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Short Communication: The potential of Sulfate Reducing Bacteria of ex-coal mine sediment pond as sulfate reducing agents of acid land in Samarinda, Indonesia

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Abstract. *Kusumawati E, Sudrajat, Purnamasari I, Panggabean BC, Apriyanti M. 2017. Short Communication: The potential of Sulfate Reducing Bacteria of ex-coal mine sediment pond as sulfate-reducing agents of acid land in Samarinda, Indonesia. Bonorowo Wetlands 7: 79-82.* The study aims to determine the effect of pH medium on the growth of sulfate-reducing bacteria taken from the e-coal mine sediment pond and determine its potential as a reducing sulfate agent of acid ex-coal mine land in Samarinda East Kalimantan, Indonesia. This study used six SRB isolated from an ex-coal mine sediment pond in Samarinda. The SRB potency test in reducing sulfate was conducted by growing the SRB on Postgate liquid medium at different pHs of 2, 4, and 6 by adding acid soils on each treatment. The results showed that sulfate reducing bacteria isolated from ex-coal mine sediment pond in Samarinda, i.e., sp.1 (*Desulfococcus* sp.), sp.2 (*Desulfotomaculum* sp.), sp.3 (*Desulfobacter* sp.), sp.4 (*Desulfobulbus* sp.), sp.5 (*Desulfobacterium* sp.) and sp.6 (*Desulfotomaculum* sp.) had potential as sulfate reducing agent of acid land. The efficiency of sulfate reduction was 89%, 91%, and 91% in the pH of 2, 4, and 6, respectively. This indicated that the highest sulfate reduction is in the medium with pH 4 and 6. In addition, sp.5 (*Desulfobacterium* sp.) growing on medium at pH 4 had the best sulfate reduction efficiency (93%) compared with other SRB isolates.

Keywords: pH of the medium, sulfate reducing bacteria, acid land

INTRODUCTION

Mining activities such as coal mining can provide economic benefits and cause environmental and soil ecosystems damages (Tala'ohu et al., 1995). One element of the causes of the impact is the generated waste as a byproduct of the remnant of the mining and processing, which are often in large volume and various kinds. United Nations Environment Programme (UNEP) classifies the impacts of mining activities, including the destruction of habitat and biodiversity at the mine site, the landscape change or loss of land use, and B3 waste and chemicals (Fahruddin 2010). The most severe mining activity problem is the phenomenon of acid mine drainage (AMD) or acid rock drainage (ARD) due to the oxidation of sulfur minerals. This will give a series of interrelated effects, namely decreasing pH, disturbing the availability and the balance of soil nutrients, and increasing the solubility of micronutrients which generally is a metal element (Havlin et al. 1999).

One environmentally friendly method is bioremediation, namely, a process to restore the environment using microorganisms as contaminants eliminating. A group of microbes that can improve the quality of post-mine land is sulfate-reducing bacteria (SRB). In metabolic activities, SRB can convert sulfate to H2S. This gas will immediately bind to the metals found in many post-mining land and precipitate in the form of metal sulfides reductive (Hards and Higgins 2004). However, the main problem often encountered in applying microorganisms for bioremediation is the decrease or the loss of potential microbial. To improve the effectiveness of microorganisms in bioremediation, the following two strategies can be done. First, bio-stimulation is a technique to add specific nutrients to stimulate the activity of local (indigenous) microbes. Atlas and Bartha (1992) stated that the bio-simulation process has successfully controlled the oil spills in water and the contamination of hydrocarbons (PAHs) in the soil. Lieberg and Cutright (1999) stated that nutrients often used in this technique are phosphorus and nitrogen. Second, bio-augmentation is a technique to introduce specific microbes in the remediated area. In addition, environmental influences such as pH, temperature, and soil moisture were also very influential in determining the success of the bioremediation process.

