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Faculty of Lanscape Architecture and Environmental Engineering (FALTL)
Universitas Trisakti, Jakarta, Indonesia
in associated with Ikatan Ahli Teknik Penyehatan dan Teknik Lingkungan Indonesia (IATPI)

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USE OF ANOVA STATISTICAL METHOD IN EVALUATION OF TOFU WASTEWATER USED FOR *SPIRULINA* CULTURE MEDIUM ENRICHED WITH UREA AND $NaHCO_3$

Mustamina Maulani[1*], Gabriella Jasmine[1], Rosmalia Dita Nugraheni[2], Maman Djumantara[1], Asri Nugrahanti[1], Bayu Satiyawira[1], Cahaya Rosyidan[1], Lisa Samura[1], Harin Widiyatni[1], P Pauhesti[1]

[1]Petroleum Engineering, Faculty of Earth Technology and Energy, Universitas Trisakti, Jakarta, 11440, Indonesia
[2]Research School of Earth Sciences, Australian National University (ANU), ACT, Canberra, Australia

*Corresponding author: mustamina@trisakti.ac.id

ABSTRACT

Indonesia has a large amount of liquid waste originating from the tofu industry. Currently, the treatment of tofu industrial wastewater is carried out using both anaerobic and aerobic methods, but both methods still have several weaknesses. In this study, the tofu industrial wastewater was utilized as a culture medium for *Spirulina* sp. to provide economic value from wastewater that can be used as bioethanol, pharmaceuticals, and food products rich in omega 3, chlorophyll, carotenoids. Aim: The growth of *Spirulina* sp. is closely related to the availability of macro and micronutrients as nutrients and the influence of environmental conditions, so this study was aimed to see the best variation of the addition of urea and $NaHCO_3$ as additional nutrients to maximize growth and cell density of *Spirulina* sp. with tofu industrial wastewater media. **Methodology and Results:** This study was done by culvitating *Spirulina* sp in the growth media, measuring the Optical Density (OD), and analyzing quantitatively and using ANOVA on IBM SPSS Statistics version 20. The study indicated that adding urea and $NaHCO_3$ to *Spirulina* sp. had no effect on cell density and growth rate. Treatment with addition of urea 0.36 g/500 ml without additional of $NaHCO_3$ had the highest growth rate, 0.00852/day, and the highest cell density value on *Spirulina* sp. growth. **Conclusion, significance, and impact study:** The tofu liquid waste can be used as a new alternative used as fertilizer because in the liquid tofu waste, it provides the nutrients needed by *Spirulina* sp.

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- ANOVA
- *Spirulina* sp.
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1. INTRODUCTION

Nowadays, the development of *Spirulina* sp. cultivation products is proliferating. In its utilization, *Spirulina* sp. can be used as wastewater bioremediation that makes the waste safer to be discharged into water bodies. Besides that, it can be used as food and organic products because it contains omega 3, chlorophyll, carotenoids, protein by 50-70% of its dry weight, and other ingredients, can be used as a product beauty treatment, and bioethanol as an energy source. Thus, later *Spirulina* sp. can play an important role in biotechnology. Therefore, it should be investigated further (Christwardana & Nur, 2013; Fakhri *et al.*, 2020; Hadiyanto & Nais, 2019). They made various forms of making the cultivation of *Spirulina* sp. It is crucial to continue further research and develop into one of the aquaculture industries. *Spirulina* is one of the cyanobacteria or bacteria containing chlorophyll and can act as organisms that can carry out photosynthesis to make their food. The spiral shape contains high phycocyanin, so the color is blue-green. Body shape *Spirulina* sp. The thread-like structure is made up of 1-12 m diameter cylindrical with thin cell walls. *Spirulina* filaments are self-contained and may move around freely. *Spirulina* sp. is a natural food for shrimp and fish larvae with high nutritional value. *Spirulina* can grow well in lakes, freshwater, seawater, and soil media. *Spirulina* can also grow in media with high alkalinity (pH 8.5–11), whereas other microorganisms are not able to grow well under these conditions. The lowest temperature for *Spirulina platensis* to live is 15^{o}C, and the optimal growth is 35-40^{o}C (Christwardana & Nur, 2013). *Spirulina* is a cosmalite microalga that can be cultivated on different media. *Spirulina* growth requires nutrients that can come from chemicals or solutions from decay of organic material or waste.

Hariyati in Indrastuti *et al.,* (2014) explained that the protein content in *Spirulina* sp. ranges from 63-68%, carbohydrates 18-20%, and fat 2-3%. With this high protein content, *Spirulina* sp. has a potential protein source for living beings, both humans and livestock. The mineral content in *Spirulina* differs from one another depending on the type of growth medium. In general, Spirulina cultivation can use freshwater or seawater. *Spirulina* cultivated in seawater contains higher minerals than in freshwater or brackish water. Seawater contains high salts such as NaCl, KCl, and MgCl. Algae that live in seawater have higher phycocyanins, polysaccharides, and inositol. Although it contains high salt, sodium content that is too high is considered unsuitable for human health due to blood pressure problem (hypertension). $NaHCO_3$ and Na_2CO_3 are used

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to reduce mineral salts. Seawater *Spirulina* has a slower growth rate than freshwater *Spirulina.* Seawater *Spirulina* has a fishy smell like seaweed, so some consumers are uncomfortable with the smell. This fishy smell results from the mineral content in Spirulina (Christwardana & Nur, 2013).

Freshwater *Spirulina* is often used as human food and pharmaceutical ingredients because the sodium content in freshwater spirulina is lower than seawater, so it is safe to use. In freshwater media, $NaHCO_3$, phosphate, and urea were added to influence the growth rate of *Spirulina* sp. (Christwardana & Nur, 2013). Freshwater *Spirulina* had a higher growth rate of around 0.16/day and yielded 1.23-1.34 g/L dry biomass. Meanwhile, seawater *Spirulina* has a lower growth rate and produces a biomass of around 10.3 g/m^2/day (Ambarsari *et al.,* 2017). Freshwater *Spirulina* does not have a fishy smell because it has a lower mineral content than seawater *Spirulina.*

