IOP Conference Series: Earth and Environmental Science

Table of contents

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← Previous issue Next issue →

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Preface

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PREFACE

The Faculty of Engineering of Universitas Negeri Semarang, Indonesia organized the 11th EIC. The conference this year, with the theme "Applied Green Technology for Environment Conservation Through Continuous Engineering Innovation," was successfully held on September 22nd, 2022. This year's annual conference was also held digitally through Zoom meeting and streamed on YouTube like in the previous year due to the effects of COVID-19 and government travel limitations.

The Academic Vice Rector of Universitas Negeri Semarang launched the conference and provided a quick overview of the institution. Next, in the plenary session, four keynote speakers from Taiwan, Malaysia, and Indonesia gave their speeches. Each of them spokes for 45 minutes, followed by a 15-minute Q&A period. Each speaker attended the Zoom meeting in order to present their speech. Also, this session was facilitated by knowledgeable and skilled moderators from the Faculty of Engineering at UNNES. From the beginning of the opening ceremony to the conclusion of the plenary session, more than 900 attendees enthusiastically joined a Zoom meeting.

Following the plenary session, nine Zoom meeting rooms were assigned to the 128 presenters from Indonesia, Malaysia, Thailand, and Taiwan depending on the contents of the manuscript in order to hold a parallel session presentation. A moderator ran the presentation and the Q&A session in each room. Presenters were allotted 10 minutes for their presentation and 5 minutes for questions and answers. All of the presenters and participants in each room had excellent discussions thus increased participants' understanding of the subject delivered. Idea sharing was also promoted through the sessions.

The committee, partner, keynote speakers, presenters, participants, and everyone else who helped make this virtual conference a success were all thanked deeply despite the pandemic circumstances. Without any notable issues, all of the attendees joined in and participated throughout the entire session. The best presenter from each parallel room was named at the conclusion to recognize their tremendous effort in organizing the presentation. All keynote speakers, presenters, and conference attendees received a certificate from the committee following the conference as identification of their involvement.

This document is a compilation of the 53 presenters' accepted manuscripts. It presents the findings from research as well as concepts, data, and applications pertaining to green technology theory, design, development, implementation, testing, and evaluation. In this proceeding, various engineering-related subjects are presented. The following areas are where green technology is used:

1) Biodegradable Materials
2) Biomass Conversion
3) Biotechnology and Bioprocess
4) Disaster Resilience Infrastructure
5) Energy Efficiency
6) Energy Management System
7) Environmental Monitoring
8) Green Chemicals
9) Green Construction
10) Green Materials

11) Green Technology in Building
12) Green Technology System
13) Green Transportation
14) Intelligent Control System
15) Natural Disaster Mitigation
16) Renewable and Sustainable Materials
17) Renewable Energy
18) Renewable Resources
19) Sustainability in the Built Environment
20) Sustainable Architecture
21) Waste Treatment

The goal of this proceeding is to contribute to the advancement of green technology. Also, we wish everyone reading this proceedings pleasure and success in expanding an understanding of engineering research. We value everyone's dedication and hard work and anticipate that the conference will be even more successful the following year.

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Adhi Kusumastuti, Ph.D
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Peer Review Statement

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Peer Review Statement

All papers published in this volume have been reviewed through processes administered by the Editors. Reviews were conducted by expert referees to the professional and scientific standards expected of a proceedings journal published by IOP Publishing.

- **Type of peer review:** Single Anonymous
- **Conference submission management system:** Morressier
- **Number of submissions received:** 129
- **Number of submissions sent for review:** 128
- **Number of submissions accepted:** 82
- **Acceptance Rate (Submissions Accepted / Submissions Received × 100):** 63.6
- **Average number of reviews per paper:** 4.99
- **Total number of reviewers involved:** 26
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  Name: Prof. Dr. Samsudin Anis
  Email: samsudin_anis@mail.unnes.ac.id
  Affiliation: Universitas Negeri Semarang

Biotechnology and Bioprocess

IOP Conference Series: Earth and Environmental Science

PAPER • OPEN ACCESS

Bioremediation of iron and manganese heavy metal polluted soil by mixed culture of *Acetobacter tropicalis* and *Lactobacillus fermentum*

To cite this article: H T Nugraha *et al* 2023 *IOP Conf. Ser.: Earth Environ. Sci.* **1203** 012006

Bioremediation of iron and manganese heavy metal polluted soil by mixed culture of *Acetobacter tropicalis* and *Lactobacillus fermentum*

