2020)	Air purification by CO_2 absorption/O_2 production

[a]NA: Not assessed (If MBF is used as a double-skin façade, natural ventilation can be provided potentially)
[b]e.g., moss-based living walls can produce electricity (Moss Voltaics—The Institute for Advanced Architecture of Catalonia, n.d.)
[c]If MBF is used as a double-skin façade, it can have the capacity to act as a wind barrier for the building
[d]More O_2 pressure levels can result in a thicker atmosphere and less absorbed sun radiation

fossil fuel depletion, and their critical role in exacerbating global warming, and UHI have led to biofuel production as an environmentally friendly solution for human life. Meanwhile, microalgae are regarded as a valuable source of the third- and fourth-generation biofuel. Microalga biomass can be transformed into biogas, bioethanol, biomethane, biohydrogen, and biodiesel as a significant attention point for renewable energy sources, which can contribute greatly to UHIM. They have superiority to the first (food corps) and second generations (waste and energy corps) due to high lipid content, more oil production, and high photosynthesis efficiency (Naik et al., 2010; Elrayies, 2018). Microalga-based biodiesel is not toxic and is acceptable for transportation of marine ecosystems and mining enclosures, which are regarded as very sensitive environments (Chisti, 2008). The research investigated different microalgae species suitable for biofuel production like *Botryococcus*, *Chlorella*, *Nannochloropsis*, *Scenedesmus*, and *Schizochytrium* thanks to their high lipid production under optimum cultivation conditions (Nautiyal et al., 2014). The net energy ratio (NER) of microalgae production, which indicates the ratio of total produced energy over energy content of PBR construction, material, and demanded energy for plant operation, can be calculated as (Jorquera et al., 2009):

$$NER = Net\ Energy\ Ratio = \frac{\sum Energy\ produced\ (lipid\ or\ bimass)}{\sum Energy\ requirement} \tag{11}$$

Besides, it is of great importance to evaluate the relationship between the NER produced by biomass and the consequent GHG emission to analyze the life cycle assessment of the process. Hence, net GHG emissions can be used to feature the potential of the system to address global warming and UHI, which can be calculated based on the equation (Wei et al., 2023):

$$NET\ GHG\ emission = Total\ GHG\ emissions - GHG\ fixation\ by\ microalgae \tag{12}$$

Compared to fossil fuel, which consists of carbon fixed through millions of years and produces excessive CO_2, biochemical industry based on green productions from renewables releases CO_2 back to the fields through plants and algae photosynthesis process (Koller et al., 2012). This process can contribute to UHIM by keeping the produced CO_2 in a balanced carbon flux. Figure 5 indicates a schematic diagram of the global carbon cycle in the bio/chemical industry and surplus CO_2 production in UHI intensification.

As mentioned before, along with biomass production, MBF has the potential to meet the thermal needs of the host building by directly transferring produced heat within the system to building units. To provide thermal symbiosis between MBF and the host building, Kerner et al. (2019) developed a control logic organized to regulate defined temperature setpoints for space/water heating/storage, culture medium, borehole heat exchanger, and source buffer storage as well as variable measurement systems. It was to control the heat produced within the PBR and meet users' thermal demand with optimal cultivation conditions and extracted heat. The

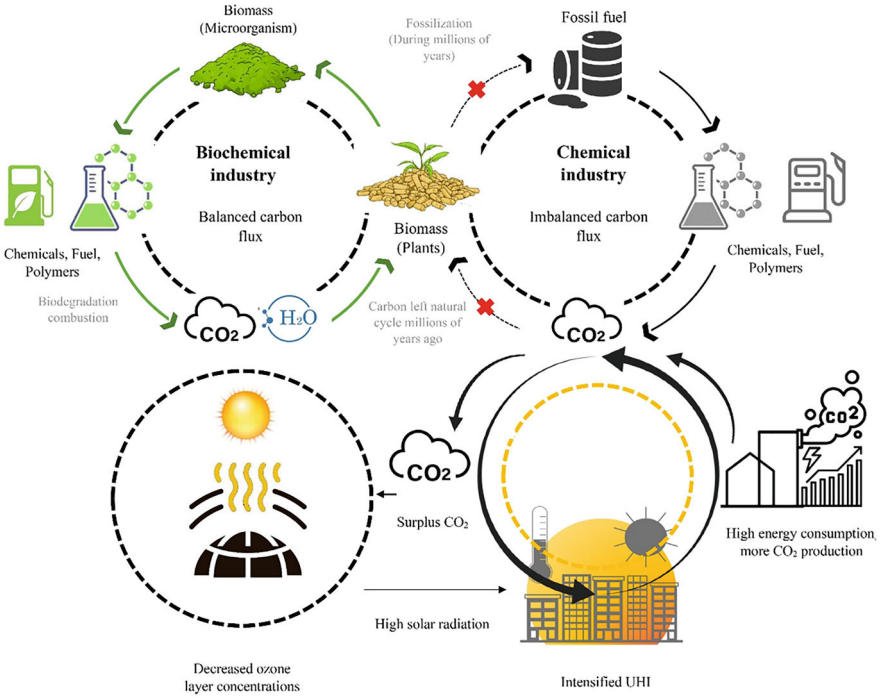


Fig. 5 Schematic diagram of the carbon cycle and its role on UHI. (Authors based on Malli (2011))

170 m^2 MBF provided 29 MWh heat extraction throughout the one-year operation, which led to 37 MWh total heat supply via a heat pump. The system is off during winter time owing to low sun radiation in Hamburg, Germany, where the system is established, and negligible heat production via MBF. Based on the results, MBF surpasses common solar energy systems as a consequence of their great potential for microalgae cultivation, biomass production, and 38% mean energy conversion efficiency. Pruvost et al. (2016) investigated biomass production and energy exchange between MBF and the host building. They stated that thermal symbiosis between the building and the PBR can considerably reduce the energy required for microalgae culture compared to a self-standing PBR regarding overheating as a problematic issue. It should be emphasized that thermal symbiosis between PBR and the building via heat exchanger to use extracted heat for building needs or via conduction, convection, or radiation is a critical issue to address UHI. Since overheated PBR is not only detrimental for microalgae cells but also can intensify ambient air temperature, exacerbate UHI, and increase building cooling load in cooling-needed climate conditions. Accordingly, it is important to adopt a climate-based MBF design to avoid elevated ambient air temperature and address the UHI phenomenon.

4 Conclusion

UHI as an adverse consequence of industrialization and urbanization has an unfavorable effect on human life. Hence, adopting practical solutions to meet the challenges of this phenomenon has drawn the attention of scientists, dramatically. Meanwhile, building envelope plays a considerable role in UHIM by directly reducing ambient air temperature and providing energy-efficient building, which leads to less fossil fuel consumption and consequently less CO_2 emission. Integrating a microalgae culture system with the building envelope as a cutting-edge green façade technology enhances the sustainably oriented performance of the host building like energy saving and daylight control. However, its contribution to UHI as part of urban surfaces has not been addressed by previous research, comprehensively. The present study investigates the role of MBF in UHI alleviation in comparison to the common green wall systems to elucidate its potential in enhancing the sustainability performance of the buildings. Hence, after evaluating the role of the building envelope in UHIM, the potential of MBF in contributing to reducing city overheating was categorized into two scales: acting as urban surface and building scale. Although MBF has great potential in UHIM by CO_2 biofixation and O_2 production for urban and building environments, still studies have not regarded this system as an air-temperature-reducing envelope technology like the green wall does directly by evapotranspiration. It is mainly due to the fact that the common form of symbiosis between microalgae culture systems and building façade can be realized through closed-loop PBR systems where ignorable evaporation occurs.

Regarding energy saving on building scale, MBF contributes to UHIM by providing adaptable shading, insulation, and energy production. While green walls control solar radiation through vegetation and their leaves, PBR-integrated buildings help daylight control by absorbing solar radiation for the photosynthesis process, which results in denser concentration and less light penetration. Furthermore, by providing an air cavity between plant layers and buildings and within the foliage, green walls act as a thermal insulator to control heat transfer. However, this characteristic depends on the thickness of the PBR reservoir, its different layers, and the microalgae medium in the MBF panels. Acting as a solar-thermal cell, MBF meets the energy demand of the host building by converting absorbed solar radiation to heat and produces biomass for biofuel production via the photosynthesis process. While most of the common green walls are not conceived as energy-producing systems, some systems can contribute to energy production depending on the cultivated vegetation and the provided facilities.

The greatest future potential is quantifying the role of MBF in UHI alleviation. Also, future research can evaluate and define which type of UHI can be affected more by MBF systems, especially in comparison with the other façade technologies. Evaluating the potential of different microalgae species in different climate zones can be the major focus of future studies, as well.

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Bioremediation of Effluents in Pilot-Scale Microalgae Cultivation Systems: A Case Study



Ana Sánchez-Zurano, Rebecca Nordio, Eduardo Iniesta López, Enrique Rodriguez Miranda, Francisco José Hernández-Fernández, José Luis Guzmán, and Francisco Gabriel Acién Fernández

Abstract The use of microalgae-bacteria consortia for effluent treatment is an emerging and promising technology that has seen continuous development over the past few decades. To optimize and efficiently apply this technology, it is essential to understand the biology surrounding these systems, in which microalgae, heterotrophic bacteria, and nitrifying bacteria play a fundamental role. These relationships are governed by environmental parameters (light, temperature) and operational parameters (pH, culture deep, air, and CO_2 injection) used in treatment systems. Therefore, understanding the influence of these parameters while achieving proper control strategies and automation presents a significant challenge. In southern Spain, in Almería, at the IFAPA Research Center in collaboration with the University of Almería, pilot-scale bioreactors of different designs and sizes have been operating for years to treat wastewater. Specifically, this case study focuses on the study of two identical 80 m^2 raceway reactors operated continuously, one with wastewater and the other with a mineral medium based on chemical fertilizers. Although the results are promising, this technology still faces challenges, such as the complete removal of organic matter present in these effluents. In the future, coupling this technology with others, such as microbial fuel cells, is proposed to not only treat wastewater but also produce valuable biomass and energy.

Keywords Microalgae-bacteria consortia · Wastewater treatment technologies · Nitrifying bacteria · Operational parameters · Pilot-scale systems

A. Sánchez-Zurano (✉) · E. I. López · F. J. Hernández-Fernández
Department of Chemical Engineering, University of Murcia, Murcia, Spain
e-mail: azurano@um.es

R. Nordio · F. G. Acién Fernández
Department of Chemical Engineering, University of Almería, Almería, Spain

CIESOL Solar Energy Research Centre, Joint Centre University of Almería-CIEMAT, Almería, Spain

E. R. Miranda · J. L. Guzmán
Department of Informatics, University of Almería, Almería, Spain

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1 Introduction

The use of microalgae for treating effluents has emerged as a promising and sustainable approach in wastewater management (Ramírez Mérida & Rodríguez Padrón, 2023). Microalgae-based treatment systems are based on the natural abilities of algae and bacteria to recover nutrients and remove organic pollutants from wastewater. These systems are versatile and can be applied to treat a wide range of effluents, including domestic sewage, industrial wastewater, agricultural leachate, centrate, and animal slurry (Abdelfattah et al., 2023). Beyond treatment, the production of microalgae coupled with wastewater treatment offers additional benefits, such as biomass production for fertilizers, biofuels, and other valuable bioproducts (Sarma et al., 2021). As environmental concerns and regulatory pressures increase, the integration of microalgae in wastewater treatment processes presents a viable solution to enhance water quality while contributing to resource recovery and environmental sustainability (Silva, 2023).