The growth of *Spirulina* sp. is closely related to the availability of macro and micronutrients as nutrients and the influence of environmental conditions (Isnansetyo and Kurniastuty, in Caturwati, 2019; Hadiyanto & Nur, 2012). To survive, *Spirulina* sp. need additional nutrients, including macronutrients and micronutrients. *Spirulina* sp. requires macronutrients such as Nitrogen, Phosphate, Carbon, Hydrogen, Oxygen, Calcium, Magnesium, Natrium, and Potassium, and also micronutrients such as Fe, Mg, Cu, Zn, B, and cyanocobalamin (Sari *et al.,* 2012). Isnansetyo and Kurniastuty in Caturwati (2019) also mention that the elements N, P, and S play a role in protein formation, K elements are involved in carbohydrate metabolism, Fe and Na elements are involved in chlorophyll production, while Si and Ca elements are involved in the construction of cell walls. Vitamin B12 is used to stimulate growth through photosynthetic stimulation, while Juneja *et al.,* (2013) stated that for *Spirulina* sp. to grow, at least five nutrients are required:

a) Nitrogen

   Nitrogen is required for the production of proteins and nucleic acids. About 7%-20% dry weight of algae contains nitrogen. Nitrogen is an integral part of ATP and is an energy carrier in cells. Nitrogen deficiency can cause enzyme changes in algal cells which are indicated by lipid synthesis and decreased chlorophyll synthesis, causing the cells to have an excess of carotenoids.

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b) Carbon

Another nutrient is Carbon that plays a role in photosynthesis for algae growth and reproduction. The carbon could be used for respiration, as a source of power, or as a component of other cells. Reducing carbon supply can lead to reduced algae growth. Algae has autotrophic growth, so need carbon in the form of CO_2, carbonate, or bicarbonate. On the otherhand, for heterotrophic growth they need acetate or glucose.

c) Phosphate

Phosphate is a macronutrient that plays a vital role in the growth and metabolism of algal cells. Phosphate is part of DNA and RNA, all live cells need this macromolecule. In addition, phosphate is associated with the formation of phospholipids.

d) Phosphorus

Phosphorus is one of the critical components needed for the growth and development of algal cells. Phosphorus normally makes up about 1% of the dry weight of algae. The Calvin-Benson cycle's synthesis and regeneration of substrates are reduced as a result of the phosphorus definition, as is the level of light usage necessary for the carbon fixation process.

e) Trace Metals

Algal cells contain trace metals in small amounts, but they are important components in physiological activities. Iron (Fe), manganese (Mn), cobalt (Co), zinc (Zn), copper (Cu), and nickel (Ni) are some of the essential trace metals for algae. Trace metals deficiency can limit algal development and obstruct photosynthesis.

In addition, the tofu industry is one of the growing industries in the food sector. This growth happened as one of the answers to meeting the community's need for high tofu consumption, which reached 8,216 kg per person per year in 2021 (Badan Pusat Statistik, 2020). Tofu is produced through the process of coagulating (precipitation) of soymilk protein, the ingredients used are ($CaSO_4$), vinegar (CH_3COOH) and $MgSO_4$. Producing tofu in the industry includes sorting, soaking, peeling off the skin, washing, milling, boiling, and filtering processes. Wastewater from tofu manufacture is the liquid waste that can cause environmental pollution (Sayow *et al.,* 2020).

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Tofu industrial waste is one of the industrial wastes that has not been widely utilized, while the waste is estimated to contain many elements that can be used for cultivation activities of microalgae plant species, especially *Spirulina* sp. In the liquid tofu waste, it provides the nutrients needed by *Spirulina* sp. Most of the wastewater from the tofu industry is channeled directly without prior treatment to sewers, rivers, and other receiving water bodies. This causes liquid tofu waste is often a problem for the surrounding environment. This habit results from the lack of knowledge of the craftsmen who know the importance of environmental cleanliness. In addition, looking at the nutrients contained in tofu liquid waste, it can be used as a source of nutrition for microalgae. One of the most popular types of microalgae in the world is *Spirulina platensis*. *Spirulina* has the potential to be used as a food source. In the cultivation of 1 acre *Spirulina*, the protein yield is 20 times that of a single acre of soybeans or corn, and 200 times that of beef. In addition, *Spirulina* contains a small amount of fat (ranging from 6-7%), primarily unsaturated fat (Effendi *et al.,* 2021; Syaichurrozi & Jayanudin, 2016).

One source of adequate nutrition for *Spirulina* sp. is liquid tofu waste. In its production activities, waste is about 40% of the total 100 kg of soybean production. Several activities can produce liquid waste during the process, namely soaking soybeans, washing soybeans and equipment for the tofu production process, filtering, pressing, and molding into blocks. The failure in making tofu so that the tofu is not suitable for consumption contributes to the increase in the production of liquid waste. Then when viewed from the handling side, the majority of liquid waste by tofu industry entrepreneurs is still not up to the quality standards for release into the waterbodies. This waste is often directly channeled into rivers and other irrigation canals in speeding up the process. Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and Total Suspended Solids (TSS) are indeed high in tofu industry wastewater. Based on the quality standards of wastewater for tofu industry, COD, BOD and TSS are 150 mg/L, 300 mg/L and 200 mg/L respectively. Therefore, waste directly discharged into water bodies is destructive to the ecosystem of water bodies and causes environmental pollution (Mulana *et al.,* 2014; Simanjuntak *et al.,* 2021). The utilization of this liquid waste is expected to be a growth media of *Spirulina* sp. The maximum density of *Spirulina* sp. are able to be monitored through optical density (OD) measurements because the OD value

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describes the population density of *Spirulina* sp. Of course, adequate media is needed, and macro and micronutrients are needed so that *Spirulina* sp. can grow properly (Zuli Pratiwi *et al.,* 2020).

Urea and $NaHCO_3$ are two substances that can be added to the mix as supplementary nutrients to help *Spirulina* sp. survive. Urea is employed as a nitrogen (N) source, whereas $NaHCO_3$ is used as a carbon (C) source. These two compounds have the advantage that they are easy to obtain and have a more economic value compared to other nitrogen and carbon sources (Caturwati, 2019). Therefore, in this study, optimization of the use of liquid tofu waste from the Harapan Maju factory, Cimanggis, will be carried out as a medium for cultivating *Spirulina* sp. enriched with variations of concentrations of urea and $NaHCO_3$ so that it can increase the economic value of tofu waste. The sample was taken from the factory because this research continues the Community Services program titled "Utilization of Liquid Waste Tofu Harapan Maju Cimanggis Depok, West Java as a Culture Media for *Spirulina* sp.". Therefore, the goal of this research is to find out what conditions are best for *Spirulina* sp. to grow in on tofu waste media with the addition of urea and $NaHCO_3$.

