Bioremediation of Mine Waste Using Sulphate Reducing Bacteria againts Rice Plant Bioindicator

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***Abstract*—The effects of mine waste toxicity are very low acidity, high sulfuric ion and heavy metal contents. The aimed at studying and knowing the ability of SRB isolates in reducing sulfate ions, increasing pH and deposition of heavy metals in overburden and the ability of SRB in reducing the effects of mining waste toxicity on bioindicators. Compiled based on a complete randomized design of two factors. The first factor of SRB inoculum type, isolate K 06, K18, and K 28 and without SRB inoculum. The second factor: gamal, rice straw, blotong and manure. Parameters observed were pH, sulfate content, heavy metal content of Ni in overburden waste, water and bioindicators. The results reveal that the treatment of SRB K18 and gamal have the best effect on the growth of rice crops those are 63.25 cm high, 24.17 pieces of leaves, number of tiller 6.08 and dry weight of plant 21.48 g. While treatment without inoculum and blotong gave the lowest growth in plants. The decline of Ni content on overburden by SRB K18 and type of the lowest blotong is 1810.69 ppm. The lowest sulfate content of soil is 43.22 ppm treated with SRB K 28 with manure.**

***Index Terms*—overburden, heavy metals, sulfate reducing bacteria, organic matter, plant rice**

I. INTRODUCTION

Waste generated from the mining business will cause environmental problems including air, water and soil environment. The impacts are not only occurring at the mine site but also are often far out of the mine site itself. Wastes from these mines may be slurry or tailings and overburden. Mining often causes exposure to minerals containing sulfur such as pyrite, pyrolyte, chalkoprite, arsenopyrite, and cobaltite. The oxidation of these minerals will produce sulfuric acid and will be accelerated by the presence of Thiobacillus ferrooxidans. Decrease in pH will cause the solubility of existing minerals increases. Water drainage from tailings can contain 4200 mg sulfate per liter and 1860 mg Fe per liter. High sulfate content and heavy metal solubility are the main limiting factors for plants grown on soil that have low or acidic pH [1].

Sulfate reducing bacteria can reduce sulphate ions to sulphides, so that these bacteria can be utilized to overcome mining waste. In the process of reduction of sulfate ions, other than produced hydrogen sulfide (H_2S), it also released hydroxyl ions (OH^-). The more sulfate ions are reduced, the more hydroxyl ions are produced, thus the pH increases [2]. The resulting sulphide will react with such dissolved metals to form a sulfide metal that precipitates then its toxicity re duced. [3].

The resulting sulphide will react with dissolved metals to form a sulfide metal that precipitates, resulting in reduced toxicity.

Sulfate reducing bacteria grown in anoxic sediments or base parts of marine sediments may have properties and characteristics that are different from sulphate reducing bacteria that grow in normal environments [4]. Thus, these bacteria have the potential to be utilized in dealing with unfavorable conditions in mine wastes, either for the management of waste relating to plants or to the life of other organisms, i.e. by reducing the solubility of sulfate ions, hydrogen ions and heavy metal ions.

II. MATERIALS AND RESEARCH METHODS

This research was conducted at Soil Laboratory and Greenhouse Faculty of Agriculture University Muslim Indonesia. The study was conducted from March to August 2015.

A. *Propagation of Sulfate Reducing Bacteria*

Isolate SRB collection of Soil Laboratory of UMI grown on liquid medium that is media of Postgate B [3]. The media composition per liter is 3.5 g of sodium lactate, $MgSO_4.7H_2O$ 2.0 g, NH_4Cl 0.2 g, KH_2PO_4 0.5 g, $CaSO_4$ 0.2 g, $FeSO_4.7H_2O$ 0.5 g, yeast extract 1, 0 g, ascorbic acid 0.1 g, 0.1 N NaOH and 0.1 N HCl to determine the pH of the media. Liquid media that have been made first autoclaved for 20 minutes at a temperature of 121^{o}C, pressure 1 atm. The screw tube contains a liquid medium, inoculated with sulphate reducing bacteria. After that it was incubated in a temperature incubator 35^{o}C. When the black is formed the sulphate reducing bacteria has grown and as an indicator of sulfate reduction in the media.