Based on the introduction, the problem in this research is how the influence of pH of the medium on the sulfate reduction activity of sulfate reducing bacteria isolated from ex-coal mine sediment pond in Samarinda on acid land (TMT) on the location of the ex-coal mine is. The purpose of this study was to determine the effect of medium pH on the potential sulfate reducing bacteria isolated from ex-coal mine sediment pond in Samarinda as a sulfate reducing agent in acid land (TMT) on the location of the ex-coal mining is. The obtained information will develop bacteria as a potential environmentally friendly bioremediation agent.

MATERIALS AND METHODS

Sampling

Sulfate-reducing bacteria (SRB) were isolated from an ex-coal mine sediment pond in Samarinda, East Kalimantan. Bacteria were isolated on Postgate liquid medium (Atlas and Park, 1993) containing (g/l) Na lactate (3.5), $Mg.SO_4$ (2.0), NH_4Cl (0.2), KH_2PO_4 (0.5), $FeSO_4.7H_2O$ (0.5) and pH 4 and sterilized at 121ºC with 1 atmosphere pressure for 15 minutes. SRB growth was characterized by the emergence of colonies in dark brown to black at the bottom of the tube.

Sulfate-reducing bacteria activity test on liquid media

Six SRB isolates result from the isolation of bacteria from ex-coal mine land in Samarinda. The isolates comprised of a mixture of six isolates based on the early identification, i.e., sp.1 (*Desulfococcus* sp.), sp.2 (*Desulfotomaculum* sp.), sp.3 (*Desulfobacter* sp.), sp.4 (*Desulfobulbus* sp.), sp.5 (*Desulfobacterium* sp.) and sp.6 (*Desulfotomaculum* sp.). Each pure isolate SRB (1 ml) was inoculated into a liquid Postgate medium enriched with 2 N sulfuric acid solution as much as 5% (v/v). The cultures were incubated in 25 ml tubes filled up to total volume. Each SRB isolate was used to treat with a consortium of all bacterial treatments. Experiments were carried out in a completely randomized design with three replications. The sulfate measurements were done at the beginning and twenty days of treatment. The control group was treated with Postgate B medium enriched with 2 N sulfuric acid solution as much as 5% (v/v) without SRB inoculation. The efficiency of each treatment was calculated using the formula stated by Widyati (2006), namely:

<u>(Initial sulfate concentration) - (final sulfate concentration) x 100%</u>
(Initial concentration)

Sulfate-reducing bacteria activity test against acid ex-coal mine soil with pH of 2, 4, 6

The composition of bacteria used in the trial experiments test was similar to SRB in Postgate liquid media. SRB isolates used in this study are maintained in a Postgate liquid medium. Each of these pure SRB isolates (1 ml) was inoculated into a liquid Postgate media with different pH, i.e., pH 2, 4, and 6. Each media was previously filled with 5 grams of acid ex-coal mine soils. The cultures were incubated in a 10 ml screw-capped tube filled to the brim. Each SRB isolate was used to treat with a consortium of all bacterial treatments. Experiments were carried out in a completely randomized design with three replications. The measurements of pH and sulfate were done at the beginning of treatment and after twenty days of treatment. The control group was treated with Postgate B medium enriched with 2 N sulfuric acid solution as much as 5% (v/v) without SRB inoculation. The efficiency of each treatment was calculated using the formula stated by Widyati (2006), namely:

<u>(Initial sulfate concentration) - (final sulfate concentration) x 100%</u>
(Initial concentration)

RESULTS AND DISCUSSION

SRB activity test results on the liquid Postgate media can be seen in Table 1. Table 1 shows that the treatment not inoculated with SRB sulfate concentrations only slightly decreased while the sulfate in the treatment inoculated with SRB sulfate concentrations varied on the 20th day after incubation. When it was calculated with the formula of efficiency of Widyati (2006, 2007), the lowest efficiency value was obtained at sp.1 and sp.3 with the amount of 74%, while the highest efficiency value was obtained at sp.5 with 91%, but on the controls which were not inoculated with SRB, the efficiency only decreased by 1% within 20 days. In this study, the sulfate concentration decreased because the SRB can use sulfate as an electron acceptor for metabolic activities (Doshi 2006). Because the sulfur accepts electrons, it will change to sulfide so that its concentration in the culture decreases.