H T Nugraha, A Rinanti*, A Wijayanti, S Aphirta

Environmental Engineering Department, Faculty of Architecture Landscape and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia 11450
astririnanti@trisakti.ac.id

Abstract. The research aim was to determine the growth response of mixed cultures of *Acetobacter tropicalis* and *Lactobacillus fermentum* in Stone Mineral Salt solutions (SMSs) and soil containing Acid Mine Drainage (AMD) in controlled batches. The first stage was the cultivation in SMSs followed by preparation of the pollutant source and sensitivity test. The second phase was the removal of heavy metals from iron and manganese in AMD by mixed bacterial cultures in SMSs with the contact time for 48, 96, 144, 192 hours, and pH 2, 3, 5, 7. The third stage was the removal on soil media with the same contact time. Research result showed that the sensitivity test of bacterium was resistant to heavy metals iron and manganese. The highest removal efficiency of iron and manganese was 94.89% at a contact time of 96 hours, while the highest removal efficiency at pH 7 was 93.79%. The removal of iron and manganese heavy metals on soil had a removal efficiency of 69.91% at a contact time of 144 hours. This study showed that a mixed culture of *Acetobacter tropicalis* and *Lactobacillus fermentum* could be an effective bacterium in removing heavy metals from iron and manganese in AMD-contaminated soil.
Keywords. Bioremediation, Acid Mine Drainage, Iron and Manganese Heavy Metal Removal, *Acetobacter tropicalis, Lactobacillus fermentum*

1. Introduction

As the mining industry develops, problems and impacts will also increase, such as environmental pollution, especially on the soil around mining sites. Polluted soil due to industrial activities can disrupt the biodiversity in the soil and affect the environmental balance. Wastewater containing heavy metals with highly acidic properties is referred to as Acid Mine Drainage (AMD) [1]. Acid Mine Drainage occurs due to the natural oxidation of minerals sulfides such as pyrite, chalcopyrite, pyrrhotite, arsenopyrite, which when exposed to air and water will produce acid waste that dissolves heavy metals so that it has the potential to pollute the environment [2]. Mining soil that is exposed to water will cause the dissolution of minerals and metals, causing a decrease in pH in the soil which causes reduced plant fertility [13].

Mining soil that is exposed to water will cause the dissolution of minerals and metals, causing a decrease in pH in the soil which causes the problem of reduced plant fertility. The problem of AMD pollution can be overcome by increasing the acidity level and removing dissolved metals. There are two events in processing AMD, namely with active processing and passive processing. Active processing is more suitable for mining areas that are still active, because it requires regular addition of reactants, while passive methods are more suitable for mining areas that have stopped operating [3]. AMD processing carried out with active processing is generally carried out by chemical methods or physical methods. The neutralization process is carried out by utilizing chemicals $Ca(OH)_2$ (hydrated lime) or $CaCO_3$ (calcium carbonate), NaOH (caustic soda) or Na_2CO_3 (soda ash) while physical methods include coagulation, flocculation, and sedimentation. Processing of physical or chemical methods is an effective method for processing AMD, but this treatment requires relatively high maintenance and operational costs and is feared to cause secondary pollution [4,3]

Passive treatment with bioremediation-based biology is a natural, economical, sustainable approach that can restore contaminated soil, surface water, and groundwater, with the help of microorganisms such as bacteria, fungi, algae, protozoa. Passive treatment methods with bioremediation are considered environmentally friendly because they utilize naturally occurring microbes in soil and groundwater that do not pose a harmful threat to surrounding humans [5,6,7,14].

Therefore, the solution needed to answer these problems is to preserve commodities in an effective and simple way. One of the innovations is doing UV irradiation and cooling. The purpose of this study was to determine the growth response of a mixed culture of bacteria *Acetobacter tropicalis* and *Lactobacillus fermentum* against heavy metal contaminants of iron and manganese in liquid and solid media containing heavy metals iron and manganese and determine the efficiency of removal of heavy metals from iron and manganese by mixed culture of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria in liquid and solid media.