2. RESEARCH METHODOLOGY

This study was conducted at Laboratory of Reservoir Fluid Analysis at Universitas Trisakti. The analysis was done in three stages, which are pre-research, research, and data collection, processing, and analyzing stage.

2.1 Pre-Research Stage

The pre-research stage analyzed waste BOD, COD, total carbon, nitrogen, and phosphorus. The synthetic medium used is a modification of the *Spirulina* growth medium used by Syaichurrozi & Jayanudi (2016) with the addition of optimum waste at a concentration of 6% v/v, with a volume of 60 ml tofu liquid waste and 840 ml water. After that, the addition of the optimum amount of urea and $NaHCO_3$ for the *Spirulina* sp. growing medium was done. During the first pre-research stage, the addition of urea and $NaHCO_3$ refers to the research method (Caturwati & Setyati, 2020). However, the researchers modified the urea and $NaHCO_3$ doses to obtain the following amounts:

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Table 1 Variations in urea and $NaHCO_3$ supply

No	Treatment	Urea (g/500 ml)	$NaHCO_3$ (g/500 ml)
1	L (control)	0.0	0.0
2	A	0.36	0.0
3	N	0.0	0.043
4	I	0.36	0.043

2.2 Research

The pre-research stage analyzed waste BOD, COD, total carbon, nitrogen, and phosphorus. The synthetic medium used is a modification of the *Spirulina* growth medium used by Syaichurrozi & Jayanudi (2016) with the addition of optimum waste at a concentration of 6% v/v, with a volume of 60 ml tofu liquid waste and 840 ml water. After that, to create a growth medium for *Spirulina* sp., the addition of the optimum amount of urea and $NaHCO_3$ was done. In the first pre-research stage, the addition of urea and $NaHCO_3$ refers to the research method (Caturwati & Setyati, 2020). However, the researchers modified the urea and $NaHCO_3$ doses to obtain the following amounts.

2.2.1 Preparation

1) Preparation of Tools and Materials

   At this stage, the researcher prepares all the tools and materials used.

2) Equipment Sterilization

   Equipment sterilization is done to avoid contamination of unwanted microorganisms growing in cultivation. Before the sterilization process, researchers washed the tools in 500 ml culture bottles, 8 liters plastic jars, aeration hose, and water stone with soap and flowing water. The apparatuses were physically sterilized using boiling water and chemical sterilized using 70% alcohol.

3) Cultivating Media

   a. Tofu wastewater is filtered using a filter and a T200 size screen printing cloth and then put into an 8 liters size jar (Figure 1).

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b. Measuring 300 ml of tofu wastewater and put into a 500 ml culture bottle.

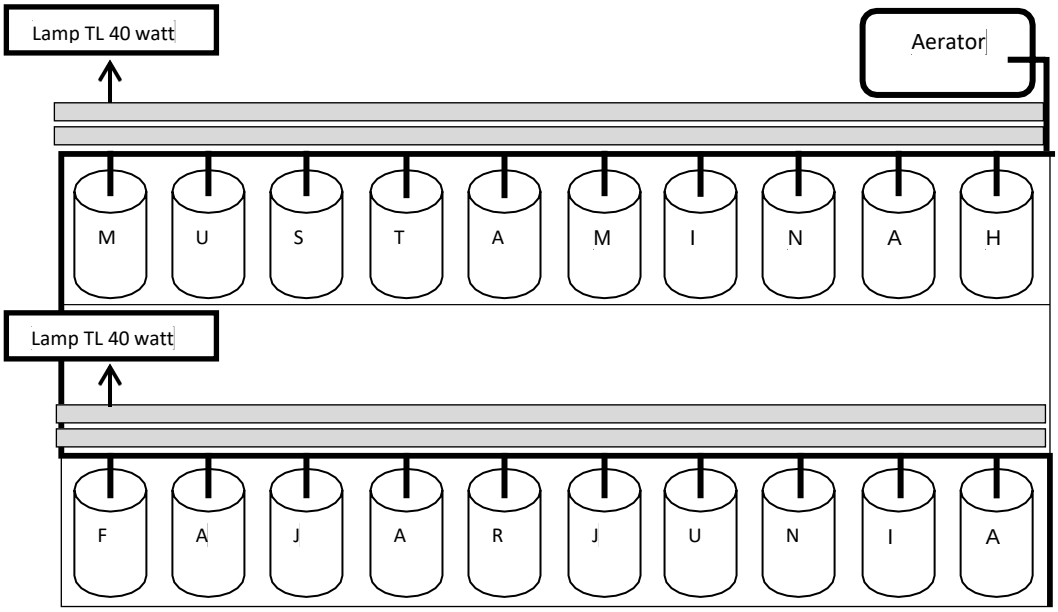

Figure 1 Cultivation media

2.2.2 Implementation

1) Culvitation

The culvitation stage was carried out using a 500 ml culture bottle containing 300 ml of the growth medium, adjusted to the initial environmental conditions, namely pH, temperature, salinity, aeration, and lighting. Culture of *Spirulina* sp. 200 ml of growth medium was added to 300 ml so that the ratio of media and inoculum was 3:2. This study used inoculants aged ten days.

2) Optical Density (OD) Measurement

OD measurements were carried out to measure the growth density of *Spirulina* sp. in each treatment. *Spirulina* sp. culture on day 0 (ready for cultivation), a 5 ml of each replication was taken to obtain the 0^{th} density (OD_0) on day 0 (t_0), and so on until the 10^{th} day of cultivation. *Spirulina* sp. samples for each treatment were put into a disposable 1.5 ml cuvette. The absorbance was measured using a Shimadzu UV-1800 spectrophotometer with a wavelength of 680 nm using a computer and UV Probe application version 2.62 Shimadzu Photometric method. The blanks used were tofu wastewater, vitamin B12, and *Spirulina* sp.

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The replication in each treatment was measured once to adjust the number of replications. Later, the average was taken to obtain the final OD. The growth pattern curve was made by measuring the OD every 24 hours for ten days of cultivation.

2.2.3 Data Collection, Processing, and Analyzing

The data was acquired using a Shimadzu UV-1800 Spectrophotometer with a wavelength of 680 nm to take OD measurements every 24 hours for 10 days of cultivation to see the curve of the growth pattern of *Spirulina* sp. for each treatment. Researchers used the equation Hirata *et al.,* (Kawaroe *et al.,* 2015).