SRB activity test was also conducted on samples of coal mine acid soil to measure the content of sulfate in the soil using ex-coal mine soil with varying pH, namely pH 2, 4, and 6. The measurement results on sulfate content changes on ex-coal mine land by SRB activity can be seen in Table 2.

Data in Table 2 show that sulfate reduction occurred in all treatments. The efficiency test on sulfate reduction is done by SRB activity in reducing ex-coal mine sulfuric acid soil after 20 days of treatment. It results that it is more significant at pH 4 and 6 than at pH 2. The sulfate reduction efficiencies ranging from pH 2, pH 4, pH 6 are respectively 89%, 91%, and 91%. This shows that sulfate reduction is most significant at pH 4 and 6. Table 1 also shows that the controls remain sulfate reduction occurred when the control contained only sterile media and acid soil, coal mines without getting additional isolates SRB. This indicates that in acid soil samples, there is also the content of indigenous sulfate-reducing bacteria in those samples. It was alleged that the sulfate-reducing bacteria are more efficient to reduce sulfate at pH 2 than at pH 4 and pH 6, so the efficiency of the control sulfate reduction treatment at pH 2 is 92%, which is higher than at pH 4 (87%) and pH 6 (91%).

Table 1. The concentration of sulfate at the beginning and the end of the test was done by the activity of sulfate to reduce bacteria in a liquid medium Postgate

Treatment	Initial sulfate concentration in the medium (mg/L)	Final sulfate concentration (mg/L)	Reduction of sulfate (mg/L)	Efficiency (%)
Control	128,668	127,405	1,263	1%
Sp.1 (*Desulfococcus sp.*)	127,835	33,450	94,385	74%
Sp.2 (*Desulfotomaculum sp.*)	128,630	31,479	97,151	76%
Sp.3 (*Desulfobacter sp.*)	126,878	32,704	94,174	74%
Sp.4 (*Desulfobulbus sp.*)	129,365	32,944	96,421	75%
Sp.5 (*Desulfobacterium sp.*)	128,043	12,023	116,020	91%
Sp.6 (*Desulfotomaculum sp.*)	127,772	14,092	113,680	89%
Consortium	129,507	32,129	97,377	75%

Table 2. Initial to final sulfate concentration on sulfate reducing bacteria activity test against coal mine acid soil with pH medium using different liquid Postgate medium

pH	Treatment	Initial sulfate concentration in the medium (mg/L)	Final sulfate concentration (mg/L)	Reduction of sulfate (mg/L)	Efficiency (%)
2	Control	202,437	15,561	186,876	92
	Sp.1 (*Desulfococcus sp.*)	220,080	24,090	195,990	89
	Sp.2 (*Desulfotomaculum sp.*)	212,917	23,106	189,810	89
	Sp.3 (*Desulfobacter sp.*)	234,273	23,726	210,547	90
	Sp.4 (*Desulfobulbus sp.*)	216,287	21,489	194,798	90
	Sp.5 (*Desulfobacterium sp.*)	215,313	29,115	186,199	86
	Sp.6 (*Desulfotomaculum sp.*)	222,023	28,318	193,705	87
	Consortium	210,480	20,580	189,900	90
					89
4	Control	196,563	24,627	171,936	87
	Sp.1 (*Desulfococcus sp.*)	224,930	19,381	205,549	91
	Sp.2 (*Desulfotomaculum sp.*)	207,207	17,841	189,366	91
	Sp.3 (*Desulfobacter sp.*)	212,080	18,229	193,851	91
	Sp.4 (*Desulfobulbus sp.*)	205,117	15,867	189,250	92
	Sp.5 (*Desulfobacterium sp.*)	219,733	16,313	203,421	93
	Sp.6 (*Desulfotomaculum sp.*)	249,217	22,382	226,834	91
	Consortium	215,213	23,404	191,809	89
					91
6	Control	213,857	19,319	194,538	91
	Sp.1 (*Desulfococcus sp.*)	206,230	21,570	184,660	90
	Sp.2 (*Desulfotomaculum sp.*)	248,897	22,241	226,656	91
	Sp.3 (*Desulfobacter sp.*)	189,877	19,147	170,730	90
	Sp.4 (*Desulfobulbus sp.*)	207,483	15,999	191,484	92
	Sp.5 (*Desulfobacterium sp.*)	189,453	16,898	172,555	91
	Sp.6 (*Desulfotomaculum sp.*)	202,990	15,891	187,099	92
	Consortium	200,970	15,153	185,817	92
					91