2. Research Method

2.1 Cultivation of Mixed Culture of Acetobacter tropicalis and Lactobacillus fermentum bacteria
Cultivation of mixed cultures of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria was carried out by batch or limited culture using SMSs liquid media. Cultivation in mixed culture is carried out to grow a mixed culture of bacteria to reach exponential conditions. Cultivation of this mixed bacterial culture is carried out by taking into account the environmental conditions suitable for the mixed bacterial culture, namely temperature, contact time, and pH. Cultivation of this mixed culture was carried out in a 250 mL erlenmeyer container with the use of 80% of the capacity. Cultivation of mixed bacterial cultures on media containing AMD was carried out by adding 10% (v/v) AMD to the media. The composition of the cultivation of mixed bacterial cultures without the addition of AMD in liquid media was 75% SMSs media, 5% molasses as an additional carbon content, and 20% mixed bacterial cultures. Cultivation of mixed bacterial cultures in SMSs media containing AMD had a composition of 70% SMSs media, 5% molasses, 5% AAT, and 20% mixed bacterial cultures.

2.2 Bacterial Mixed Culture Sensitivity Test to Acid Mine Drainage
This sensitivity test was conducted to determine the sensitivity of a mixed culture of bacteria *Acetobacter tropicalis* and *Lactobacillus fermentum* to heavy metals iron and manganese present in Acid Mine Drainage (AMD). This sensitivity test was carried out in Nutrient Agar (NA) solid media exposed to a mixed culture of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria by adding disc paper containing AMD.

2.3 Test for Removal of Heavy Metals of Iron and Manganese in Liquid Media SMSs
The removal of iron and manganese in the liquid medium of SMSs was carried out in two stages with the aim of obtaining the optimum conditions in terms of contact time (hours) and pH. The heavy metal removal test in liquid media has two stages, First phase is optimization of pH, and second phase is optimization of contact time. In the first phase iron and manganese removal test, the optimization of pH. The independent variable is pH with variations of 2, 3, 5, 7 while the fixed variable is temperature with room temperature of 30o C, 10% AAT concentration (v/v), and contact time for 96 hours. The highest removal of iron and manganese from one of these pH variations was used as a suitable pH for the growth of mixed cultures of bacteria *Acetobacter tropicalis* and *Lactobacillus fermentum* to remove iron and manganese. This pH will be the optimum pH for mixed cultures of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria to remove iron and manganese. In the first phase iron and manganese removal test, the optimization of pH. The independent variable is pH with variations of 2, 3, 5, 7 while

the fixed variable is temperature with room temperature of 30° C, 10% AMD concentration (v/v), and contact time for 96 hours. The highest removal of iron and manganese from one of these pH variations was used as a suitable pH for the growth of mixed cultures of bacteria *Acetobacter tropicalis* and *Lactobacillus fermentum* to remove iron and manganese. This pH will be the optimum pH for mixed cultures of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria to remove iron and manganese.

2.4 Test for Removal of Iron and Manganese in Solid Media Contaminated Soil AMD

The removal of iron and manganese in AD by mixed cultures of *Acetobacter tropicalis* and *Lactobacillus fermentum* in solid media was carried out in soil media that had been exposed to AMD. Tests for removal of iron and manganese on solid media were not carried out in stages, because environmental conditions were controlled such as temperature and pH for the growth of mixed cultures of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria. In the optimization of contact time, the independent variables at the contact time (hours) were 48, 96, 144, and 192 hours while the fixed variables were room temperature and optimum pH of the research in liquid media. The highest removal of iron and manganese from one of these contact time variations was used as a suitable and optimum contact time for the growth of mixed cultures of bacteria *Acetobacter tropicalis* and *Lactobacillus fermentum* in removing heavy metals of iron and manganese.

3. Results and Discussion

Cultivation of mixed cultures of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria carried out with the addition of AMD had a higher number of bacterial cells than those without AMD. This shows that the mixed culture of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria can utilize the heavy metals contained in AMD as a source of nutrients. Seen in the growth curve, at 72 hours the number of bacteria increased higher reaching 210 cells/mL and the number of bacteria decreased at 120 hours to 198 cells/mL.

In Figure 1. it can be seen that the increase in the number of mixed culture cells of bacteria *Acetobacter tropicalis* and *Lactobacillus fermentum* in solid media continued for up to 168 hours, reaching 359 cells/mL. This phase is called the exponential phase of a mixed culture of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria in their growth, while at 264 hours the mixed culture of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria reached a stationary phase leading to death, because the number of cells in the soil had decreased, which reached 340 cells/mL.

