Restoration of soil contaminated by mine acid using *Thiobacillus* sp. and *Clostridium* sp. effective bacteria

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Abstract. The mining industry in Indonesia in recent years has grown rapidly, causes an emerging problem of environmental mine acid pollution on the land around the mining site. The most dangerous effect of mine acid is the high accumulation of metals in land re-vegetation. The method for recovering land contaminated by mine acid that used in this study is bioremediation using effective bacteria *Thiobacillus* sp. and *Clostridium* sp. The core of this research is to find optimum environmental conditions (contact time, pollutant concentration, and bacterial concentration) to degrade mine acid, which is that effective bacteria contacted with soil contaminated by mine acid under optimum environmental conditions with variations of time. The results showed that the optimum environmental conditions for effective bacteria to degrade mine acid were 48 hours contact time, 60% pollutant concentration and 40% bacterial concentration. The results of this study is the effective bacteria efficiently recover soil contaminated by mine acid within 48 hours as evidenced by an increase in pH from 2 to 6, a decrease in Fe content from 3.6 mg/L to 0.27 mg/L, and decreased Mn levels from 3.2 mg/L, to 0.31 mg/L.

1. Introduction

A solution that contains sulphate acid produced from mineral oxidation in mining process is known as mine acid. The most dangerous negative impact of mine acid is the high accumulation of metals in soil and water, which causes disruptions on land revegetation efforts [1]. Besides that, mine acid can also endanger the health of the society because the people that live nearby mining areas are commonly known to utilize rivers that run through mining areas as their water source.

Pollutant compound processing can be effectively and safely produced through biological method conduction by utilizing organisms [2,3]. One available biological method is known as bioremediation by using microorganism to neutralize pollutant and avoid soil and water pollutions [4]. Decomposition and mineralization often resulted from integration between chemical and microorganism [5]. Minerals solubility in acid condition, mineral deposition in anaerobe condition, metal ions absorption by bacteria and algae, and metal organ complex reshuffling are the examples of indirect microorganism’s participations [6].

One mine acid polluted soil bioremediation alternative is by utilizing sulphate reductor bacteria. Swamp and rice field sediment addition as substrate of sulphate reductor bacterial growth in mine acid able to increase its pH, lower its sulphate content and improve sulphate reductor growth in 30 days [7]. Because of that, a research by utilizing bacteria-owned enzyme is required to degrade acid content in mine acid so that soil pollution can be decreased without producing any negative effect on the
environment and with a relatively low cost. This research was conducted to test effective ability of bacteria such as Clostridium sp. and Thiobacillus sp. to remove mine acid pollutant so that it can be applied in mine acid water pollution in Indonesian mining industry, because Clostridium sp. and Thiobacillus sp. effective bacteria is proven to be able to grow in such extreme condition and can reduce several pollutant compound [8-10].

2. Research methodology

2.1. Mine acid pollutant characterization
To conduct water quality characteristics observation on coal mining environment, the guidance utilized in this research is the Decision of Ministry of Environment Number 113 Year 2003 regarding Waste Water Quality for Coal Industry or Activity.

2.2. Media Stone Mineral Salt (SMSs) solution producing
SMSs media is produced by mixing 0.5 grams of CaCO₃; 2.5 grams of NH₄NO₃; 1 gram of Na₂HPO₄.7H₂O; 0.5 grams of KH₂PO₄; 0.5 grams of MGSO₄.7H₂O; and 0.2 grams of MnCl₂.7H₂O with a liter of aquades.

2.3. Clostridium sp. and Thiobacillus sp. bacteria cultivation
Clostridium sp. and Thiobacillus sp. bacteria cultivation was conducted with a batch or limited on using SMSs media. Bacteria cultivation is aimed to grow bacteria in optimum situation. Bacteria cultivation can be conducted by mixing 150 ml of SMSs media, 20 ml of Clostridium sp. bacteria, 20 ml of Thiobacillus sp. bacteria, molasses (carbon source) and 20 ml of mine acid.

2.4. Contact time optimization in liquid media
The first step to determine optimum temperature determination is by preparing 100 ml of SMSs media in an Erlenmeyer flask added with 300 ppm of mine acid pollutant. The mine acid pollutant was measured with 30% of effective bacteria concentration in a total volume of 80 ml solution. The mine acid pollutant then measured with a pH indicator paper to obtain original pH. After that, add 30% of effective bacteria concentration from 80ml of total solution volume. The solution is let alone in a different contact time variation, which are 48, 96, 144, 192, and 240 hours in room temperature. In every treatment, incubation is conducted in shaker with a speed of 180 rpm. After finishing these steps, the next step is to conduct mine acid pollutant pH decrease by using pH meter measurer, and also measuring bacteria growth speed by using Petrof Hausser Chamber method.

