Potential sulfate reducing bacteria for decrease Fe and Mn determination agents on ex-coal mining soil in Samarinda

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Abstract. The aim of this study was to determine the potential of sulfate reducing bacteria (SRB) as reducing agents for decrease Fe and Mn in acid soils of coal mines using five isolates of isolated SRB from post-coal mining sediments in Samarinda. The SRB potential test in reducing Fe and Mn was carried out by growing SRB on liquid Postgate media with varying pH of 3, 4 and 5 with the addition of mine acid soil to each treatment. Each of the pure SRB isolates (1 ml) was inoculated into liquid Postgate media with different pH, namely pH 3, 4 and 5 which had previously been filled with 5 grams of acidic soil after coal mining which had been mashed and sterilized at 121°C for 15 minutes. The culture was incubated in a threaded tube of 10 ml volume to full. The results showed that five isolates of SRB from post-coal mine sediments in Samarinda were Desulfococcus sp., Desulfotomaculum sp., Desulfobacter sp., Desulfothlibus sp. and Desulfobacterium sp. have potential as reducing agents for decrease Fe and Mn in Postgate B liquid media which have been added with coal mine acid soils. The best efficiency of Fe reduction by Desulfobacterium sp. at pH 3 is 6% and the best Mn reduction by bacteria Desulfotomaculum sp. at pH 5 is 4%.

1. Introduction
The development of the mining industry in Indonesia is very rapid because it is still a mainstay for the national and regional economies [1]. One of the provinces in Indonesia which has the potential of abundant coal mining and gold is East Kalimantan. The Directorate General of Mineral and Coal until February 2018 has issued thousands of Mining Business Licenses spread across Indonesia and recorded 1,037 companies that have mining business licenses, namely as many as 878 companies have carried out production activities, while 159 companies are still in the exploration stage [2].

Mining systems that are commonly carried out in Indonesia are open pit mining, cleaning all plants on the ground and removing soil and overburden to a place [3]. Open pit is a type of strip mining where the excavated material is deep in the ground and requires overburden removal and excavation. The stages of mining activities generally include exploration, construction of access and energy generation road infrastructure, construction of employee settlements and worker base camps, overburden and rock waste disposal, extraction of minerals, processing of minerals, tailings disposal, and mine reclamation and closure [4].

The tendency to increase the role of coal in national energy supply will be a serious environmental problem in the future. This can be seen in various coal mining activities that are very synonymous with activities of natural and environmental destruction. Many post-coal mining land is left after
exploitation. The loss of surrounding vegetation due to deep excavation is real damage occurring in the field. If the land is not rehabilitated, it will cause land to die and damage the existing ecosystem. Other than that, there is a decrease in nutrients, soil pH and thin layer of topsoil, as well as an increase in the content of toxic elements for plants. Such lands certainly need repairs so that they can be reused for agricultural businesses [1].

Thus, the problem that must be overcome is to improve soil conditions after coal mining. One method that is environmentally friendly is bioremediation, which is a process using microorganisms, fungi, green plants or enzymes produced to restore environmental conditions by eliminating contaminants. The microbial group that can be used to improve soil quality after coal mining is sulfate reducing bacteria (SRB). This SRB is thought to play an important role in the process of reforming organic matter, sulfate reducing, iron reducing, and being able to isolate iron and pyrite. In its metabolic activity, the SRB can reduce sulfate to H₂S. This gas will soon bind to metals that are mostly found in ex-mining land and are precipitated in the form of reductive metal sulphide [5].

This situation attracts researchers to find out the potential of SRB from isolation from post-coal mining sediments in Samarinda as reducing agents for Fe and Mn in coal mining acid in Samarinda. The purpose of this study was to determine the potential of sulfate reducing isolates from post-coal mining sediments in Samarinda as reducing agents of Fe and Mn in acid soils of coal mines in Samarinda.