To calculate the growth rate, the researchers employed quantitative descriptive methods and simple statistics to calculate the average value of Optical Density (OD). The ANOVA test on IBM SPSS Statistics version 20 was used to do quantitative data analysis to determine the most effective addition of nutrients for *Spirulina* sp. growth. The X variable in this study represents the addition of urea and $NaHCO_3$, whereas the Y variable represents the Optical Density (OD) value. Normality and homogeneity tests were performed before to the ANOVA test to check that the data were regular and homogeneous. The following are the normalcy and homogeneity test hypotheses:

a. If the significant value is (p) > 0.05, the data is normally distributed and homogeneous.
b. If the significant value is (p) < 0.05, the data is not normally distributed and not homogeneous.

The ANOVA test can be performed when the data is normally distributed. The ANOVA test hypotheses are as follows:

- Ho: *Spirulina* sp. OD and growth rate were unaffected by the addition of urea and $NaHCO_3$.
- Hi: The OD and growth rate of *Spirulina* sp. are affected by the addition of urea and $NaHCO_3$.

If the treatment being tested has a significant effect, then Duncan's multiple area tests are continued to determine the differences between treatments.

3. RESULTS AND DISCUSSION

3.1 *Spirulina* sp. Cell Density

From the normality and homogeneity tests results it is known that the cell density data (OD or Optical Density) of *Spirulina* sp., it is regular and homogeneous if p > 0.05, each shown in Table 2.

Table 2 Normality test results of *Spirulina* sp. density

Parameter		Understandardized Residual
N		44
Normal Parameters[a,b]	Mean	Std. 0E-8
	Deviation	.13289463
Most Extreme Differences	Absolute	.123
	Positive	.066
	Negative	-.122
Kolmogorov-Smirnov Z		.766
Asymp. Sig, (2-tailed)		.326

[a] *Normally distributed*
[b] *Calculated from data*

Table 3 Homogeneity test results of *Spirulina* sp. density

Levene Statistic	df1	df2	Sig.
.648	3	40	.524

The results obtained are different from the ANOVA test's analysis results. There is a significant value of 0.122 (p > 0.05) in this equation. As a result, additional tests are not possible. The addition of nutrients in the form of urea and $NaHCO_3$ had no effect on the cell density of *Spirulina* sp., according to these findings. Therefore, differences in the nutrient composition of the growing media can affect the rise in density. Meanwhile, the density of microalgae can decrease if the nutrients in the growth media are not sufficient for microalgae growth until the last day of cultivation. The results of the ANOVA test can be seen in the Table 4.

Table 4 ANOVA test result on *Spirulina* sp. density

Parameter	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.112	3	.040	1.860	.122
Within Groups	.713	40	.020		
Total	.825	43			

3.2 *Spirulina* sp. Growth Rate

The calculation of *Spirulina* sp.'s growth rate per day was used to quantify the speed of growth of S*pirulina* sp. daily using the formula Hirata *et al.* in Kawaroe *et al., (*2015). The calculation results of the growth rate of *Spirulina* sp. can be seen in Table 5.

Table 5 *Spirulina* sp.'s growth rate on control variable and three treatments

Sample	Day										X
	1	2	3	4	5	6	7	8	9	10	
L	0.0241	0.0156	0.0112	0.0085	0.0061	0.00350	-0.0003	-0.0011	-0.0098	-0.0094	0.01807
A	0.0241	0.0265	0.0099	0.0098	0.0046	-0.0002	-0.0002	-0.0001	0.0005	0.0113	0.00852
N	0.0275	0.0037	0.0165	0.0129	0.0032	0.0011	0.0020	-0.0041	-0.0110	-0.0070	0.00448
I	0.0241	0.0252	0.0129	0.0003	0.0070	0.0018	0.0029	0.0020	0.00420	0.0013	0.00817

X = average
L = control variable
A = addition of urea 0.36 g/500 ml without additional of $NaHCO_3$
N = addition of $NaHCO_3$ 0.043 g/500 ml without additional of urea
I = addition of urea 0.36 g/500 ml and addition of $NaHCO_3$ 0.043 g/500 ml

The growth rate (k) of *Spirulina* sp. was measured in different ways for each treatment. Treatment A has the highest average growth rate of 0.00852/day, followed by treatment I at 0.00817/day, while the control treatment has the lowest average growth rate of 0.01807/day, followed by treatment N at 0.00448/day. In the meantime, results on *Spirulina* sp. growth rate are normal and homogenous ($p > 0.05$) for the normality and homogeneity tests, as shown in the Table 6.

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Table 6 The results of the *Spirulina* sp. growth rate test

Parameter		Understandardized Residual
N		40
Normal Parameters[a,b]	Mean	0E-8
	Std. Deviation	.012031247
Most Extreme Differences	Absolute	.130
	Positive	.130
	Negative	-.103
Kolmogorov-Smirnov Z		.860
Asymp. Sig, (2-tailed)		.430

[a] *Normally distributed*
[b] *Calculated from data*

Table 7 Homogeneity results of *Spirulina* sp.

Levene Statistic	df1	df2	Sig.
.115	3	33	.940

The ANOVA test revealed a significant value of 0.940 (p>0.05) in the analysis (Table 7). As a result, no additional experiments may be conducted. The addition of nutrients in urea and $NaHCO_3$ did not affect the growth rate of *Spirulina* sp., according to the ANOVA test results. With the possibility of being influenced by other factors. The results of the ANOVA test can be seen in Table 8.

Table 8 ANOVA test result on *Spirulina* sp.

Parameter	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.463	.700
Within Groups	.003	33	.000		
Total	.003	37			

The observations of the growth rate of *Spirulina* sp. obtained have different results from the observations of density. The results obtained have different results from the density observations. The highest growth rate in this study was obtained in treatment A while the highest density was obtained in treatment I. This was because the concepts of density and growth rate were different. Density is the growth of microalgae which can be expressed as an

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increase in the number, density, and cell population. While the growth rate is the rate of increase of a biomass growth per unit time. In this study, the unit of density used was cells/ml, while the growth rate used units of cells/day (Prayitno, 2016).

4. CONCLUSION

Based on the results, it can be concluded that providing the nutrients urea and $NaHCO_3$ has no effect on the cell density and growth rate of *Spirulina* sp. However, the growth rate of *Spirulina* sp. varied depending on the supply of urea and $NaHCO_3$. As it can be seen from the experiment, treatment A showed the highest growth rate, namely 0.00852/day.