After extensive evaluation and validation in laboratory settings, numerous studies have advanced the use of microalgae for wastewater treatment to the pilot scale, mainly employing raceway but also other photobioreactors such as tubular and thin-layer. Scaling up photobioreactor processes presents higher complexity compared to conventional bioreactors. Optimizing this technology at the pilot scale and achieving its practical application in industrial systems require addressing several critical factors. These include the biological complexities of the system, particularly the interactions between microalgae, bacteria, viruses, and fungi. These interactions are influenced by environmental and operational factors that affect population dynamics and nutrient recovery from wastewater (https://doi.org/10.1016/j.jenvman.2024.122385).

Scaling up these processes also necessitates the development of control strategies and models to optimize and automate photobioreactors (Porras Reyes et al., 2024). This chapter will focus on these considerations and highlight the case of 100 m^2 raceway reactors in Almería, Spain, which have been operated for 2 years using urban wastewater. These reactors have yielded valuable experimental data crucial for technological advancement. Additionally, it will discuss real-world applications where companies are already implementing this technology with promising results.

Furthermore, the chapter will explore future enhancements through the integration of microbial fuel cells (MFCs) with microalgae systems. MFCs offer a promising approach for recovering nutrients from wastewater as bioenergy. Coupling these technologies can enable simultaneous wastewater treatment and the recovery of biomass and bioenergy. The development of wastewater treatment with microalgae has been progressing for decades, and it is the moment to realize its full potential, contributing to the objectives outlined in the 2030 Agenda.

2 Status Update on Microalgae-Involved Wastewater Treatment Processes

2.1 Biological Interactions: The Key to Success

In natural environments, algae interact with microorganisms through the phycosphere, the area immediately surrounding algae with a high microorganism density. The term "phycosphere" was coined in 1972 to describe a zone that extends outward from an algal cell or colony for an undefined distance, where extracellular products are excreted. Today, the phycosphere is seen as a hub for exchanging various chemical substances between microalgae and microorganisms, including growth-promoting compounds, organic carbon, algicidal substances, antibiotics, and gaseous compounds (Bell & Mitchell, 2016). These exchanges dictate relationships among microalgae, such as mutualism, commensalism, antagonism, parasitism, and competition (Ashraf et al., 2023).

Through the phycosphere, algae and microorganisms form a functional unit called a holobiont. An algal holobiont is an ecological system where microalgae and its microbiota (bacteria, viruses, fungi, etc.) create an integrated unit with specialized symbiotic interactions essential for both. This concept also applies to using algae for treating effluents, where algae interact with various microorganisms. Specific types and mechanisms of holobiont interactions affect their maintenance, stabilization, and establishment, and stress conditions can destabilize the functional unit, leading to system failure (Ren et al., 2022). Therefore, understanding the interactions in wastewater treatment systems with microalgae is crucial for industrial success. Many interactions benefit nutrient recovery and biomass production, while others can be detrimental to these goals.

In wastewater treatment, a key aspect of algal interactions with microorganisms is the formation of microalgae-bacteria consortia, particularly regarding assimilation, competition, and nutrient recovery. Though once seen as contaminants (Fuentes et al., 2016), bacteria significantly enhance nutrient recovery when interacting with algae (Wirth et al., 2020). Among these consortia, one fundamental bacterial group emerge heterotrophic bacteria.

Within these partnerships, microalgae harness sunlight to perform photosynthesis, using carbon dioxide (CO_2) as a carbon (C) source. This process releases dissolved oxygen (DO), which is then utilized by heterotrophic bacteria to oxidize organic matter. In turn, these bacteria produce CO_2, which microalgae readily absorb, thus completing the cycle (Dai & Wang, 2024). This symbiotic relationship has been recognized for over half a century, dating back to Oswald's observations in open ponds (Oswald et al., 1953). Nowadays, there are industrial-scale wastewater treatment systems based on this technology, allowing for the purification of organic matter and the recovery of N and P present in biomass form.

Apart from organic matter, high concentrations of N appear in wastewater in various forms. Nitrifying bacteria, subdivided into ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB), play a key role with algae. AOB

oxidize $N\text{-}NH_4^+$ to $N\text{-}NO_2^-$, and NOB convert $N\text{-}NO_2^-$ to $N\text{-}NO_3^-$. Algae can consume both $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$, but prefer $N\text{-}NH_4^+$, creating a specific relationship between algae and AOB. Different authors describe an antagonistic relationship between AOB and algae, as both organisms can assimilate N in the form of NH_4^+ and C in the form of CO_2. However, others suggest this relationship can be beneficial for AOB because they use the O_2 released during photosynthesis for ammonia oxidation (Bankston et al., 2020). This is demonstrated by the similar behavior of AOB, NOB, and algae with O_2 concentrations: they are active at moderate O_2 levels but inhibited by higher concentrations (Sánchez-Zurano et al., 2022) (https://doi.org/10.1016/j.jenvman.2024.122385).

Other operational parameters, such as culture depth, play a role in the relationship between algae and nitrifiers. Lower depths allow more light, promoting algae growth, but excessive solar radiation inhibits nitrifying bacteria, with NOB more affected than AOB. This can lead to nitrite accumulation at light intensities above 225 $\mu mol \cdot m^{-2} \cdot s^{-1}$(Vergara et al., 2016). In contrast, using reactors with greater height enhances the presence of shaded areas, which in turn promotes the growth of nitrifiers. This results in an increase in N in the form of $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ within the cultures (Sánchez-Zurano et al., 2021c).

The accumulation of N in microalgae-based wastewater treatment systems challenges both purification and algae growth. Specifically, $N\text{-}NO_2^-$ can inhibit algal growth starting at 5 $mg \cdot L^{-1}$. While not a permanent inhibitor, it reduces microalgae activity. Short-term exposures likely don't significantly affect photosynthesis, but continuous exposure has cumulative effects (González-Camejo et al., 2020). Therefore, understanding the role of AOB and NOB is essential for achieving effective wastewater treatment.

Denitrification is significant in wastewater treatment, using $N\text{-}NO_2^-$/ $N\text{-}NO_3^-$ as electron acceptors instead of DO to oxidize organic matter, primarily under anoxic conditions. Although anoxic conditions are uncommon in microalgae cultures, microalgae respiration can further deplete dissolved oxygen, especially at night or in the dark (Holmes et al., 2020).

For adequate phosphorous removal are essential the interactions between algae and polyphosphate-accumulating organisms (PAOs). These bacteria assimilate phosphate as polyhydroxyalkanoates (PHA) under anaerobic conditions and release $P\text{-}PO_4^{-3}$ during the aerobic stage. Conversely, algae can directly accumulate polyphosphate. Under certain environmental and operational conditions, microalgae can perform the luxury phosphorus uptake phenomenon, which involves the uptake of phosphorus by microalgal cells beyond what is required for growth, storing the excess phosphate within the biomass as polyphosphate (>1% P dry weight) (Slocombe et al., 2020). Additionally, algae can retain their polyphosphate granules for several days, whereas bacteria tend to quickly re-release their stored phosphorus. In mixed cultures of algae and bacteria, one advantage is that the phosphate released by PAOs under aerobic conditions can be assimilated by algae, contributing to complete P removal (Zhang et al., 2021).

In addition to the bacterial groups described, studies have referenced the potential co existence of microalgae along with photosynthetic bacteria, specifically phototrophic purple bacteria (PPB). The photoheterotrophic metabolism of PPB allows them to utilize light as an energy source while using organic compounds as sources of carbon and electrons, facilitated by bacteriochlorophyll pigments (Lu et al., 2019). Within the category of PPB, two distinct groups can be identified: purple sulfur bacteria (PSB) and purple non-sulfur bacteria (PNSB), which often coexist in the same environment.

The co existence of microalgae and PPB is especially interesting, as microalgae enable high levels of N recovery, while PPB exhibits high C assimilation and rapid growth (Sepúlveda-Muñoz et al., 2022). However, establishing and maintaining these relationships in algae cultures is challenging, as PPB conducts photosynthesis under anoxic conditions. High DO concentrations negatively impact PPB by decreasing their growth rate, leading to pigment loss and diminished photosynthetic capability. Under these conditions, PPBs are outcompeted by faster-growing aerobic organisms. This results in a gradual decline in PPB abundance, while PPB shows some resilience at lower DO concentrations (0–0.5 $mg{\cdot}L^{-1}$). However, in algae cultures DO could reach more than 20 $mg{\cdot}L^{-1}$ (Capson-Tojo et al., 2021).

While algae-bacteria interactions have been a focal point in effluent treatment processes involving algae, there is currently significant interest in studying algae interactions with other organisms, especially those exhibiting parasitic behavior. This shift in focus is attributed to the fact that the technology of effluent treatment with microalgae has already been tested at pilot and industrial scales. However, it faces challenges related to maintaining stable cultures over time and preventing system failures due to biological interactions. Like any other living organism, algae are susceptible to parasitic infections. Parasitism is the most common consumer strategy, and numerous biotic interactions are driven by parasites such as viruses, pathogenic bacteria, and parasitic eukaryotes. Special attention is focused on parasitic fungi, including chytrids and aphelids. They are typically associated with negative effects due to their morbidity and mortality impacts on host populations (Grossart et al., 2019).

2.2 Environmental and Operational Parameters

As described in the previous section, the key to the success of microalgae-based effluent treatment lies in the various biological interactions that occur. These interactions and the organisms' activity are in turn governed by the environmental and operational factors under which the systems operate (Acién et al., 2016). In summary, it can be stated that available light is the primary parameter that determines algae growth in bioreactors when operating under photoautotrophic conditions. Furthermore, this activity, determined by light, will be modulated by the influence of temperature, dissolved oxygen (DO), pH, concentration of main nutrients, dilution rate (if operated continuously), and depth of the cultures (which in turn is related to the light available in the cultures) (Solimeno et al., 2015).

The availability of light within the culture is determined by the average irradiance, which is influenced by the solar radiation reaching the reactor surface and the reduction of light intensity caused by the biomass. This reduction depends on factors such as biomass concentration, the light attenuation properties of the biomass, and the depth of the water (Grima et al., 1994). The average irradiance within the culture significantly affects biological system performance, impacting both wastewater treatment capacity and biomass production. Two primary scenarios are explored: one emphasizes maximizing wastewater treatment capacity, with microalgae primarily serving as DO producers, while the other prioritizes resource recovery, focusing on microalgae biomass production. In the former, high water depths (30–40 cm) are necessary for efficient organic matter degradation by bacteria, whereas in the latter, low water depths (10–20 cm), favoring increased light availability, are utilized to stimulate greater microalgae biomass growth (Acien et al., 2023).

Thus, the availability of light, determined by the depth of the culture, will influence the activity of both microalgae and bacteria, as well as the assimilation and recovery of nutrients. However, these processes will also be influenced by temperature, which depends on the location of the photobioreactors and the season. Microalgae production in outdoor photobioreactors could experience temperature fluctuations between 10 and 45 °C in temperate regions (Ras et al., 2013). Indeed, most microalgae species are capable of carrying out photosynthesis and cellular division over a wide range of temperatures, with optimal conditions between 25 and 35 °C (Rodríguez-Miranda et al., 2022), while bacteria prefer higher temperatures.