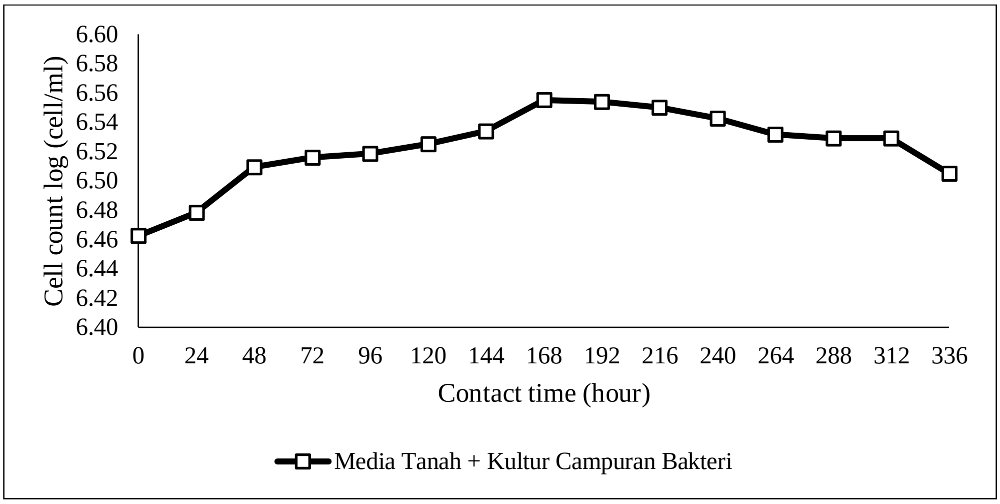

Figure 1. Growth Curve of Mixed Culture of *Acetobacter tropicalis* and *Lactobacillus fermentum Bacteria* in Solid Media Contaminated Soil AMD

Based on Figure 2. it can be seen that the mixed culture of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria was able to remove iron and manganese at a very acidic pH as characteristic of AMD with the removal percentage reaching 86.53%. The highest of iron and manganese occurred at pH 7, which is a neutral pH for the growth of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria with a removal efficiency of 93.79%, based on [12], endosulfan bioremediation on various soil layers with a concentration level of 2 mg/g was conducted on the pH level of 7, while the lowest removal efficiency occurred at pH 3, which was 83.96%. From the results of the removal efficiency, it can be seen that a mixed culture of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria can stay alive and help remove iron and manganese by utilizing these metals as additional nutrients as evidenced by the level of removal efficiency above 80% at acidic to neutral pH. and proved [10] where the mixed culture of these bacteria has excellent survival in the acidic pH range, which is 1.5 – 3.5. Refer to [11] bioremediation has the capability to function synergistically in utilizing heavy metals as a source of nutrient to expand its allowance by 50%.

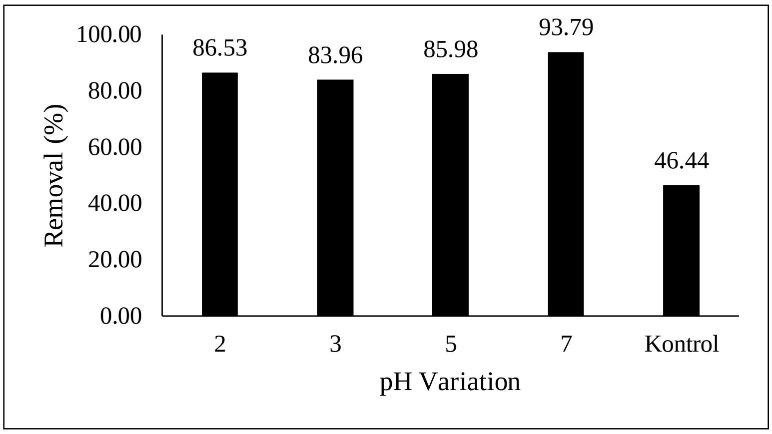

Figure 2. Removal of Heavy Metals Iron and Manganese (%) on Variation of pH in Liquid Media SMSs