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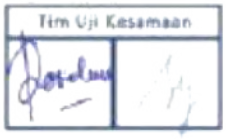
USE OF ANOVA STATISTICAL METHOD IN EVALUATION OF TOFU WASTEWATER USED FOR SPIRULINA CULTURE MEDIUM ENRICHED WITH UREA AND NaHCO3

by Mustamina Maulani



Use of ANOVA Statistical Method in Evaluation of Tofu Wastewater used for Spirulina Culture Medium Enriched with Urea and $NaHCO_3$
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USE OF ANOVA STATISTICAL METHOD IN EVALUATION OF TOFU WASTEWATER USED FOR *SPIRULINA* CULTURE MEDIUM ENRICHED WITH UREA AND $NaHCO_3$

Mustamina Maulani[1*], Gabriella Jasmine[1], Rosmalia Dita Nugraheni[2], Maman Djumantara[1], Asri Nugrahanti[1], Bayu Satiyawira[1], Cahaya Rosyidan[1], Lisa Samura[1], Harin Widiyatni[1], P Pauhesti[1]

[1]Petroleum Engineering, Faculty of Earth Technology and Energy, Universitas Trisakti, Jakarta, 11440, Indonesia
[2]Research School of Earth Sciences, Australian National University (ANU), ACT, Canberra, Australia

*Corresponding author: mustamina@trisakti.ac.id

ABSTRACT

Indonesia has a large amount of liquid waste originating from the tofu industry. Currently, the treatment of tofu industrial wastewater is carried out using both anaerobic and aerobic methods, but both methods still have several weaknesses. In this study, the tofu industrial wastewater was utilized as a culture medium for *Spirulina* sp. to provide economic value from wastewater that can be used as bioethanol, pharmaceuticals, and food products rich in omega 3, chlorophyll, carotenoids. **Aim:** The growth of *Spirulina* sp. is closely related to the availability of macro and micronutrients as nutrients and the influence of environmental conditions, so this study was aimed to see the best variation of the addition of urea and $NaHCO_3$ as additional nutrients to maximize growth and cell density of *Spirulina* sp. with tofu industrial wastewater media. **Methodology and Results:** This study was done by culvitating *Spirulina* sp in the growth media, measuring the Optical Density (OD), and analyzing quantitatively and using ANOVA on IBM SPSS Statistics version 20. The study indicated that adding urea and $NaHCO_3$ to *Spirulina* sp. had no effect on cell density and growth rate. Treatment with addition of urea 0.36 g/500 ml without additional of $NaHCO_3$ had the highest growth rate, 0.00852/day, and the highest cell density value on *Spirulina* sp. growth. **Conclusion, significance, and impact study:** The tofu liquid waste can be used as a new alternative used as fertilizer because in the liquid tofu waste, it provides the nutrients needed by *Spirulina* sp.

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- ANOVA
- *Spirulina* sp.
- Cultivation
- Tofu wastewater
- Waste utilization

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1. INTRODUCTION

Nowadays, the development of *Spirulina* sp. cultivation products is proliferating. In its utilization, *Spirulina* sp. can be used as wastewater bioremediation that makes the waste safer to be discharged into water bodies. Besides that, it can be used as food and organic products because it contains omega 3, chlorophyll, carotenoids, protein by 50-70% of its dry weight, and other ingredients, can be used as a product beauty treatment, and bioethanol as an energy source. Thus, later *Spirulina* sp. can play an important role in biotechnology. Therefore, it should be investigated further (Christwardana & Nur, 2013; Fakhri *et al.*, 2020; Hadiyanto & Nais, 2019). They made various forms of making the cultivation of *Spirulina* sp. It is crucial to continue further research and develop into one of the aquaculture industries. *Spirulina* is one of the cyanobacteria or bacteria containing chlorophyll and can act as organisms that can carry out photosynthesis to make their food. The spiral shape contains high phycocyanin, so the color is blue-green. Body shape *Spirulina* sp. The thread-like structure is made up of 1-12 m diameter cylindrical with thin cell walls. *Spirulina* filaments are self-contained and may move around freely. *Spirulina* sp. is a natural food for shrimp and fish larvae with high nutritional value. *Spirulina* can grow well in lakes, freshwater, seawater, and soil media. *Spirulina* can also grow in media with high alkalinity (pH 8.5–11), whereas other microorganisms are not able to grow well under these conditions. The lowest temperature for *Spirulina platensis* to live is 15°C, and the optimal growth is 35-40°C (Christwardana & Nur, 2013). *Spirulina* is a cosmalite microalga that can be cultivated on different media. *Spirulina* growth requires nutrients that can come from chemicals or solutions from decay of organic material or waste.

Hariyati in Indrastuti *et al.*, (2014) explained that the protein content in *Spirulina* sp. ranges from 63-68%, carbohydrates 18-20%, and fat 2-3%. With this high protein content, *Spirulina* sp. has a potential protein source for living beings, both humans and livestock. The mineral content in *Spirulina* differs from one another depending on the type of growth medium. In general, Spirulina cultivation can use freshwater or seawater. *Spirulina* cultivated in seawater contains higher minerals than in freshwater or brackish water. Seawater contains high salts such as NaCl, KCl, and MgCl. Algae that live in seawater have higher phycocyanins, polysaccharides, and inositol. Although it contains high salt, sodium content that is too high is considered unsuitable for human health due to blood pressure problem (hypertension). $NaHCO_3$ and Na_2CO_3 are used

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to reduce mineral salts. Seawater *Spirulina* has a slower growth rate than freshwater *Spirulina*. Seawater *Spirulina* has a fishy smell like seaweed, so some consumers are uncomfortable with the smell. This fishy smell results from the mineral content in Spirulina (Christwardana & Nur, 2013).

Freshwater *Spirulina* is often used as human food and pharmaceutical ingredients because the sodium content in freshwater spirulina is lower than seawater, so it is safe to use. In freshwater media, $NaHCO_3$, phosphate, and urea were added to influence the growth rate of *Spirulina* sp. (Christwardana & Nur, 2013). Freshwater *Spirulina* had a higher growth rate of around 0.16/day and yielded 1.23-1.34 g/L dry biomass. Meanwhile, seawater *Spirulina* has a lower growth rate and produces a biomass of around 10.3 g/m^2/day (Ambarsari *et al.*, 2017). Freshwater *Spirulina* does not have a fishy smell because it has a lower mineral content than seawater *Spirulina*.