Algal and bacterial processes can alter the pH, causing acidification through NH_4^+ assimilation and nitrification (Rossi et al., 2020), or basification by photosynthetic CO_2, which can raise the pH of cultures to over 9.5–10. Such high pH levels can be harmful, as both algae and bacteria generally thrive in neutral to slightly alkaline conditions (7.5–9.5) (Sánchez-Zurano et al., 2021a, 2022). Unlike temperature, pH is easily controlled through CO_2 injections into the system, preventing the rise in pH in the cultures because of the photosynthetic activity and avoiding N losses by N-NH_3 stripping and P precipitation (Arbib et al., 2013; Posadas et al., 2015).

Dissolved oxygen (DO) also plays a crucial role, as microalgal cultures often exhibit DO levels between 100% and 400% air saturation, or even higher. Regarding its effect on algae, it has been shown that at elevated levels, DO can inhibit photosynthesis and inflict photochemical damage on the photosynthetic apparatus and other cellular components (Peng et al., 2013). Also DO could change the role of the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) by increasing photorespiration activity (light-dependent consumption of oxygen and production of CO_2), which reduces cell growth, and potentially cause the entire culture to collapse (Kazbar et al., 2019; Raso et al., 2012).

Concerning bacterial activity, it has been reported that DO concentrations far above air saturation can have inhibitory effects due to the diffusion of oxygen through the membranes, causing oxidative stress in cells (Baez & Shiloach, 2014). However, this inhibitory effect seems to be related only to long-term exposure to

dissolved oxygen oversaturation. Additionally, studies have shown contradictory results in this regard, suggesting that the impact of dissolved oxygen may be more closely related to the specific consortium established. To reduce the impact of dissolved oxygen (DO) on culture growth, implementing an aeration or stirring mechanism is essential (Pawlowski et al., 2015).

In addition to these parameters, it is essential to consider nutrient concentrations in the effluent. These concentrations will determine the growth of algae and bacteria, as detailed in the previous section. Through nutrients, symbiotic or competitive relationships can emerge. For instance, algae and AOB may engage in competition for C inorganic and/or N-NH_4^+ (Casagli et al., 2021a). The composition of wastewater is primarily influenced by the activities prevalent in the area, such as agriculture, industry, or farming. A significant portion of wastewater originates from various sources like homes, industries, and businesses (Silva, 2023). Understanding wastewater composition is crucial, especially considering the specific requirements of microalgae biomass, which necessitates a C/N/P ratio of 100/14/2.

In a wastewater treatment plant (WWTP), three main types of wastewater are distinguished: firstly, wastewater after primary treatment (with a composition of 100/21/4); second, wastewater after secondary treatment (with a composition of 100/25/12); and third, the centrate from anaerobic digestion (with a composition of 100/207/5), notable for its high contaminant concentration. Upon observation, it's clear that only wastewater from primary treatment matches the needed C/N/P ratio. The other types have too much nitrogen and phosphorus or not enough carbon. This shows that microalgae in these cultures need more carbon, like CO_2 or bicarbonate, to fully use the nitrogen and phosphorus in the medium (Acién et al., 2016). In addition to considering the composition, it's essential to note that while most of the P is P-PO_4^{-3}, the majority of N in wastewater exists as N-NH_4^+. Ammonium levels exceeding 160 mg·L^{-1} can become inhibitory for microalgae (Scarponi et al., 2021).

2.3 *Control Strategies and Modeling in Microalgae-Based Wastewater Systems*

The development of mathematical models that describe the phenomena occurring within photobioreactors contributes not only to a better understanding of the system itself but also to the development of new control strategies and various optimization approaches for production and resource reduction. However, the format and complexity of these models can vary greatly depending on the desired application.

One of the initial approaches to modeling microalgae production systems is based on the basic description of the dynamics of the processes involved in the system, known as first-principles modeling. From this perspective, modeling microalgae biomass production photobioreactors is achieved through the description of mass balances within the reactor and the chemical reactions of its elements. Modeling the dynamics of the phenomena occurring inside a photobioreactor and estimating the

elements within it is a complex process that involves a certain degree of parameter uncertainty, making calibration difficult. Using freshwater with fertilizers as a reference for microalgae biomass production, some examples serve as a basis for the initial development of the system (Fernández et al., 2014, 2016).

However, when the microalgae production process is combined with wastewater treatment, the model's complexity increases significantly. It becomes necessary to include the growth dynamics of new organisms (bacteria) and their interaction with the elements present in the medium (Casagli et al., 2021b; Nordio et al., 2024; Sánchez-Zurano et al., 2021b; Solimeno et al., 2017).

From a control perspective, it is not always necessary to develop and apply such a detailed model. As mentioned, the most critical variables affecting the growth of microalgae in the reactor are solar radiation, pH, DO, temperature, and nutrient concentration in the medium. Of these, solar radiation depends on the location and cannot be controlled, while nutrient input depends on the wastewater inlet. Therefore, the controllable variables are pH, DO, and temperature. However, temperature control is not conventional, as it is energy demanding and requires heat exchangers, though alternatives are being studied to avoid using these devices (Rodríguez-Miranda et al., 2021) and design methods to minimize energy losses. Consequently, pH and DO are the most commonly controlled variables due to their ease of manipulation through CO_2 injection or aeration, respectively.

Although the algae-bacteria consortia along with wastewater treatment phenomena may vary compared to a clean microalgae culture, in terms of control, the design and application of strategies are equivalent.

There are primarily two control approaches: classical control and advanced control. Classical control is characterized by being manual or using the on/off strategy, which involves fully opening the gas injection valve (CO_2 or air) when the controlled variables (pH and DO) exceed a certain value. In the case of pH, this control method causes oscillations and high consumption.

On the other hand, advanced control encompasses a series of strategies aimed at maintaining the control variable within specific values. One of the most commonly used approaches is PID control (proportional-integral-derivative), which computes the error between the system variable and a desired reference value and generates a signal proportional to this error. For pH, depending on the error between its current value and the desired value, the gas injection valve opening is adjusted to keep pH as close as possible to the reference. For designing such controllers, a simplified model of the system, known as a black-box model, is used, relating the manipulable variable mathematically to its effect on the system (influence of CO_2 or air injection on pH and DO) (Rodríguez-Miranda et al., 2020; Sánchez et al., 2022). This control strategy can be combined with another method called event-based control, where the control signal is applied to the valve only when the variable (pH or DO) deviates from established limits (Pawlowski et al., 2015; Rodríguez-Miranda et al., 2019a, b).

Another strategy applied to pH is model-based control, where the signal applied to the CO_2 injection valve depends on a model designed to reflect the changing pH dynamics. These simplified models are obtained through measured data from the system and can be used for developing predictive or adaptive controls based on system conditions (Caparroz et al., 2023; Otálora et al., 2023; Pataro et al., 2023).

Regarding DO control, due to its dynamics, it is impossible to maintain a specific value over time as it fluctuates between daytime and nighttime periods. However, optimal controls can be applied for aeration when the system demands it, calculating the necessary air flow rate to maintain a reference value during daytime (Barceló-Villalobos et al., 2022).

There are some notable differences in pH and DO control between wastewater reactors and those with freshwater. In the case of wastewater reactors, due to CO_2 production by bacteria, the amount of gas injected for pH control is reduced. Additionally, due to DO consumption by bacteria, accumulation levels are not as high as in freshwater microalgae reactors. However, during the night period, anoxic conditions can be reached, making it crucial to maintain the aeration provided by the dissolved oxygen control over nighttime to avoid this scenario.

These control strategies allow for adjusting and maintaining different operating points in photobioreactors. Thus, operational conditions regarding pH and DO can be set not only to optimize microalgae growth but also, conversely, to maximize bacterial growth to clean water and reduce nutrient concentration, depending on the application and desired objective.

3 The Case Study of Pilot Scale in Almería

At the forefront of sustainable biotechnology, the SABANA project seeks to transform the agricultural, wastewater treatment, and aquaculture industries, offering sustainable solutions for crop nutrition, waste remediation, and fish feed production. As part of this initiative, up to three industrial facilities were installed and are being operated, one additional demonstration facility being installed in IFAPA Research Centre in Almería to produce commercial-scale amounts of microalgae biomass for evaluation in various markets, serving as a platform for advancing microalgae biotechnology in Europe.

Among the research lines proposed by the research center, wastewater treatment has emerged as a key focus area, with particular emphasis on the coupling of biomass production and water remediation. This technology has been successfully tested at pilot-scale levels in raceway reactors of 80 m^2 (12 m^3), located in IFAPA Research Centre, demonstrating the robustness and reliability of this innovative process, particularly as a potential replacement for conventional secondary treatment methods (Nordio et al., 2023). These reactors have been operated continuously for 3 years with raw wastewater from the University of Almería (UAL) and the municipality wastewater treatment plant. Notably, the technology has shown significant efficacy in reducing N, P, and COD concentrations in wastewater.

3.1 Raceway Reactors and Culture Media

The raceway reactor is the typical photobioreactor design chosen for microalgae cultivation in wastewater treatment. Indeed, closed photobioreactors are normally used to produce high-added value products from microalgae biomass, such as nutraceuticals or food ingredients, considering their capital and running costs. On the contrary, raceways ensure low costs and easy manageability, and they offer the possibility of treating a high amount of water per surface utilized (Morillas-España et al., 2021).

Raceway reactors are typically composed of three main parts: the channels, the paddlewheel, and the sump (Fig. 1). The paddlewheel is connected to an electrical engine, ensuring the culture recirculation on the channels. Furthermore, the sump is used for the gas injections and the gas-liquid mass transfer. The raceway reactors available at the University of Almería have a surface of 80 m^2, and a total volume of 12 m^3. They are composed of a double channel of 40 m connected with 180 bends, while the sump has a volume of 0.6 m^3 (0.9 diameter × 1.2 height) where gas diffusers are used for the flow of compressed air and CO_2.

Furthermore, the reactors are provided with a series of probes that include pH, DO, temperature, and culture depth. One pH and one DO probe are installed after the reactor sump for the culture monitoring, while four additional sensors (two for pH and two for DO) are at the end of the second channel and are used for the development of advanced control strategies. All the sensors are connected to a programmable logic controller (PLC) and a supervisory control and data acquisition (SCADA) system, allowing for continuous monitoring of the culture status and to a collection of online data (sample time of 1 s) (Fig. 2).

To conduct experiments under conditions as close as possible to those of real wastewater treatment industrial plants, the culture medium chosen was untreated urban wastewater, collected directly from the University of Almería. The wastewater was initially stored in a 10 m^3 dark tank and then pumped to the reactor using a



Fig. 1 Pilot-scale raceways located in the SABANA demo plant, Almería, Spain

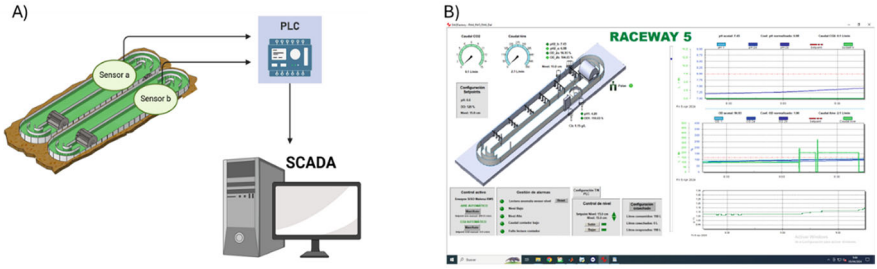


Fig. 2 Online measures systems. (**a**) Scheme of sensor-PLC-SCADA system for culture monitoring; (**b**) example of SCADA monitoring

pressure pump through an independent pipe. To ensure a consistent supply of wastewater to the raceways, during specific periods of the year, the wastewater was sourced from the AQUALIA S.A. Primary Wastewater Plant.