2.5. Pollutant concentration optimization in liquid media
The first steps to determine mine acid concentration variation is by preparing SMSs media in a 100 ml Erlenmeyer and add various mine acid pollutant concentration of 50, 100, 300, and 500 ppm. These mine acid then further measured by utilizing pH indicator paper to obtain original pH. After that, add 30% of effective bacteria concentration in 80 ml of solution total volume. The solution is let alone in a room temperature. In every treatment, an incubation inside a shaker with 180 rpm speed within optimum time obtained from the previous step. After finishing these steps, the next step is to conduct measurement on mine acid pH decrease by using pH meter, and to measure bacteria growth speed by using Petrof Hausser Chamber method.

2.6. Effective bacteria concentration optimization in liquid media
The first step to determine effective bacteria concentration is by preparing a SMSs media in a 100 ml Erlenmeyer flask added with mine acid pollutant with optimum concentration obtained from the previous step. This mine acid pollutant can be measured by utilizing pH indicator paper to obtain original pH. After that, add effective bacteria from 80 ml total solution volume with different concentration variations, which are 10%, 20%, 30%, and 50%. The solution then let alone in room temperature. In
every treatment, an incubation inside a shaker with 180 rpm speed within optimum time obtained from the previous step. After finishing these steps, the next step is to conduct measurement on mine acid pH decrease by using pH meter, and to measure bacteria growth speed by using Petrof Hausser Chamber method.

2.7. Contact time optimization in soil media
The first step to determine contact time variations optimization is to prepare soil media in a glass square-shaped reactor with 23 x 18 x 8 cm with a 5 mm diameter hole at the bottom side and add mine acid pollutant with optimum concentration obtained from mine acid pollutant concentration optimization step. The pollutant is further measured with pH indicator paper to obtain original pH. After that, add active bacteria of 80 ml solution total volume with optimum concentration obtained from the effective bacteria concentration optimization. The solution is let alone in a room temperature. This step is conducted in with a different time variation of 48, 96, 144, 192 and 240 hours. After finishing these steps, the next step is to conduct measurement on mine acid pH decrease by using pH meter, and to measure bacteria growth speed by using Petrof Hausser Chamber method.

3. Results and discussion
Picture 1 shows a constant pH escalation until the end of contact time. The value of pH shown in t-0 is 2 which is very acidic and continue to escalate until t-5 with 240 hours of contact time that shows a value of 5.20. Meanwhile control graphic in early observation (t-0) shows pH value of 2 until at t-5 with 240 hours contact time the pH value only reach 2.28, which is still very acidic. Escalation of pH in mine acid happened because of effective bacteria activity in reducing mine water acidity.

![Figure 1](image-url)  
**Figure 1.** Value of pH in every contact time variation.

The result of bacteria measurement with Petrof Hausser Chamber method on t-0 is 2x105 cells/ml in various contact time variations and 1.7x105 in controlled treatments. During t-1 observation on 48 hours contact time, the number of bacteria slightly decreased to 1.3x105 cells/ml in various contact time variation and 0.4x105 cell/ml in controlled treatment. in t-3 controlled treatment until t-5 with 240 hours of contact time there are no more bacteria was growing due to lack of nutrition to help effective bacteria grow. Effective exponential bacteria happen in the 192th hours that was shown by the largest number of bacteria, which is 3.7 x105 cells/ml, before it finally dropped to 2.36 x105 cells/ml on the 240th hours of contact time.
Figure 2. Bacteria density in various contact time variations.

Figure 3 shows pH escalation on different pollutant concentrations. The pH value in 10% pollutant concentration shows a value of 5.5 and in pollutant concentration of 25%, a value of 4.1 was shown. This means that a higher pollutant concentration will produce a lower pH of mine acid.

Figure 3. Value of pH in various pollutant concentration variations (Mine acid water).

Bacteria number calculation result with Petrof Hausser Chamber method shows a slight decrease of bacteria number along with pollutant concentration addition. In 5% concentration of mine acid water, effective bacteria growth reaches $3.65 \times 10^5$ cells/ml. Meanwhile by additional 25% of mine acid concentration, effective bacteria growth slightly increased to $2.94 \times 10^5$ cells/ml (Figure 4). The decrease of bacteria number is caused by lack of nutrition needed by effective bacteria, which finally disrupts its growth rate.
4. Conclusion

*Thiobacillus* sp. and *Clostridium* sp. bacteria is proven effective to increase pH on 10% mine acid treatment as pollutant with 240 hours of contact time in a liquid media. A further research is required to test *Thiobacillus* sp. and *Clostridium* sp. effective bacteria ability to neutralize soil media that is polluted by mine acid water.

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