The growth of *Spirulina* sp. is closely related to the availability of macro and micronutrients as nutrients and the influence of environmental conditions (Isnansetyo and Kurniastuty, in Caturwati, 2019; Hadiyanto & Nur, 2012). To survive, *Spirulina* sp. need additional nutrients, including macronutrients and micronutrients. *Spirulina* sp. requires macronutrients such as Nitrogen, Phosphate, Carbon, Hydrogen, Oxygen, Calcium, Magnesium, Natrium, and Potassium, and also micronutrients such as Fe, Mg, Cu, Zn, B, and cyanocobalamin (Sari *et al.*, 2012). Isnansetyo and Kurniastuty in Caturwati (2019) also mention that the elements N, P, and S play a role in protein formation, K elements are involved in carbohydrate metabolism, Fe and Na elements are involved in chlorophyll production, while Si and Ca elements are involved in the construction of cell walls. Vitamin B12 is used to stimulate growth through photosynthetic stimulation, while Juneja *et al.*, (2013) stated that for *Spirulina* sp. to grow, at least five nutrients are required:

a) Nitrogen

   Nitrogen is required for the production of proteins and nucleic acids. About 7%-20% dry weight of algae contains nitrogen. Nitrogen is an integral part of ATP and is an energy carrier in cells. Nitrogen deficiency can cause enzyme changes in algal cells which are indicated by lipid synthesis and decreased chlorophyll synthesis, causing the cells to have an excess of carotenoids.

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b) Carbon

Another nutrient is Carbon that plays a role in photosynthesis for algae growth and reproduction. The carbon could be used for respiration, as a source of power, or as a component of other cells. Reducing carbon supply can lead to reduced algae growth. Algae has autotrophic growth, so need carbon in the form of CO_2, carbonate, or bicarbonate. On the otherhand, for heterotrophic growth they need acetate or glucose.

c) Phosphate

Phosphate is a macronutrient that plays a vital role in the growth and metabolism of algal cells. Phosphate is part of DNA and RNA, all live cells need this macromolecule. In addition, phosphate is associated with the formation of phospholipids.

d) Phosphorus

Phosphorus is one of the critical components needed for the growth and development of algal cells. Phosphorus normally makes up about 1% of the dry weight of algae. The Calvin-Benson cycle's synthesis and regeneration of substrates are reduced as a result of the phosphorus definition, as is the level of light usage necessary for the carbon fixation process.

e) Trace Metals

Algal cells contain trace metals in small amounts, but they are important components in physiological activities. Iron (Fe), manganese (Mn), cobalt (Co), zinc (Zn), copper (Cu), and nickel (Ni) are some of the essential trace metals for algae. Trace metals deficiency can limit algal development and obstruct photosynthesis.

In addition, the tofu industry is one of the growing industries in the food sector. This growth happened as one of the answers to meeting the community's need for high tofu consumption, which reached 8,216 kg per person per year in 2021 (Badan Pusat Statistik, 2020). Tofu is produced through the process of coagulating (precipitation) of soymilk protein, the ingredients used are ($CaSO_4$), vinegar (CH_3COOH) and $MgSO_4$. Producing tofu in the industry includes sorting, soaking, peeling off the skin, washing, milling, boiling, and filtering processes. Wastewater from tofu manufacture is the liquid waste that can cause environmental pollution (Sayow *et al.*, 2020).

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Tofu industrial waste is one of the industrial wastes that has not been widely utilized, while the waste is estimated to contain many elements that can be used for cultivation activities of microalgae plant species, especially *Spirulina* sp. In the liquid tofu waste, it provides the nutrients needed by *Spirulina* sp. Most of the wastewater from the tofu industry is channeled directly without prior treatment to sewers, rivers, and other receiving water bodies. This causes liquid tofu waste is often a problem for the surrounding environment. This habit results from the lack of knowledge of the craftsmen who know the importance of environmental cleanliness. In addition, looking at the nutrients contained in tofu liquid waste, it can be used as a source of nutrition for microalgae. One of the most popular types of microalgae in the world is *Spirulina platensis*. *Spirulina* has the potential to be used as a food source. In the cultivation of 1 acre *Spirulina*, the protein yield is 20 times that of a single acre of soybeans or corn, and 200 times that of beef. In addition, *Spirulina* contains a small amount of fat (ranging from 6-7%), primarily unsaturated fat (Effendi *et al.*, 2021; Syaichurrozi & Jayanudin, 2016).

One source of adequate nutrition for *Spirulina* sp. is liquid tofu waste. In its production activities, waste is about 40% of the total 100 kg of soybean production. Several activities can produce liquid waste during the process, namely soaking soybeans, washing soybeans and equipment for the tofu production process, filtering, pressing, and molding into blocks. The failure in making tofu so that the tofu is not suitable for consumption contributes to the increase in the production of liquid waste. Then when viewed from the handling side, the majority of liquid waste by tofu industry entrepreneurs is still not up to the quality standards for release into the waterbodies. This waste is often directly channeled into rivers and other irrigation canals in speeding up the process. Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), and Total Suspended Solids (TSS) are indeed high in tofu industry wastewater. Based on the quality standards of wastewater for tofu industry, COD, BOD and TSS are 150 mg/L, 300 mg/L and 200 mg/L respectively. Therefore, waste directly discharged into water bodies is destructive to the ecosystem of water bodies and causes environmental pollution (Mulana *et al.*, 2014; Simanjuntak *et al.*, 2021). The utilization of this liquid waste is expected to be a growth media of *Spirulina* sp. The maximum density of *Spirulina* sp. are able to be monitored through optical density (OD) measurements because the OD value

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describes the population density of *Spirulina* sp. Of course, adequate media is needed, and macro and micronutrients are needed so that *Spirulina* sp. can grow properly (Zuli Pratiwi *et al.*, 2020).

Urea and $NaHCO_3$ are two substances that can be added to the mix as supplementary nutrients to help *Spirulina* sp. survive. Urea is employed as a nitrogen (N) source, whereas $NaHCO_3$ is used as a carbon (C) source. These two compounds have the advantage that they are easy to obtain and have a more economic value compared to other nitrogen and carbon sources (Caturwati, 2019). Therefore, in this study, optimization of the use of liquid tofu waste from the Harapan Maju factory, Cimanggis, will be carried out as a medium for cultivating *Spirulina* sp. enriched with variations of concentrations of urea and $NaHCO_3$ so that it can increase the economic value of tofu waste. The sample was taken from the factory because this research continues the Community Services program titled "Utilization of Liquid Waste Tofu Harapan Maju Cimanggis Depok, West Java as a Culture Media for *Spirulina* sp.". Therefore, the goal of this research is to find out what conditions are best for *Spirulina* sp. to grow in on tofu waste media with the addition of urea and $NaHCO_3$.