3.2 Biomass Production and Nutrient Recovery

In routine production operations, the raceway reactors are operated continuously with a fixed dilution rate of 0.2 day^{-1}. To test the resilience of this technology, experiments are typically conducted over long periods to assess its capacity for effective nutrient removal from the medium. Additionally, the biomass productivity and the nutrient removal capacity of the wastewater system were compared with an identical raceway reactor operated in parallel but fed with freshwater and chemical fertilizers. With this comparative analysis, it was possible to elucidate the influence of the wastewater medium on overall system performance. It also provides a deeper understanding of the principal mechanisms occurring within wastewater treatment systems, serving as a starting point for further optimization of raceway reactor operations.

Figure 3 illustrates the biomass productivity, expressed as $g{\cdot}m^{-2}{\cdot}day^{-2}$, over an extended period from April to December for both the fertilizer-fed and wastewater-fed systems. It is important to note that, despite the initial inoculation of the reactor with a specific strain (*Scenedesmus* sp.), the dominant strain was changing over time due to seasonal changes and water fluctuations. Effectively maintaining a single strain in outdoor systems is challenging, yet it may not be critical for the water remediation efficiency of the system. During this period the wastewater used was taken from the University of Almería (UAL) and for a brief period from the urban wastewater treatment plant (WWTP) in the city.

As raceway reactors operate outdoors, the primary factors influencing productivity are environmental conditions, notably solar radiation and temperature. Typical solar radiation values in Almería during the same period range from 1180 to 400 $\mu E{\cdot}m^{-2}{\cdot}s^{-1}$ on average during daylight hours between April and

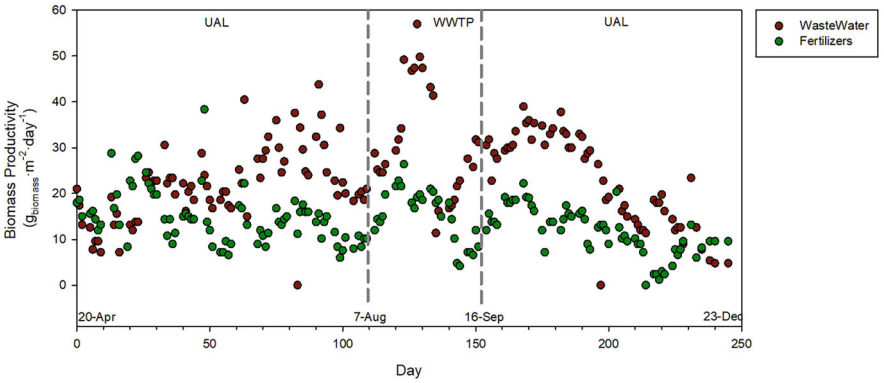


Fig. 3 Biomass productivity, over an extended period from April to December for both the fertilizer-fed and wastewater-fed systems

January. Simultaneously, temperatures can fluctuate between 10 and 30 °C, with peaks reaching 38 °C within the culture during summer. On average, these conditions can be considered close to optimal for microalgae growth.

The biomass productivity of the fertilizer-fed system varied between 9 and 30 $g{\cdot}m^{-2}{\cdot}day^{-2}$, following a trend similar to that of solar radiation. Notably, productivity peaked during spring (the period of maximum solar radiation in Almería) and gradually decreased as the colder seasons approached. Conversely, the wastewater-fed system exhibited slightly higher productivity, ranging between 9 and 40 $g{\cdot}m^{-2}{\cdot}day^{-2}$, with some peaks reaching 55 $g{\cdot}m^{-2}{\cdot}day^{-2}$. Moreover, the dynamics of biomass productivity in the wastewater system did not strictly follow the pattern suggested by solar radiation and seasonal changes, indicating that other factors, such as wastewater medium composition, may have influenced it.

These results align with other studies conducted in similar systems (Nordio et al., 2023; Sánchez-Zurano et al., 2021d), confirming that microalgae can be cultivated for extended periods in wastewater, ensuring continuous biomass production. Moreover, this study demonstrates that the raceway reactor is a suitable technology for this process.

Figure 4 shows the evolution of the N inlet and outlet of the raceway reactors in terms of $N\text{-}NH_4^+$ and $N\text{-}NO_3^-$. The $N\text{-}NH_4^+$ is the predominant N form in wastewater influents, with concentrations ranging between 100 and 300 $g_{N\text{-}NH4}{\cdot}m^{-3}$. The N content of microalgal biomass varies between 5% and 10%, with microalgae cells having the capacity to assimilate N in various forms such as ammonium, nitrate, nitrite, and organic nitrogen (e.g., urea and amino acids) (Fallahi et al., 2020). However, NH_4^+ is the preferred nitrogen form for algae due to its lower energy requirement for assimilation. Concurrently, another significant group of bacteria found in wastewater, such as nitrifying bacteria, also consumes NH_4^+. Literature reports indicate that nitrifiers compete with microalgae for NH_4^+ consumption (González-Camejo et al., 2022), but the presence of microalgae can double the N

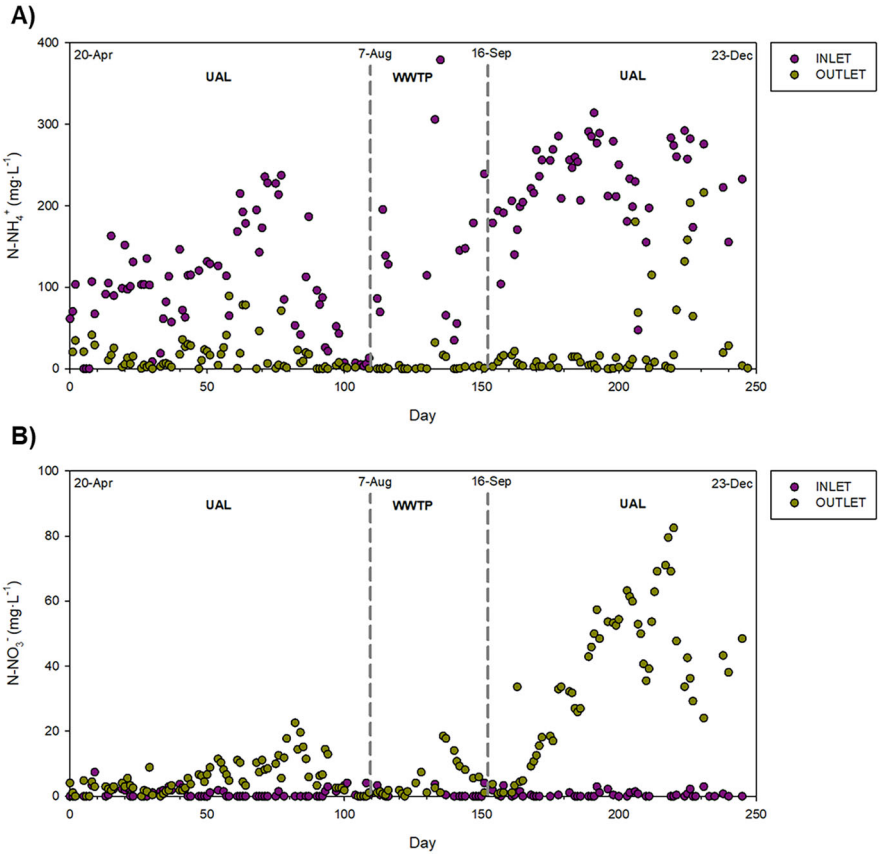


Fig. 4 The evolution of the N inlet and outlet of the raceway reactors in terms of N-NH_4^+ (**a**) and N-NO_3^- (**b**)

removal compared to nitrification alone (Bankston & Higgins, 2020). Indeed, the effluent N-NH_4^+ concentration was maintained below 50 $g_{N\text{-}NH4}{\cdot}m^{-3}$ for most of the study period (Fig. 4a), enabling a reduction of 30–90%.

Conversely, the influent NO_3^- concentration is often negligible (<3 $mg_{N\text{-}NO3}{\cdot}L^{-1}$), but it can increase up to 80 $g_{N\text{-}NO3}{\cdot}m^{-3}$ due to nitrifier activity (Fig. 4b). Proper management of nitrification and avoidance of excess N-NO_3^- accumulation are crucial, especially considering the EU legislation that limits nitrogen concentration in discharged water to 15 $mg_N{\cdot}L^{-1}$(Council Directive of May 1991 concerning urban wastewater treatment, 1991). Since microalgae consume N-NO_3^- only when N-NH_4^+ is exhausted, N-NO_3^- accumulation can be prevented by ensuring proper management of production systems to limit nitrification and favor algal activity. In this respect, from September onward, the activity of nitrifiers significantly increased compared to the spring and summer seasons, resulting in a substantial production of N-NO_3^-. This increase is related to a change in operational

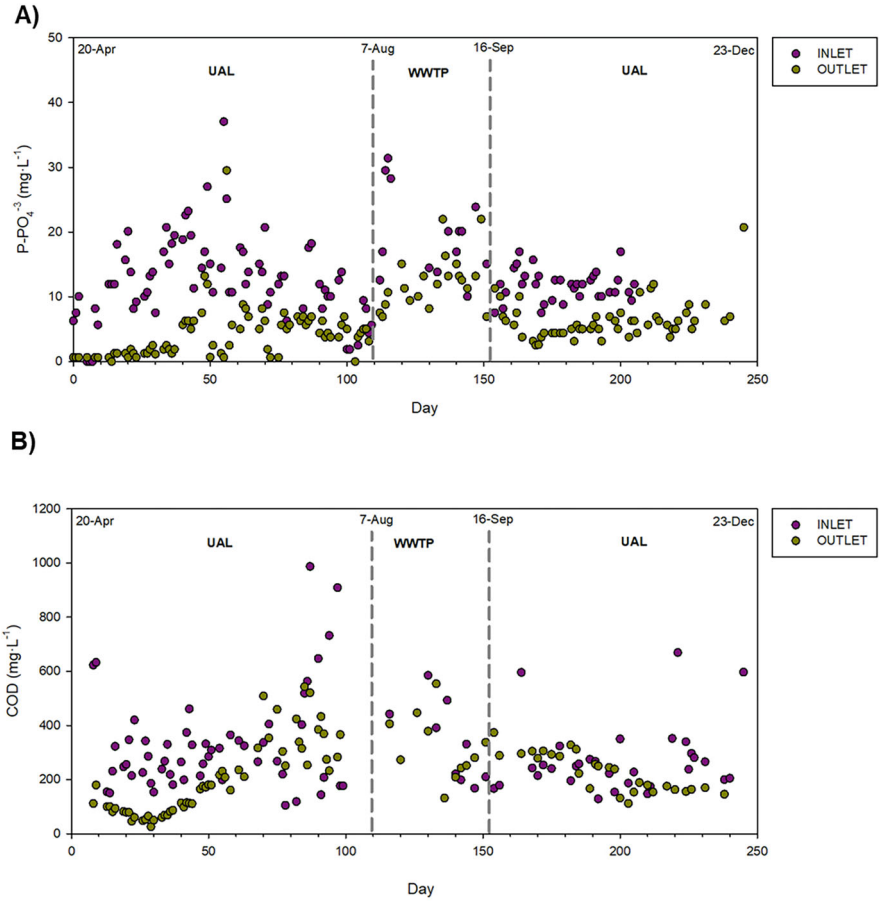


Fig. 5 The evolution of P-PO_4^{3-} (**a**) and COD (**b**) of the microalgae-bacteria system in the raceway reactors

conditions implemented in September when the airflow rate in the sump used to strip the dissolved oxygen was reduced from 200 to 50 L·min^{-1}. The decreased oxygen removal led to a decrease in the concentration of microalgae growth while favoring bacterial proliferation, particularly nitrifying bacteria.