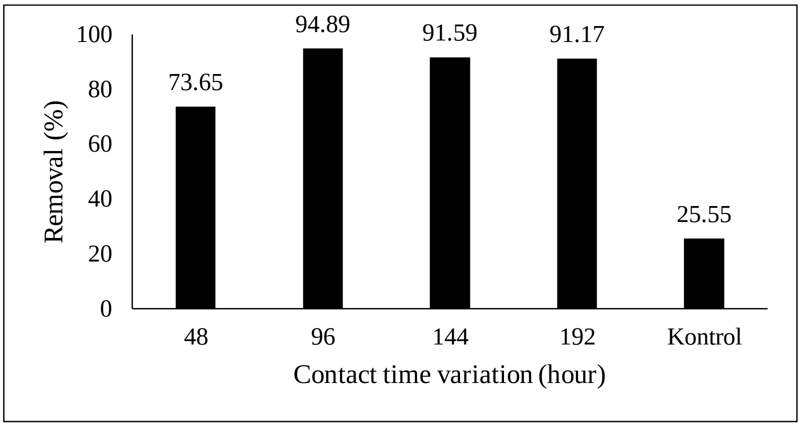

Figure 3. Removal of Heavy Metals Iron and Manganese (%) on Variation of Contact Time in Liquid Media SMSs

From these results, it can be seen in Figure 3, the highest removal of iron and manganese occurred within a contact time of 96 hours with an initial concentration of iron 8.3 mg/L and manganese 204.6 mg/L being 0.39 mg/L for a concentration the final concentration of iron and 11.44 mg/L for the final concentration of manganese so that the removal percentage was 94.89%. The same result [9] shows that the highest iron metal removal occurred on 144 hours of contact time with a removal percentage of 96% The lowest percentage of iron and manganese removal occurred at a contact time of 48 hours with the initial concentration of iron 8.3 mg/L and manganese 204.6 mg/L being the final concentration of iron 1.88 mg/L and manganese 61.49 mg. /L, so that the percentage of allowance is 73.65%. The percentage of removal efficiency for heavy metals iron and manganese continued to increase with increasing contact time up to 96 hours. At a contact time of 192 hours, the percentage of removal was reduced to 91.17%. It can be seen that the percentage of removal will continue to increase up to a contact time of 96 hours, but the addition of a contact time of more than 96 hours will not cause the removal to increase. The optimum contact time for a mixed culture of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria to remove 8.3 mg/L iron and 204.6 mg/L manganese was 96 hours.

In this study, the optimum pH in previous studies in SMSs liquid media was pH 7. The control batch was prepared with the composition of soil contaminated with AMD and rice husk as bulking agent. After analysis, the concentration of iron and manganese in soil containing AMD was 4008.13 mg/L and 456.72 mg/L. The heavy metal content of iron and manganese in the control batch after 96 hours was reduced to 3908.2 mg/L and 440.82 mg/L. Changes in the concentration of iron and manganese in the control batch showed that the physical process did not have much influence in the process of this research. The results of heavy metal removal of iron and manganese in solid media can be seen in Figure 4 which is the result of removal of heavy metals of iron and manganese in graphical form.

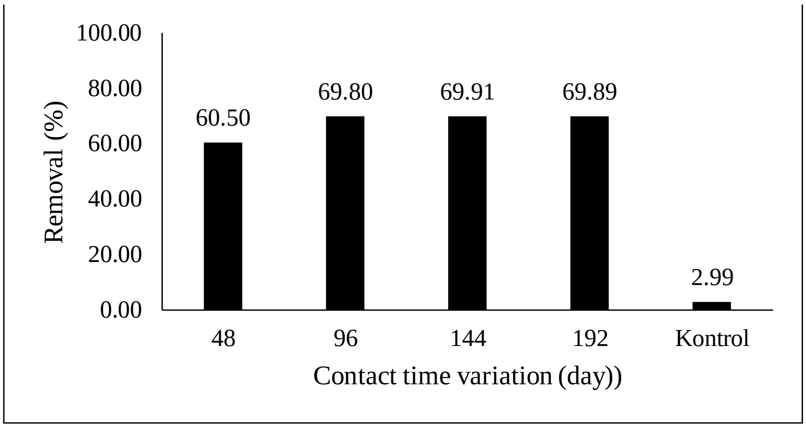

Figure 4. Removal of Iron and Manganese (%) on Variation of contact time in Solid Media of Soil Contaminated with AMD

4. Conclusion

Mixed cultures of *Acetobacter tropicalis* and *Lactobacillus fermentum* bacteria were resistant to media containing AMD (iron and manganese) as evidenced by the absence of inhibition zones. Physical and chemical factors play less role in the removal of iron and manganese. Removal of iron and manganese in solid media requires a longer contact time compared to removal of iron and manganese in liquid media due to mixed culture contact of iron and manganese more easily occur in liquid media, so that iron and manganese is more likely to be quickly removed.

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