2. RESEARCH METHODOLOGY

This study was conducted at Laboratory of Reservoir Fluid Analysis at Universitas Trisakti. The analysis was done in three stages, which are pre-research, research, and data collection, processing, and analyzing stage.

2.1 Pre-Research Stage

The pre-research stage analyzed waste BOD, COD, total carbon, nitrogen, and phosphorus. The synthetic medium used is a modification of the *Spirulina* growth medium used by Syaichurrozi & Jayanudi (2016) with the addition of optimum waste at a concentration of 6% v/v, with a volume of 60 ml tofu liquid waste and 840 ml water. After that, the addition of the optimum amount of urea and $NaHCO_3$ for the *Spirulina* sp. growing medium was done. During the first pre-research stage, the addition of urea and $NaHCO_3$ refers to the research method (Caturwati & Setyati, 2020). However, the researchers modified the urea and $NaHCO_3$ doses to obtain the following amounts:

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Table 1 Variations in urea and $NaHCO_3$ supply

No	Treatment	Urea (g/500 ml)	$NaHCO_3$ (g/500 ml)
1	L (control)	0.0	0.0
2	A	0.36	0.0
3	N	0.0	0.043
4	I	0.36	0.043

2.2 Research

The pre-research stage analyzed waste BOD, COD, total carbon, nitrogen, and phosphorus. The synthetic medium used is a modification of the *Spirulina* growth medium used by Syaichurrozi & Jayanudi (2016) with the addition of optimum waste at a concentration of 6% v/v, with a volume of 60 ml tofu liquid waste and 840 ml water. After that, to create a growth medium for *Spirulina* sp., the addition of the optimum amount of urea and $NaHCO_3$ was done. In the first pre-research stage, the addition of urea and $NaHCO_3$ refers to the research method (Caturwati & Setyati, 2020). However, the researchers modified the urea and $NaHCO_3$ doses to obtain the following amounts.

2.2.1 Preparation

1) Preparation of Tools and Materials

   At this stage, the researcher prepares all the tools and materials used.

2) Equipment Sterilization

   Equipment sterilization is done to avoid contamination of unwanted microorganisms growing in cultivation. Before the sterilization process, researchers washed the tools in 500 ml culture bottles, 8 liters plastic jars, aeration hose, and water stone with soap and flowing water. The apparatuses were physically sterilized using boiling water and chemical sterilized using 70% alcohol.

3) Cultivating Media

   a. Tofu wastewater is filtered using a filter and a T200 size screen printing cloth and then put into an 8 liters size jar (Figure 1).

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b. Measuring 300 ml of tofu wastewater and put into a 500 ml culture bottle.

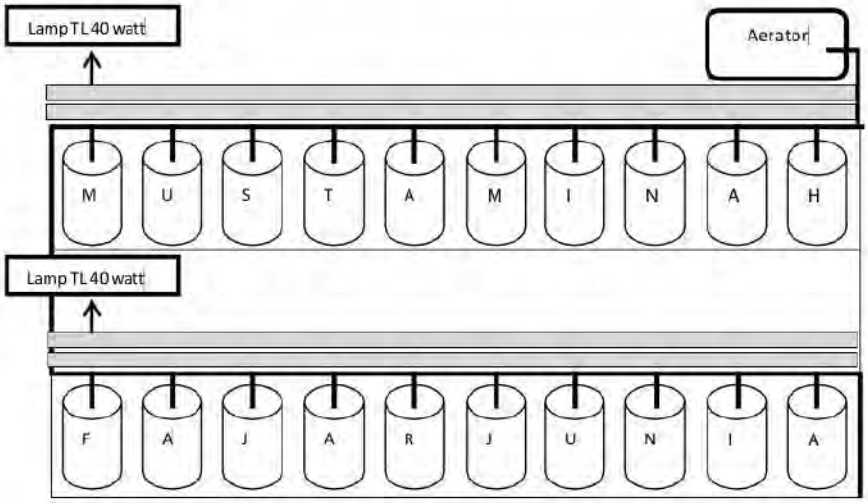

Figure 1 Cultivation media

2.2.2 Implementation

1) Culvitation

The culvitation stage was carried out using a 500 ml culture bottle containing 300 ml of the growth medium, adjusted to the initial environmental conditions, namely pH, temperature, salinity, aeration, and lighting. Culture of *Spirulina* sp. 200 ml of growth medium was added to 300 ml so that the ratio of media and inoculum was 3:2. This study used inoculants aged ten days.

2) Optical Density (OD) Measurement

OD measurements were carried out to measure the growth density of *Spirulina* sp. in each treatment. *Spirulina* sp. culture on day 0 (ready for cultivation), a 5 ml of each replication was taken to obtain the 0th density (OD_0) on day 0 (t_0), and so on until the 10th day of cultivation. *Spirulina* sp. samples for each treatment were put into a disposable 1.5 ml cuvette. The absorbance was measured using a Shimadzu UV-1800 spectrophotometer with a wavelength of 680 nm using a computer and UV Probe application version 2.62 Shimadzu Photometric method. The blanks used were tofu wastewater, vitamin B12, and *Spirulina* sp.

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The replication in each treatment was measured once to adjust the number of replications. Later, the average was taken to obtain the final OD. The growth pattern curve was made by measuring the OD every 24 hours for ten days of cultivation.

2.2.3 Data Collection, Processing, and Analyzing

The data was acquired using a Shimadzu UV-1800 Spectrophotometer with a wavelength of 680 nm to take OD measurements every 24 hours for 10 days of cultivation to see the curve of the growth pattern of *Spirulina* sp. for each treatment. Researchers used the equation Hirata *et al.*, (Kawaroe *et al.*, 2015).