In making sulfate reduction, SRB uses sulfate as an energy source, i.e., as an electron acceptor, and uses organic materials as a carbon source (C). The carbon acts as a donor, the electrons in the metabolism, and the cell's building blocks (Groudev et al. 2001). Djurle (2004) adds that the SRB uses electron donor H_2 and the C source (CO_2), obtained from organic materials. The reaction of sulfate reduction by SRB, according to Van Houten et al. (1994, 1995), is as follows:

$$SO_4^{2-} + H_2 + 2H+ \rightarrow H_2S + 4H_2O$$

In this research, the initial pH medium used is 2, 4, and 6; after being treated with the increase in the pH, it ranges 5-6. This shows that the SRB effectively increases the pH in each treatment. This occurrence may be because sulfuric acid is so strong that adding this compound into the environment will influence acidity (pH). The more the reduction of sulfate, the higher the PH. Increasing pH for SRB activity reducing the sulfate will have a double impact, i.e., to produce H_2S and bicarbonate ion (HCO). H_2S results from reducing sulfate (sulfate decreases, the pH will increase), and bicarbonate acts to increase the pH

(Frank 2000). Based on the normality test to find out whether a value is a significantly different comparator or equal to the average value of each treatment, if the sample average sig <0.05, there were no significant differences in the samples with comparative values, and the data could not be received. The normality test showed abnormal results, so a non-parametric data analysis was used. The Kruskal-Wallis test analyzed data to analyze the sulfate reduction using a single factor, namely pH or SRB course. The test results showed that the sulfate reduction in pH or SRB treatment alone is not significantly different because sig is > 0.05, and the data is rejected. Then, the data analysis was done by Friedman test to determine the effect of treatment in two factors: pH and SRB. The results of the Friedman test showed that the reduction of sulfate to the treatment of two factors, namely pH and SRB, was significantly different because sig was <0.05.

In conclusion, after doing this research, we concluded that six isolates of sulfate reducing bacteria isolated from ex-coal mine sediment pond in Samarinda, namely sp.1 (*Desulfococcus* sp.), sp.2 (*Desulfotomaculum* sp.), sp.3 (*Desulfobacter* sp.), sp.4 (*Desulfobulbus* sp.), sp.5 (*Desulfobacterium* sp.) and sp.6 (*Desulfotomaculum* sp.) have potential as sulfate reducing agent in ex-coal mine acid soil (TMT) with efficiency sulfate reduction ranging from pH 2, pH 4, pH 6 respectively 89%, 91%, and 91%. This indicates that the sulfate reduction is highest in the medium with pH 4 and 6. It is also noted that sp.5 (*Desulfobacterium* sp.) on medium with pH 4 has the best ability of sulfate reduction efficiency compared to other SRB isolates at 93%. These results align with the Postgate activity test on sulfate reduction in liquid media, showing that treatment with sp.5 (*Desulfobacterium* sp.) gives the best results, i.e., 91% compared with other treatments.

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