Besides N, microalgae require P to produce ribosomal RNA, assimilating it directly into the biomass as P-PO_4^{3-}. This marks a significant difference from activated sludge processes, which typically exhibit low P removal efficiency, and they are often enriched with polyphosphate-accumulating organisms (PAOs) to enhance biological phosphorus removal (Fallahi et al., 2021). In contrast, microalgae can uptake Ps and, under specific operational conditions, accumulate it. The primary mechanism for P removal is microalgal assimilation, initially dominated by adsorption followed by assimilation (Rezvani et al., 2020). Figure 5a illustrates the P-PO_4^{3-} inlet and outlet concentrations in the system. While the influent P concentration varies between 10 and 30 $mg_P \cdot L^{-1}$, with peaks reaching 40 $mg_P \cdot L^{-1}$, the

effluent concentration consistently remained below 15 $mg_P \cdot L^{-1}$. Despite the high removal achieved by the system, it was unable to consistently maintain phosphorus concentrations below the 2 $mg \cdot L^{-1}$ limit imposed by the EU for water discharge.

Finally, Fig. 5b illustrates the COD concentration inlet and outlet of the system. The influent maintains a consistent COD content of approximately 300 $mgO_2 \cdot L^{-1}$, which is reduced by 50–200 $mgO_2 \cdot L^{-1}$. The COD represents both soluble and particulate organic matter, and its reduction is primarily due to the activity of heterotrophic bacteria. On the contrary, the heterotrophic growth of microalgae is considered negligible, as the organic carbon in wastewater typically exists in complex forms like proteins and fibers, which are not directly consumed by algae. Instead, algae utilize CO_2. Considering the European legislation that sets the COD concentration limit at 125 $mg \cdot L^{-1}$, the system is only able to consistently reduce organic matter below this value during specific periods of the year when heterotrophic activity is enhanced.

In conclusion, this case study demonstrates that microalgae-based wastewater treatment can effectively reduce nutrient content in water through synergistic interactions with bacteria. However, effectively reducing N, P, and COD below EU thresholds requires proper management of the remediation systems to promote microalgae growth, reduce nitrification, and enhance COD reduction. These systems are biologically complex, and directly linking operational conditions to the dynamics of microbial populations experimentally remains a challenge.

3.3 Modeling the Biological System

Conducting year-long experiments allows for the evaluation of system dynamics and responses to sudden changes in wastewater composition, environmental conditions, and diverse raceway operations. A particularly significant challenge is posed by the variability in nutrient concentrations of the influent wastewater, which highly influences the overall biological system. Despite long-term pilot-scale experiments providing valuable data for system evaluation, they are both time- and resource-intensive. Consequently, once the primary mechanisms involved in the biological process have been identified, the development of proposed simulation tools can represent an adequate solution.

Indeed, mathematical models are capable of describing the biological system and they can become an invaluable tool for simulating various scenarios in a short time. Furthermore, they can serve to carry out optimization studies, to enhance the nutrient removal from water and the algal production. Achieving these objectives requires the development of "comprehensive" biological models that incorporate the influence of numerous environmental and operational variables on the biological system, such as solar radiation, temperature, pH, and wastewater composition. In recent years, research has been partially focused on this topic, leading to the development of some algae-bacteria models (Casagli et al., 2021b; Reichert et al., 2001; Sánchez-Zurano et al., 2021b; Solimeno et al., 2019; Zambrano et al., 2016). However, most of these models have been validated only at the laboratory scale or using synthetic

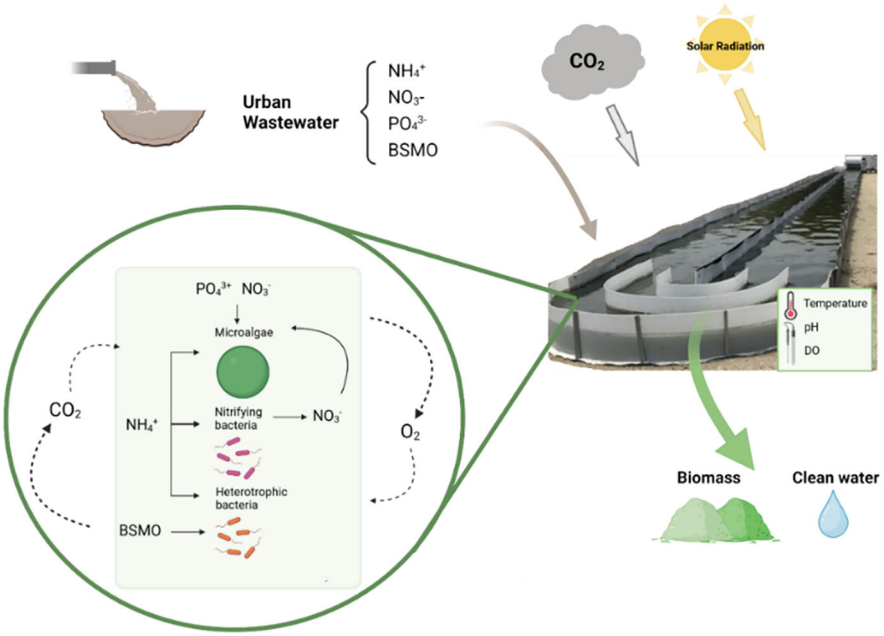


Fig. 6 Scheme of the ABACO-2 concept

wastewater. In contrast, validation over extended periods, at a pilot scale, and using real wastewater are essential for assessing their robustness and potential as tools for bridging the gap between laboratory observations and industrial production.

In this framework, a novel algae-bacteria model named ABACO-2 was developed and underwent extensive validation over 8 months using data from a pilot-scale raceway reactor operated with untreated urban wastewater (Nordio et al., 2024). Figure 6 illustrates the model concept and objectives. The main microbial populations considered were microalgae, nitrifying bacteria (NOB and AOB grouped), and heterotrophic bacteria. The ABACO-2 model aims to assess the interactions among these populations and the dynamics of nutrient concentrations in the medium. As part of the model concept, the aim is to describe the microalgal growth and photosynthesis during which they produce oxygen and simultaneously consume inorganic carbon, N, and P. Concurrently, there is the description of the growth of bacteria that consume oxygen and release inorganic carbon, which is subsequently utilized by microalgae. Specifically, heterotrophic bacteria oxidize the readily soluble organic matter (BSMO), estimated as a fraction of the total COD. Conversely, nitrifying bacteria compete with microalgae for the consumption of $N\text{-}NH_4^+$, releasing $N\text{-}NO_3^-$ during the nitrification process. Additionally, microalgae can consume nitrate when $N\text{-}NH_4^+$ concentrations are low, as it is their preferred nitrogen form due to its lower energy requirement for assimilation.

The model encompasses seven biological processes (Table 1) including the growth of microalgae on NH_4^+ and NO_3^-, the growth of nitrifying and heterotrophic

Table 1 Processes of the ABACO-2 model

n.	Process	Process rate [$g \cdot m^{-3} \cdot day^{-1}$]
1	Microalgae growth on NH_4	$\mu_{max,alg} \cdot \mu(I_{av}) \cdot \overline{\mu(T) \cdot \mu(pH)} \cdot \overline{\mu(O_2)_{alg}} \cdot \mu(N-NH_4) \cdot \mu(P-PO_4) \cdot X_{alg}$
2	Microalgae growth on NO_3	$\mu_{max,alg} \cdot \mu(I_{av}) \cdot \overline{\mu(T) \cdot \mu(pH)} \cdot \overline{\mu(O_2)_{alg}} \cdot \mu(N-NO_3) \cdot \mu(P-PO_4) \cdot \left(1 - \overline{\mu(N-NH_4)}\right) \cdot X_{alg}$
3	Microalgae decay	$m \cdot X_{alg}$
4	Nitrifying bacteria growth	$\mu_{max,nit} \cdot \overline{\mu_{nit}(T) \cdot \mu_{nit}(pH) \cdot \mu_{nit}(O_2) \cdot \mu_{nit}(N-NH_4)} \cdot X_{nit}$
5	Nitrifying bacteria decay	$\theta_{nit} \cdot m_{nit} \cdot X_{nit}$
6	Heterotrophic bacteria growth	$\mu_{max,het} \cdot \overline{\mu_{het}(T) \cdot \mu_{het}(pH) \cdot \mu_{het}(O_2) \cdot \mu_{het}(N-NH_4) \cdot \mu_{het}(BSMO)} \cdot X_{het}$
7	Heterotrophic bacteria decay	$\theta_{het} \cdot m_{het} \cdot X_{het}$

bacteria, and their respective decay. Moreover, the model incorporates the effects of multiple variables on microbial growth, such as temperature, pH, dissolved oxygen, and nutrient concentration.

A key innovation of the ABACO-2 model compared to existing algae-bacteria models is the introduction of nutrient consumption yields, in contrast to stoichiometric yields. Classical models like ASM assume a constant biomass composition and, consequently, constant nutrient uptake from the medium. However, this assumption may not hold for microalgae, as they can accumulate certain components through a phenomenon known as "luxury uptake." As already mentioned, microalgae can uptake phosphorus beyond their growth requirements, storing it as polyphosphate (Slocombe et al., 2020). Modeling this phenomenon is complex, as it can be influenced by numerous factors such as light, temperature, oxygen, and medium composition. However, accurately representing biomass composition is fundamental to estimating nutrient removal from the medium with high accuracy, and it represents a fundamental difference from classical bacterial models. As a first step in this direction, the ABACO-2 model replaced the constant stoichiometric coefficients with yield coefficients that are functions of the medium composition and salinity, following the recommendations of Zurano et al. (2021).

Figures 7 and 8 present the validation simulation results from applying the ABACO-2 model in terms of biomass concentration and nutrient evolution; PO_4^{3}, NO_3^{-}, BSMO (readily COD biodegradable), and NH_4^{+}. The experimental data, represented as dots, were collected over 8 months and correspond to those presented in the previous section. The continuous line represents the simulation, while the colored shading indicates the model uncertainty at 95%. Consequently, it can be considered a valuable tool for simulating and studying microalgae production in wastewater, as well as tracking nutrient dynamics within cultures.