2. Materials and Methods
The material used in this study was Postgate B media [6] containing (g/l) Na lactate (3.5), MgSO₄ (2.0), NH₄Cl (0.2), KH₂PO₄ (0.5), FeSO₄. 7 H₂O (0.5), 0.1 N HCl, 0.1 N NaOH, distilled water, 95% alcohol, spirits, and acid soils after coal mining. Another material used in this study was five isolates of SRB from pond sediments after coal mining in Jl. Pelita 7, Kecamatan Sambutan, Samarinda. Experiment using a completely randomized design consisting of:

| P1 = Control | = Postgate B + 5 gr acid soils after coal mining |
| P2 = sp. 1 (Desulfococcus sp.) | = Postgate B + 5 gr acid soils after coal mining + 1 ml SRB |
| P3 = sp. 2 (Desulfotomaculum sp.) | = Postgate B + 5 gr acid soils after coal mining + 1 ml SRB |
| P4 = sp. 3 (Desulfobacter sp.) | = Postgate B + 5 gr acid soils after coal mining + 1 ml SRB |
| P5 = sp. 4 (Desulfobulbus sp.) | = Postgate B + 5 gr acid soils after coal mining + 1 ml SRB |
| P6 = sp. 5 (Desulfobacterium sp.) | = Postgate B + 5 gr acid soils after coal mining + 1 ml SRB |

3. Stages of Research
Sterilization of tools and materials. The test tube, Erlenmeyer, blue tip, Petri dish and Postgate B media with varying pHs of 3, 4 and 5 were sterilized in the autoclave for 15 minutes with a pressure of 1 atm at 121°C.

Making Postgate B media with various pH treatments 3, 4, and 5. The main composition of Postgate B media for one liter of liquid media consisted of (g / l) Na lactate (3.5), MgSO₄ (2.0), NH₄Cl (0.2), KH₂PO₄ (0.5), FeSO₄. 7 H₂O (0.5) was put into a 1000 ml Erlenmeyer flask, added aquadest to the tera mark. The media was arranged with NaOH and HCl solution to obtain pH 3, 4 and 5 for each treatment. Homogenized and sterilized was done at 121°C 1 atmospheric pressure for 15 minutes.

Testing the activity of samples of acid soil after coal mining. The SRB isolates used in this study were maintained in liquid Postgate media. Each of the pure SRB isolates (1 ml) was inoculated into liquid Postgate media with different pHs. It was pH 3, 4 and 5 which had previously been filled with 5 g of acidic soil after coal mining which had been mashed and sterilized at 121°C for 15 minutes. The
culture was incubated in a threaded tube of 10 ml volume to full. The experiment was carried out in a complete randomized design with 3 replications. Measurements of Fe and Mn were carried out at the beginning and twenty days. As a control, the treatment of postgate B media with different pHs was pH 3, 4 and 5 but not inoculated with SRB. Bioremediation efficiency was calculated to find out what percentage of pollutants that can be reduced during treatment by using formula [7], as follows:

\[
\frac{(\text{initial concentration}) - (\text{final concentration})}{\text{(initial concentration)}} \times 100\%
\]

4. Result and Discussion

This research was conducted to determine the potential of sulfate reducing bacteria (SRB) from isolation from post-coal mine sediments as reducing agents for Fe and Mn in acid soils of coal mines in Samarinda. After working on the research the results are as follows:

Table 1. The efficiency (%) of sulfate reducing bacterial isolates in reducing Fe and Mn levels in each treatment on a laboratory scale

| pH | Inoculum                        | Efficiency treatment of Fe (%) | Efficiency treatment of Mn (%) |
|----|---------------------------------|------------------------------|--------------------------------|
| 3  | Control                         | 0                            | 0                              |
|    | Sp 1 (Desulfooccus sp.)         | 2                            | 2                              |
|    | Sp 2 (Desulfotomaculum sp.)     | 1                            | 1                              |
|    | Sp 3 (Desulfo bacter sp.)       | 0                            | 3                              |
|    | Sp 4 (Desulfobulbus sp.)        | 0                            | 2                              |
|    | Sp 5 (Desulfobacterium sp.)     | 6                            | 1                              |
| 4  | Control                         | 0                            | 0                              |
|    | Sp 1 (Desulfooccus sp.)         | 2                            | 1                              |
|    | Sp 2 (Desulfotomaculum sp.)     | 1                            | 1                              |
|    | Sp 3 (Desulfo bacter sp.)       | 0                            | 2                              |
|    | Sp 4 (Desulfobulbus sp.)        | 0                            | 1                              |
|    | Sp 5 (Desulfobacterium sp.)     | 0                            | 1                              |
| 5  | Control                         | 0                            | 0                              |
|    | Sp 1 (Desulfooccus sp.)         | 0                            | 1                              |
|    | Sp 2 (Desulfotomaculum sp.)     | 1                            | 4                              |
|    | Sp 3 (Desulfo bacter sp.)       | 0                            | 2                              |
|    | Sp 4 (Desulfobulbus sp.)        | 1                            | 2                              |
|    | Sp 5 (Desulfobacterium sp.)     | 0                            | 1                              |