To calculate the growth rate, the researchers employed quantitative descriptive methods and simple statistics to calculate the average value of Optical Density (OD). The ANOVA test on IBM SPSS Statistics version 20 was used to do quantitative data analysis to determine the most effective addition of nutrients for *Spirulina* sp. growth. The X variable in this study represents the addition of urea and $NaHCO_3$, whereas the Y variable represents the Optical Density (OD) value. Normality and homogeneity tests were performed before to the ANOVA test to check that the data were regular and homogeneous. The following are the normalcy and homogeneity test hypotheses:

a. If the significant value is (p) > 0.05, the data is normally distributed and homogeneous.
b. If the significant value is (p) < 0.05, the data is not normally distributed and not homogeneous.

The ANOVA test can be performed when the data is normally distributed. The ANOVA test hypotheses are as follows:

- Ho: *Spirulina* sp. OD and growth rate were unaffected by the addition of urea and $NaHCO_3$.
- Hi: The OD and growth rate of *Spirulina* sp. are affected by the addition of urea and $NaHCO_3$.

If the treatment being tested has a significant effect, then Duncan's multiple area tests are continued to determine the differences between treatments.

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3. RESULTS AND DISCUSSION

3.1 *Spirulina* sp. Cell Density

From the normality and homogeneity tests results it is known that the cell density data (OD or Optical Density) of *Spirulina* sp., it is regular and homogeneous if $p > 0.05$, each shown in Table 2.

Table 2 Normality test results of *Spirulina* sp. density

Parameter		Unstandardized Residual
N		44
Normal Parameters[a,b]	Mean	Std. 0E-8
	Deviation	.13289463
Most Extreme Differences	Absolute	.123
	Positive	.066
	Negative	-.122
Kolmogorov-Smirnov Z		.766
Asymp. Sig. (2-tailed)		.326

[a] *Normally distributed*
[b] *Calculated from data*

Table 3 Homogeneity test results of *Spirulina* sp. density

Levene Statistic	df1	df2	Sig.
.648	3	40	.524

The results obtained are different from the ANOVA test's analysis results. There is a significant value of 0.122 ($p > 0.05$) in this equation. As a result, additional tests are not possible. The addition of nutrients in the form of urea and $NaHCO_3$ had no effect on the cell density of *Spirulina* sp., according to these findings. Therefore, differences in the nutrient composition of the growing media can affect the rise in density. Meanwhile, the density of microalgae can decrease if the nutrients in the growth media are not sufficient for microalgae growth until the last day of cultivation. The results of the ANOVA test can be seen in the Table 4.

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Table 4 ANOVA test result on *Spirulina* sp. density

Parameter	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.112	3	.040	1.860	.122
Within Groups	.713	40	.020		
Total	.825	43			

3.2 *Spirulina* sp. Growth Rate

The calculation of *Spirulina* sp.'s growth rate per day was used to quantify the speed of growth of *Spirulina* sp. daily using the formula Hirata *et al.* in Kawaroe *et al.*, (2015). The calculation results of the growth rate of *Spirulina* sp. can be seen in Table 5.

Table 5 *Spirulina* sp.'s growth rate on control variable and three treatments

Sample	Day										X
	1	2	3	4	5	6	7	8	9	10	
L	0.0241	0.0156	0.0112	0.0085	0.0061	0.00350	-0.0003	-0.0011	-0.0098	-0.0094	0.01807
A	0.0241	0.0265	0.0099	0.0098	0.0046	-0.0002	-0.0002	-0.0001	0.0005	0.0113	0.00852
N	0.0275	0.0037	0.0165	0.0129	0.0032	0.0011	0.0020	-0.0041	-0.0110	-0.0070	0.00448
I	0.0241	0.0252	0.0129	0.0003	0.0070	0.0018	0.0029	0.0020	0.00420	0.0013	0.00817

X = average
L = control variable
A = addition of urea 0.36 g/500 ml without additional of $NaHCO_3$
N = addition of $NaHCO_3$ 0.043 g/500 ml without additional of urea
I = addition of urea 0.36 g/500 ml and addition of $NaHCO_3$ 0.043 g/500 ml

The growth rate (k) of *Spirulina* sp. was measured in different ways for each treatment. Treatment A has the highest average growth rate of 0.00852/day, followed by treatment I at 0.00817/day, while the control treatment has the lowest average growth rate of 0.01807/day, followed by treatment N at 0.00448/day. In the meantime, results on *Spirulina* sp. growth rate are normal and homogenous ($p > 0.05$) for the normality and homogeneity tests, as shown in the Table 6.

Table 6 The results of the *Spirulina* sp. growth rate test

Parameter		Understandardized Residual
N		40
Normal Parameters[a,b]	Mean	0E-8
	Std. Deviation	.012031247
Most Extreme Differences	Absolute	.130
	Positive	.130
	Negative	-.103
Kolmogorov-Smirnov Z		.860
Asymp. Sig. (2-tailed)		.430

[a] *Normally distributed*
[b] *Calculated from data*

Table 7 Homogeneity results of *Spirulina* sp.

Levene Statistic	df1	df2	Sig.
.115	3	33	.940

The ANOVA test revealed a significant value of 0.940 ($p>0.05$) in the analysis (Table 7). As a result, no additional experiments may be conducted. The addition of nutrients in urea and $NaHCO_3$ did not affect the growth rate of *Spirulina* sp., according to the ANOVA test results. With the possibility of being influenced by other factors. The results of the ANOVA test can be seen in Table 8.

Table 8 ANOVA test result on *Spirulina* sp.

Parameter	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	.000	3	.000	.463	.700
Within Groups	.003	33	.000		
Total	.003	37			

The observations of the growth rate of *Spirulina* sp. obtained have different results from the observations of density. The results obtained have different results from the density observations. The highest growth rate in this study was obtained in treatment A while the highest density was obtained in treatment I. This was because the concepts of density and growth rate were different. Density is the growth of microalgae which can be expressed as an

increase in the number, density, and cell population. While the growth rate is the rate of increase of a biomass growth per unit time. In this study, the unit of density used was cells/ml, while the growth rate used units of cells/day (Prayitno, 2016).

4. CONCLUSION

Based on the results, it can be concluded that providing the nutrients urea and $NaHCO_3$ has no effect on the cell density and growth rate of *Spirulina* sp. However, the growth rate of *Spirulina* sp. varied depending on the supply of urea and $NaHCO_3$. As it can be seen from the experiment, treatment A showed the highest growth rate, namely 0.00852/day.

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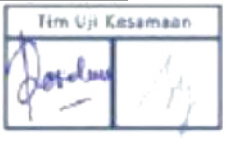

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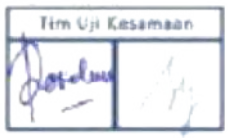

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