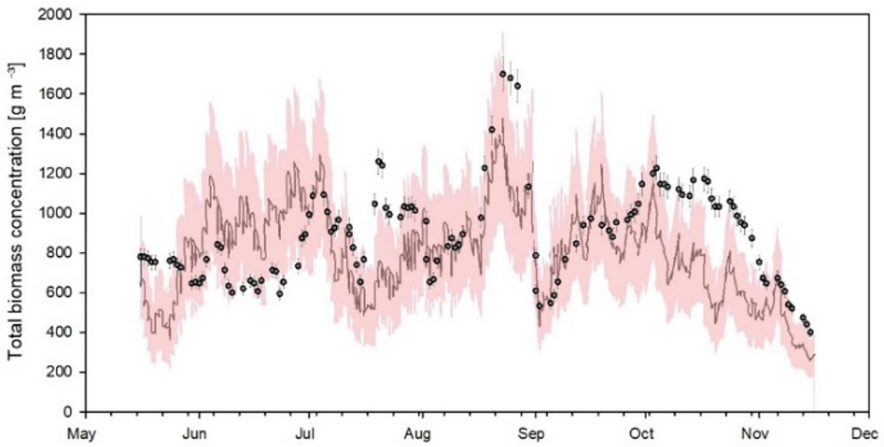


Fig. 7 The validation simulation results from applying the ABACO-2 model in terms of biomass concentration in the system fed with wastewater

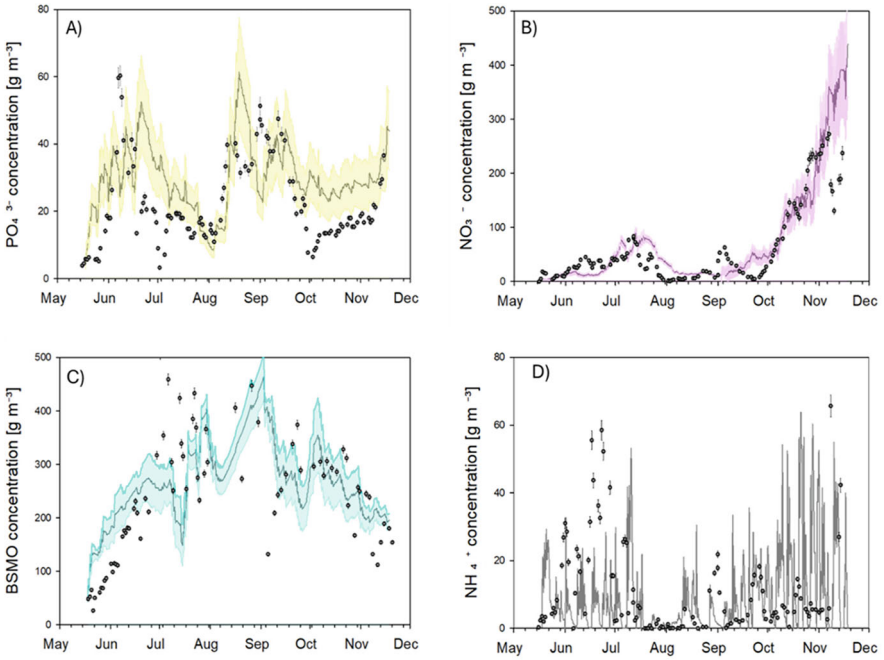


Fig. 8 The validation simulation results from applying the ABACO-2 model in terms of PO_4^{3-} (**a**), NO_3^- (**b**), BSMO (**c**), and NH_4^+ (**d**)

While the ABACO-2 model serves as a valuable tool for simulating algae-bacteria systems, its validation has been limited to pilot-scale reactors, and its effectiveness in large-scale production systems approaching industrial settings remains unproven.

To date, no algae-bacteria models have been validated for facilities exceeding 80 m^2 due to the limited number of such facilities deploying this technology. Moreover, current experimental limitations, such as the inability to differentiate between algal and bacterial biomass contributions, limit an accurate model validation. Finally, future research should be focused on the development of a standard algae-bacteria model, following the example provided by the ASMs. The development of a standard algae-bacteria model could offer significant benefits, particularly by facilitating the development of simulation tools and software, thereby promoting the industrial development of these systems. However, model standardization is contingent upon establishing standards for experimental data collection, monitoring campaigns, and production systems, which still differ from one to other facilities.

4 The Analysis of Real-World Systems

However, despite the advantages of microalgae processes for wastewater treatment, their commercial adoption remains limited. Over the past century, high-rate algal ponds have been developed worldwide. Initially researched at the University of California in the mid-twentieth century for their potential in using algal biomass for wastewater treatment, these systems have since been implemented in various countries such as Israel, France, Morocco, the United Kingdom, Spain, Australia, China, and New Zealand. They have been applied to treat different types of waste, including domestic, tannery, dairy, and piggery waste (Young et al., 2017). Apart from large facultative ponds lacking specific algae strain production, few pilot plants globally recover algae biomass from wastewater. One challenge is the extensive surface area needed, demanding enhanced process efficiency to cut costs and optimize energy use (Arbib et al., 2022).

Furthermore, an industrial application of this technology is exemplified in Chiclana (Cádiz, Spain), where a 2-hectare algae cultivation facility has been developed through the FP7 ALL-GAS project funded by the EU. This facility can treat 2000 $m^3 \cdot day^{-1}$ of wastewater, equivalent to servicing 10,000 population equivalents. Data from a consecutive year demonstrate the production potential of 200 tons per hectare per year of biomass for bioenergy and biofertilizers, along with treated water output of up to 1840 $m^3 \cdot day^{-1}$ (Aqualia (Spain), 2018). The energy balance over a year of continuous operation reveals significantly lower operational expenses compared to conventional wastewater treatment, with electricity consumption three times less. Moreover, the system generates nearly twice the energy it requires, producing efficient and sustainable biofuel, biofertilizer, and reusable water.

More recently, and as part of the European project SABANA, two new wastewater treatment plants were built in Spain by Aqualia, located at Hellín (1-ha) and Merida (2-ha). These new facilities have a capacity of up to 1000 and 2000 $m^3 \cdot day^{-1}$ respectively, including harvesting of the biomass using dissolved air flotation systems (DAFAST) and the use of biomass for biogas production and agriculture uses. One relevant advantage of these technologies is the low energy consumption, below 0.2 $kWh \cdot m^{-3}$ in comparison to 0.5 $kWh \cdot m^{-3}$ of conventional activated sludge systems. However, the major difference concerning conventional technologies is the possibility to recover nutrients as biomass, thus up to 10 tonN and 2 tonP can be recovered per hectare and year using these technologies. In these reactors, the technology has been improved to reduce the hydraulic retention time to 2 days and the requirement of surface up to 2 m^2/equivalent person, thus approaching this technology to the land requirements of conventional activated sludge processes (Fig. 9).

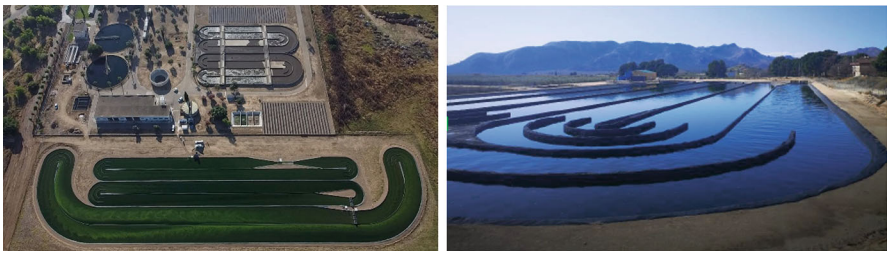


Fig. 9 Image of the microalgae-based wastewater treatment plants operated by Aqualia in Spain: Left Merida, Right Hellin

5 Future Trends: Coupling Microalgae Technology and Microbial Fuel Cells

As described throughout the chapter, the use of microalgae for effluent treatment is a highly promising technology, but it still faces challenges, especially related to (i) the use of effluents with high levels of turbidity that reduce available light in the cultivation, (ii) the use of wastewater with $N\text{-}NH_4^+$ levels above 200 $mg{\cdot}L^{-1}$, and (iii) low levels of COD removal as system is only able to consistently reduce COD below discharge limits during specific periods of the year. Therefore, this technology can be coupled with others to mitigate these issues and further increase the added value of the products obtained.

Microbial fuel cells (MFCs) have emerged as an alternative for treating wastewater due to their high efficiency in removing COD while simultaneously generating electricity (Hernández-Fernández et al., 2015; Roy et al., 2023). This technology is based on converting chemical energy from organic materials into electrical energy, recovering high-value products.

Overall, an MFC is an electrochemical device typically consisting of two compartments: the anodic and cathodic compartments, separated by a proton exchange membrane (PEM) (Hernández-Fernández et al., 2023; Rahimnejad et al., 2015). In the anodic chamber, microbial consortia break down the organic matter in the wastewater, converting it into protons, electrons, and CO_2. The electrons produced travel from the anode to the cathode via a conductive wire, while the protons move to the cathode chamber through the exchange membrane. In the cathode compartment, a terminal electron acceptor, usually O_2 (serves as the oxidant), combines with protons (H_2) and electrons to form water (H_2O), thus completing the circuit (Sonawane et al., 2022). Thus, MFCs are an eco-friendly technology for nutrient removal from different effluents. However, a significant drawback associated with the MFC operation for wastewater treatment is the inadequate nutrient removal by the bacteria in the anode chamber.

To improve this technology, photosynthetic microbial fuel cells (PMFCs) have been proposed (Khandelwal et al., 2020). In PMFCs, microalgae assist the cathode

by undergoing photosynthesis, releasing O_2 as a by-product, which can be beneficial for the process in the cathode chamber, where O_2 acts as an oxidant (Enamala et al., 2020; Sharma et al., 2024). This O_2 then acts as the terminal electron acceptor, contributing to the generation of electricity. Incorporating algae into MFCs offers other several advantages. Algae can absorb and remove a wide range of nutrients, such as $N\text{-}NO_3^-$ and $P\text{-}PO_4^{-3}$, that bacteria in the anode chamber cannot efficiently eliminate. They are also known for their ability to remove various toxins present in domestic, industrial, and commercial wastewater, thereby improving the quality of the treated water. Additionally, algae can be harvested and used as biomass for biofuel production or as raw material in other industrial processes, adding economic value. Integrating algae can make the wastewater treatment process more sustainable and energy efficient, as algae help reduce the need for additional chemicals and energy for nutrient removal. Furthermore, algae capture CO_2 during photosynthesis, contributing to the reduction of greenhouse gas emissions associated with wastewater treatment. In summary, incorporating algae into MFC systems can provide a more comprehensive and sustainable solution for wastewater treatment, improving pollutant removal efficiency while offering additional benefits such as biomass generation and CO_2 emission reduction.

At the laboratory scale, systems combining both technologies have already been developed using dual-chamber MFCs, and the results have been very promising. However, this technology needs to be scaled up and validated, suggesting the possibility of designing reactors similar to current raceways that integrate MFC technology. In this way, microalgae can grow under outdoor conditions using solar light as an energy source and provide O_2 to the MFC to act as an electron acceptor. To achieve this goal, MFC technology must become more cost effective by developing inexpensive and environmentally sustainable materials for the anode, cathode, and membrane that do not affect algae activity. This perspective of integration into pilot-scale reactors would allow for not only the recovery of nutrients from effluents in the form of biomass but also the production of bio-electricity.