SRB’s ability to reduce heavy metal content is strongly influenced by environmental factors, including bioavailability metals, pollutant concentration, electron acceptor, water content, nutrients, osmotic pressure, oxygen, pH, redox potential, soil structure, temperature, and water activity [8]. Based on the test of heavy metal in Table 1, it was shown that the decrease in Fe and Mn metal concentrations in Postgate B media were different according to the pH conditions of the media and inoculated SRB isolates. In the control treatment there was no reduction of Fe or Mn. This is because the control treatment did not inoculate any type of sulfate reducing bacteria. Table 1 also showed that the best Fe reduction efficiency was obtained by pH 3 media treatment with the addition of 6%
Desulfobacterium sp. The best Mn reduction was obtained by pH 5 media treatment and 4% of Desulfotomaculum sp. From the results obtained, it can be seen that each type of bacterium has a different optimal pH in reducing Fe and Mn.

The key factor for metal remediation is that metals are non-biodegradable but can transform through sorption, methylation, complexation and change their valence values [9]. When heavy metal ions are spread around the cell, metal ions will be bound to the elements found in the cell wall based on the ability of the chemical affinity possessed by the cell [10]. This process occurs effectively with the presence of a certain pH and the presence of other ions where heavy metals can become precipitated unsolved salts [11]. The effect of pH on bacterial growth is related to enzyme activity. Enzymes are complex molecules based on proteins produced by cells. This enzyme is needed by bacteria to catalyze reactions related to bacterial growth. When the pH becomes too high or low, the basic structure of the enzyme can change, so that the active side of the enzyme cannot bind the substrate and the enzyme activity becomes affected. Even enzymes can actually stop functioning. In this study the most optimal pH for bacterial growth in reducing heavy metals is at pH 3 and pH 5. This can be seen from the efficiency of each parameter of the heavy metals Fe and Mn analyzed.

There are several mechanisms for removing heavy metals by microorganisms. The cellular structure of a microorganism can trap heavy metal ions and subsequently sorb them onto the binding sites of the cell wall [12]. This process is called biosorption. The mechanism involves several processes, including electrostatic interaction, ion exchange, precipitation, the redox process, and surface complexation [13]. The other method is a process in which the heavy metal ions pass across the cell membrane into the cytoplasm, through the cell metabolic cycle, this is referred to as bioaccumulation. The organism that will accumulate heavy metals should have a tolerance to one or more metals at higher concentrations, and must exhibit enhanced transformational abilities, changing toxic chemicals to harmless forms that allows the organism to lessen the toxic effect of the metal, and at the same time, keep the metal contained [14].

5. Conclusion
Can be concluded that five isolates of SRB from isolation from post-coal mining sediments in Samarinda are Sp 1 (Desulfococcus sp.), Sp 2 (Desulfotomaculum sp.), Sp 3 (Desulfobacter sp.), Sp 4 (Desulfobulbus sp.) and Sp 5 (Desulfobacterium sp.) potentially as reducing agents for decrease Fe and Mn heavy metals in Postgate B media which added acid soil after coal mining. The best Fe reduction efficiency by bacteria Sp 5 (Desulfobacterium sp.) at pH 3 is 6% and the best Mn reduction by bacteria Sp 2 (Desulfotomaculum sp.) at pH 5 is 4%.

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