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Pilot-Scale Outdoor Microalgae Production: A Case Study



Rosangela Rodrigues Dias, Luiz Roberto de Olival Costa, Mariany Costa Deprá, Leila Queiroz Zepka, and Eduardo Jacob-Lopes

Abstract The results found in the laboratory may not fully reflect what happens in practice. Pilot-scale outdoor production studies are an important part of the puzzle to identify and resolve challenges that may only arise when production is scaled up and placed under a variety of climatic conditions. Therefore, in this chapter, the main objective was to evaluate a case involving microalgae cultivation outdoors on a pilot scale. The case study data was analyzed to extract insights contributing to a broader understanding of outdoor microalgae cultivation. Hot spots for outdoor microalgae cultivation are identified and conclusions are drawn about the potential of these systems for biomass productivity. This case study presents a holistic approach to exploring microalgae cultivation in real-world situations.

Keywords Algae · Biomass · Large-scale cultivation · Practical case · Technological nodes

1 Introduction

Although microalgae production is not necessarily new, it has gained more attention in recent decades due to technological advances, sustainability concerns, and applications in diverse markets. Some of the most relevant markets for microalgae are energy, chemicals and materials, food and feed, and personal care and pharmaceuticals. Fortunately, as research and development in this area advances, new possibilities are discovered, further expanding the range of applications for this unique feedstock.

In this context, bioreactors, as an indispensable part of the process, play a fundamental role in achieving scales proportional to industrial demands. Currently, it is known that microalgae are predominantly cultivated in two bioreactor

R. R. Dias (✉) · M. C. Deprá · L. Q. Zepka · E. Jacob-Lopes
Federal University of Santa Maria, UFSM, Santa Maria, RS, Brazil

L. R. de Olival Costa
MicroAlgae Biotechnology, LTDA, Holambra, SP, Brazil

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configurations: raceway ponds, which are simpler and more economical open systems, and tubular bioreactors, which are closed systems characterized by more expensive but more efficient (Chang et al., 2017).

Open systems, such as raceway ponds, allow microalgae to grow outdoors directly influenced by environmental variables such as sunlight supply and temperature. Although they are a popular option due to their simplicity and low construction and operating costs, they can be negatively impacted by environmental variables that—without adequate control—affect biomass productivity and quality. Contamination by unwanted organisms, such as other microalgal species, bacteria, and viruses, is a major vulnerability of these systems (Ahmad et al., 2021; Bani et al., 2021; Al-Dailami et al., 2022). Therefore, closed systems (mainly, tubular bioreactors) have gained popularity among bioreactor configurations. These systems can provide (i) greater control over environmental variables, (ii) lower risk of biological contamination, (iii) lower water loss through evaporation, and (iv) greater efficiency in the use of space (Banerjee et al., 2020; Deprá et al., 2021; Zarei et al., 2023).

Given the advantages attributed to tubular bioreactors, in this chapter, we will translate the results of proof-of-concept experiments to pilot-scale outdoor production. By evaluating the growth kinetics of pilot-scale tubular bioreactor, we aim to provide exquisite details that contribute to exploring industrial-scale microalgae cultivation considering real situations.

2 Case Study Presentation: From Lab to Pilot Scale

There is a vast literature on the growth kinetics of microalgae on a laboratory scale. The results are often surprising, showing biomass productivities as high as 1.5 g L^{-1} d^{-1} (Bahadar et al., 2013; Ramachandra and Saranya, 2022). However, at pilot and industrial scale, bioreactors unsurprisingly encounter challenges that impede the theoretical success achieved in the laboratory. Unfortunately, few details emerge from the operation of these systems, and, in general, it is worth highlighting that negative experiences and failures are hidden or only slightly publicized, which prevents a comprehensive view of the situation. Furthermore, many projects designed on a pilot scale face challenges that prevent the dream of an industrial plant from being off the table (Deprá et al., 2022). In this sense, the data presented in the scientific literature raises the challenge of exploring microalgae cultivation at scales that meet commercial needs and that reflect what happens in practice. Therefore, in an attempt to fill this gap, we present the case study of a pilot-scale microalgae cultivation in the southwest region of Brazil (Holambra—SP—Latitude 22°37′51.7″S and Longitude 47°04′18.5″W). This region presents, as in other regions of the world, atmospheric conditions of a tropical nature that particularly favor the commercial production of microalgae in the open air (Siqueira et al., 2020; Dias et al., 2021). Details of the pilot plant and its operation are described below.

2.1 Description and Operation of the Pilot Plant

Under simple concepts, pilot plants are small-scale pre-industrial installations used to test processes, techniques, or equipment before their implementation on a large scale, i.e., in an industrial plant. These plants are designed to replicate the conditions and procedures that will be used in the industrial plant, allowing engineers and operators to evaluate in advance the performance and viability of processes or technologies and optimize them—if necessary—reducing associated risks and costs. In this case study, the pilot plant was strategically situated in the intended location for industrial-scale operation. The tubular bioreactor plant was installed in Holambra in the state of São Paulo (Latitude 22°37′51.7″S and Longitude 47°04′ 18.5″W) in the Southeast region of Brazil, by the company MicroAlgae Biotecnologia LTDA. Figure 1 shows the tubular bioreactors installed and in operation.

The tubular bioreactors with a water column of 1.5 m were built in fiberglass with a working volume of 250 L. The species of microalgae used was *Scenedesmus obliquus*. Cultures of *Scenedesmus obliquus* were started in the laboratory. Laboratory culture conditions were cell density of 0.15 g L^{-1}, 25 °C, 150 μmol m^{-2} s^{-1}, and 12 h photoperiod. The culture medium used was modified M8A [Sodium nitrate (5.4 g L^{-1}), KH_2PO_4 (1.0 g L^{-1}), $MgSO_4.7H_2O$ (0.40 g L^{-1}), $CaCl_2.2H_2O$ (0.013 g L^{-1}), Ferric EDTA (0.116 g L^{-1}), KSC mix (0.050 g L^{-1}), and $NaHCO_3$ (0.84 g L^{-1})] with pH adjusted to 7.5 (Kliphuis et al., 2010). The gas dispersion system consisted of a circular fine-bubble gas diffuser.

Scenedesmus obliquus cultures in the tubular bioreactor in batch mode operated with an initial cell density of 0.15 g L^{-1}. Temperature and light conditions were not controlled (vulnerable to daily fluctuations). The meteorological conditions for the

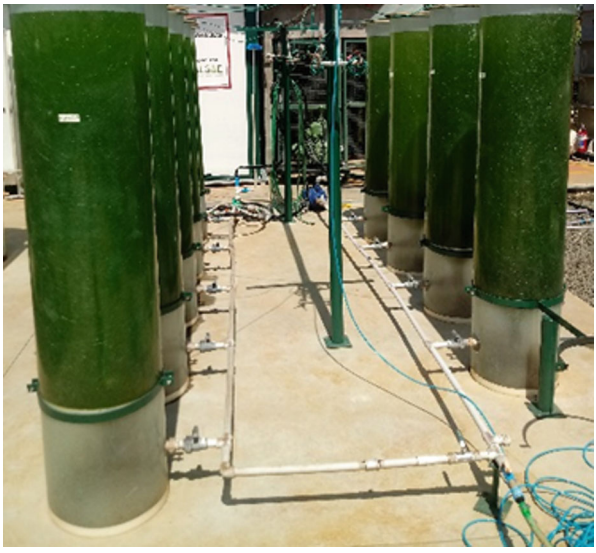


Fig. 1 Tubular bioreactor in production

Table 1 Climatological data (Climate-Data.org)

	October	November	January
Average temperature (°C)	23.1	22.8	23.9
Minimum temperature (°C)	18.3	18.8	20.2
Maximum temperature (°C)	28.7	27.8	28.4
Humidity (%)	65	72	77
Sunshine hours (h)	9.4	8.9	9.2

Table 2 Meteorological data from the pilot plant in Holambra—SP

	October	November	January
Average temperature (°C)a	29.8	33.9	27.5
Minimum temperature (°C)a	20.1	23.3	19.5
Maximum temperature (°C)a	39.6	44.6	35.6
Humidity (%)a	56.3	33.0	69.2
Sunshine hours (h)	12.0	13.0	13.0

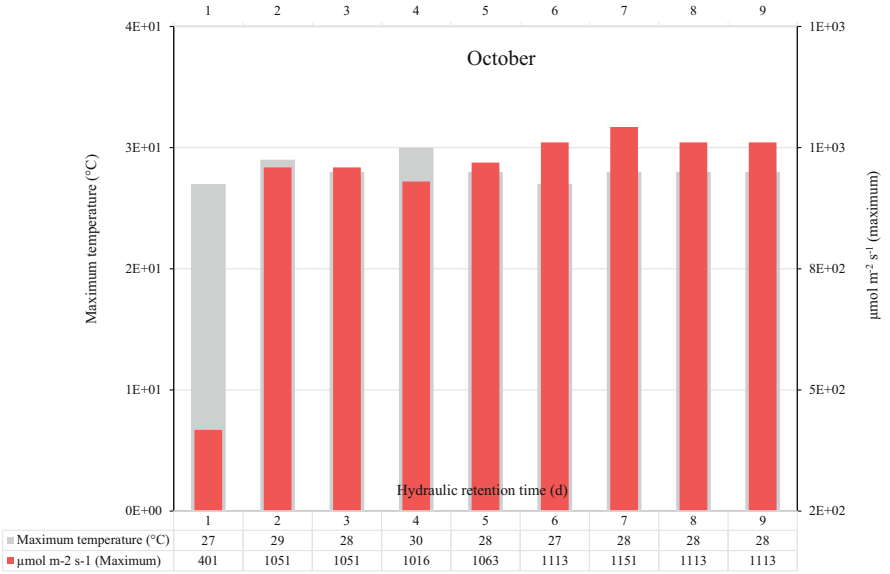
[a] Inside the greenhouse where the plant was located.

experiment period that occurred in October and November 2023 and January 2024 are shown in Tables 1 and 2. There were no pH adjustments and air injection with carbon dioxide (CO_2). The water required to feed the reactor was obtained from the local sanitation company's conventional drinking water supply. Despite the potability of the water supplied by the local company, a series filter system was installed, with porosities of 5 µm. Cell density was assessed gravimetrically by filtering a known volume of culture medium through a 0.45 µm filter and subjecting it to drying at 60 °C for 24 h. Cell density was monitored every 24 h during the microorganism's growth phases.

3 Case Study Analysis: Growth Kinetics

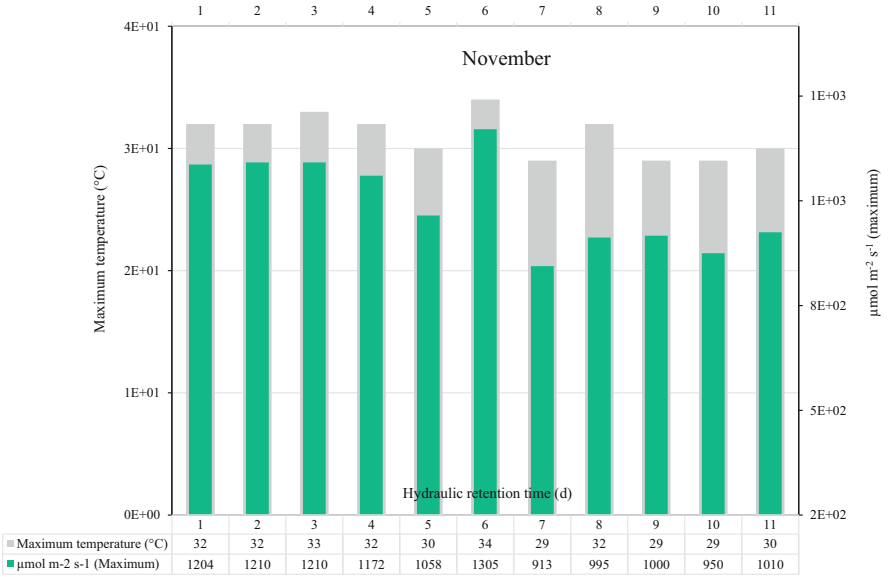
In microalgae cultivation outdoors, sunlight and temperature are subject to daily and seasonal variations inherent to geographic location. In this sense, Figs. 2, 3, and 4 present the maximum temperature recorded inside the tubular bioreactor and the maximum photosynthetic photon flux density (PPFD) on the external surface of the tubular bioreactor. These data are important because they can be correlated with data on the growth kinetics of the microalgae *Scenedesmus obliquus*.

As can be seen in Figs. 2, 3, and 4, the temperature inside the tubular bioreactor reached 35 °C. Regarding the maximum photosynthetic photon flux density, minimum values of 186 µmol m^{-2} s^{-1} and 401 µmol m^{-2} s^{-1} were recorded in January and October, respectively. These lower values correspond to the maximum flux density of photosynthetic photons on cloudy days. The highest recorded values of maximum photosynthetic photon flux density were 1660 µmol m^{-2} s^{-1} in January.



	1	2	3	4	5	6	7	8	9
Maximum temperature (°C)	27	29	28	30	28	27	28	28	28
µmol m-2 s-1 (Maximum)	401	1051	1051	1016	1063	1113	1151	1113	1113

Fig. 2 Maximum temperature inside the tubular bioreactor and maximum photosynthetic photon flux density on the external surface of the tubular bioreactor



	1	2	3	4	5	6	7	8	9	10	11
Maximum temperature (°C)	32	32	33	32	30	34	29	32	29	29	30
µmol m-2 s-1 (Maximum)	1204	1210	1210	1172	1058	1305	913	995	1000	950	1010

Fig. 3 Maximum temperature inside the tubular bioreactor and maximum photosynthetic photon flux density on the external surface of the tubular bioreactor

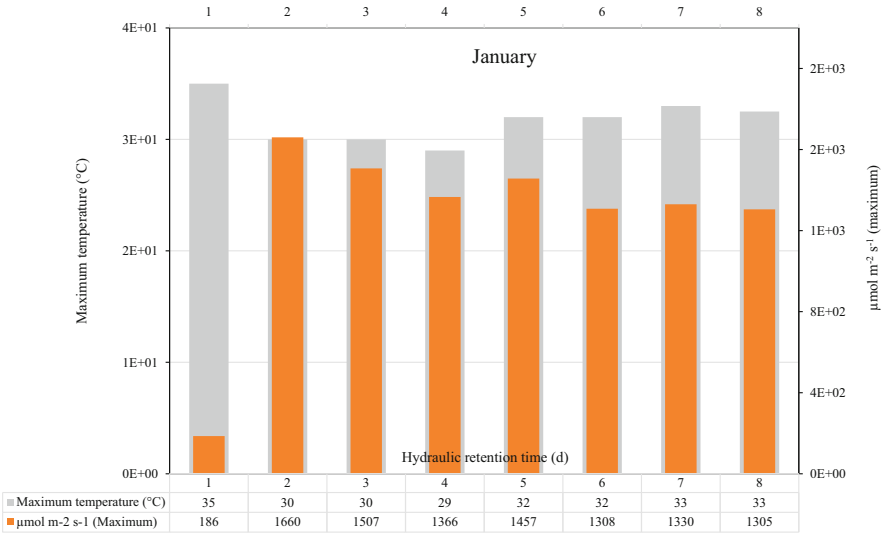


Fig. 4 Maximum temperature inside the tubular bioreactor and maximum photosynthetic photon flux density on the external surface of the tubular bioreactor

Table 3 Growth kinetics for *Scenedesmus obliquus*

Parameter	October	November	January
pH_{max}	9.0	8.8	8.9
HRT(d)	9	11	8
X_{max} (g L^{-1})	0.90	1.1	1.05
μ_{max} (d^{-1})	0.18	0.05	0.12
TG (d)	3.85	12.6	5.34
Px (peak) (g L^{-1} d^{-1})	0.07	0.03	0.08

X_{max} maximum biomass density (g L^{-1}), μ_{max} maximum specific growth rate (d^{-1}), *HRT* hydraulic retention time (d), *tg* generation time (d), P_X biomass productivity (g L^{-1} d^{-1})

Note, in the southern hemisphere, the months of October and November are spring and the month of January is summer.

Regarding microalgae growth kinetics, the main parameter that provides insights into the viability of microalgae-based processes is biomass productivity. That said, Table 3 shows the growth kinetics of *Scenedesmus obliquus* in a tubular bioreactor in the location of Holambra—SP (Latitude 22°37′51.7″S and Longitude 47°04′18.5″ W).

The microalgae *Scenedesmus obliquus* did not present an exponential growth phase (data not shown), that is, the cells did not actively reproduce, which suggests that the environmental conditions may not have been ideal for the rapid growth of the cells. It is suggested that the reasons why this occurred were (i) adverse environmental conditions, as daily fluctuations in temperature and light may have negatively

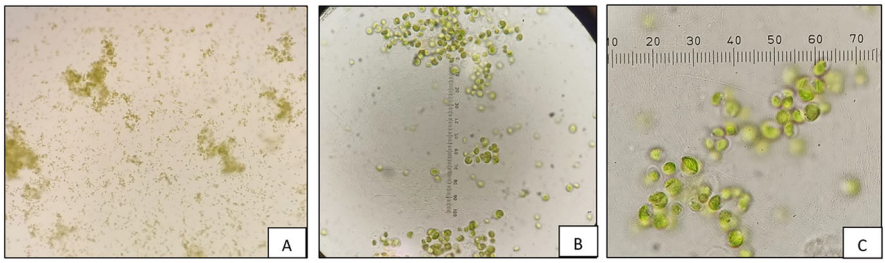


Fig. 5 Screenshots of observed samples. (**a**) 100× magnification, demonstrating the dominance of the species. (**b**) 400× magnification. (**c**) 1000× magnification, details of *Chlorella* sp., ruler: 10 lines = 10 μm

affected growth, (ii) CO_2 limitation, as there was no injection of CO_2-enriched air, which may have resulted in a lower photosynthetic rate, and (iii) competition with other organisms, including other microalgae, bacteria, and viruses.

Regarding temperature, variations within the bioreactor may have had a substantial impact on the growth and health of *Scenedesmus obliquus*. Excessive temperature fluctuations lead the cultures to abiotic stress. The variation of up to 10 °C recorded during cultivation can be considered significant. This variation, in addition to leading to thermal stress, can interfere with metabolic processes, including photosynthesis and cellular respiration. Furthermore, there may have been synergy with other stresses, such as CO_2 and light limitation, enhancing the negative effects on the culture. Regarding light, both low light intensity and daily fluctuations may have affected culture performance, leading to a lower photosynthetic rate, as the stress due to fluctuations.

On the other hand, concerning CO_2 limitation, not injecting CO_2-enriched air may have resulted in lower biomass productivity. CO_2 is essential in photosynthesis processes, in which microalgae, using light energy, convert CO_2 and H_2O into chemical energy, producing biomass. CO_2 limitation can generate a lower photosynthetic rate, slower cell growth, metabolic stress, and competition with other organisms because other species may not be as dependent on CO_2.

Regarding competition with other organisms, the qualitative analysis of the samples obtained during cultivation raised the hypothesis of the presence of bacteria and viruses, as confirmed by the dominant presence of another microalgae strain—*Chlorella*. Although measures can be taken regarding the hygiene and decontamination of bioreactors, the hypothesis was raised that contamination by another microalgae strain came from the water used, that was not autoclaved. Figure 5 shows some image captures of the slides observed under a microscope.

In short, by way of comparison, Dias et al. (2021), when evaluating the biomass productivity of *Scenedesmus obliquus* in Mopti (city in Mali) with temperature and light conditions similar to Holambra—SP, they found biomass productivity of 0.34 g L^{-1} d^{-1}. Please note, that the study was carried out in the laboratory with an injection of 15% CO_2 but, despite this, it effectively simulated the conditions and light fluctuations that occur during the day. The result presented for biomass

productivity is comparatively up to 11 times greater than the productivity found in the local climate of Holambra—SP (month of November). The results presented here for the *Scenedesmus obliquus* cultivation on a pilot scale were not satisfactory.

4 Case Study Limitations: Considerations and Recommendations

Failed experiences are not the end but rather an inevitable part of the process and a powerful catalyst for solid results. The results obtained in the microalgae pilot plant are the bottleneck that many small companies face before achieving the theoretical success achieved in laboratory cultivations. Although these microorganisms are praised for their robustness and ability to thrive in challenging environmental conditions, it is important to note that this does not mean they are invulnerable to environmental stresses.

In the case study described, it is important to highlight that the pilot plant was installed inside a greenhouse, where the internal temperature was higher than the ambient temperature and the light intensity was lower. Therefore, while the plant's location within a greenhouse may offer advantages such as protection from external elements such as rain, it also offers disadvantages. The ambient temperature inside the greenhouse reached 48 °C, directly influencing the internal temperature of the bioreactor, which reached 35 °C. These high temperatures may have exceeded the ideal range for *Scenedesmus obliquus* growth, causing thermal stress. Furthermore, the reduced passage of light, around 50% less (unpublished data), limited the availability of light energy, affecting the microalgae's ability to carry out photosynthesis. To counterbalance the effects of internal temperature and reduced light passage, adaptations to the cultivation system would be necessary, such as using refrigeration systems and the implementation of complementary lighting. These adaptations can ensure an environment conducive to the healthy growth of microalgae.

On the other hand, when it comes to contamination of the culture by microorganisms, including another microalgae strain, it is crucial to trace the contamination sources. This includes determining whether the contamination comes from water or other sources. Based on this information, it is possible to implement corrective measures that include treating water, improving hygiene and sanitation procedures, and adopting more aseptic equipment and growing practices.

In summary, this case study highlights an experience that is not negative, but rather the reality of the microalgae practice cultivation. Cultivation is a practice that needs to be rigorously monitored to ensure healthy growth and biomass productivity that is worth the economic investment. Underestimating the rigor of this activity can lead to project failure. The information inherited in this case study shows the fragility of microalgae-based businesses and can serve as a basis for understanding why commercial production, which is now over 60 years old, is progressing at such a

slow pace. Although it may seem paradoxical, this is not to discourage companies interested in investing in microalgae biotechnology, but rather to highlight the complexity and sensitivity of this practice. It is an appeal for a careful approach and investment in research to overcome challenges and explore the full potential of microalgae.

5 Conclusion

It is not news that microalgae cultivation on a pilot and industrial scale, subject to variable environmental conditions, often does not reach the theoretical maximums observed in the laboratory. This disparity between ideal and actual results on a large scale reflects the challenges faced when trying to reproduce controlled conditions in an industrial environment. Looking at more than 140 years of microalgae cultivation in the laboratory and more than 60 years of commercial cultivation, we will see that twists and turns and apparent dead ends have marked the history of research and development (R&D) efforts. However, today there is a boom in technologies that, combined with the increasing use of artificial intelligence, can offer promising prospects for the future of microalgae technology. Microalgae is a nature-based solution that, despite setbacks, can be hailed as a versatile solution to many global problems, from food and energy security to environmental protection.

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