B. *Bioremediation of Mining Waste with Bioindicator of Rice Plant*

This study was prepared based on a complete randomized factorial design. Consists of two factors,

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namely the type of sulphate reducing inoculum bacteria and the type of organic material. The first factor of the SRB inoculum type consisted of isolates K 06 (I_1), K18 (I_2), K 28 (I_3), and without the provision of SRB inoculum (control) (I_0). The second factor of organic material consists of organic gliricidia (B_1), rice straw (B_2), sugar mill waste (blotong) (B_3) and manure (B_4). The combination of these two factors obtained sixteen treatments. Each treatment was repeated three times to obtain 48 units of experiment. Rice seeds are sown in soil media and given urea, SP-36 and KCl fertilizers. The dosage of each fertilizer is 150 kg urea / ha, 100 kg SP-36 / ha, and 100 kg KCl / ha. Rice seedlings are ready to be removed after 20 days in the nursery. Rice seeds that grow healthy and homogeneous growth are planted in research pots. The post nickel mine samples taken from the Soroako nickel mine were first dried and cleaned from the remains of plants, rocks and gravel, then sieved with a 2 mm diameter hole. Furthermore, soil samples are put into pots as much as 5 kg / pot. Organic materials are given according to the treatment with a dose of 10 tons / ha or equivalent to 25 grams per pot. Mix evenly between the organic material and the soil, then flood the water as high as 10-15 cm. After flooding, the treatment of SRB isolate was applied to the isolation of K 06 (I_1), K18 (I_2), K 28 (I_3), and without the inoculum of SRB (control) (I_0). In stagnant conditions, the pot is incubated for one week. The planting of rice seeds is done after one week incubation, ie as many as 4 plants per pot. Basic fertilizer is given at planting time of 200 kg urea / ha, 150 kg KCl / ha and 150 kg SP-36 / ha. Maintenance is done on rice plants, including weeds that grow and keep the water inundation. Observation parameters include: dry weight of rice plants, Ni uptake of rice plants, Ni content in soil and water, soil sulfate and soil pH. Analysis of variance according to a complete randomized factorial design used to analyze the effect of treatment on observation parameters. To know the difference between the tested treatment was done the least significant difference (LSD) test the level of 5% [5].

III. Result and Discussion

A. Result

The observation of dry weight of rice plants showed that the highest treatment of I_2 which was 13.85 g was significantly different with no BPS (I_0) and was not significantly different from I_1 and I_2 (Table I).

The result of observation of parameter of heavy metal content of Ni, sulphate ion and pH showed that sulfate reducing bacteria treatment had significant effect on heavy metal Ni content of rice plant, overland pH but no significant effect on Ni content in the inundation water. The treatment of various types of organic matter significantly affected the heavy metal content of Ni over-burden soils, heavy water content of puddle water, and soil sulfate ion content over burden, but no significant effect on Ni content in rice crops and treated over burdened soil pH. While the interaction of both have a significant effect on heavy metal content of Ni land over burden, sulfate ion content of soil over burden, and soil pH over burden, but no significant effect on the parameter of heavy metal Ni content of rice plants and heavy water content of water inundation.

The results of Ni content analysis of rice plants in Table II showed that the highest B3I0 treatment of 477, 09 ppm was significantly different with B_2I_1, B_2I_2 and B_2I_3. And the lowest is B_2I_2 treatment with Ni content of 51,43ppm. At the SRB treatment showed that the treatment of I_2 of Ni content was lowest at 84, 93 ppm and the highest at Io treatment was 339, 69 ppm Ni.

TABLE I. Average Dry Weight of Paddy Plants Aged 42 Days

Type of Organic Material	Treatment of SRB				Average
	I_0	I_1	I_2	I_3	
	Dry Weight Rice crop (g)				
B_1	7.87	16.28	17.57	14.21	13.98_y
B_2	6.89	13.12	21.48	14.02	13.88_y
B_3	6.12	11.24	9.05	11.84	9.56_x
B_4	6,44	7.47	7.28	6.55	6.94_x
Average	6.83^a	12.03^{ab}	13.85^b	11.66^{ab}	

Information: The numbers followed by the same letter (a, b) on the line and (x, y) in the same column are not significantly different based on the 5% LSD test.

TABLE II. Average Ni Content in Rice Plant Bioindicators after Bioremediation Treatment

Type of Organic Material	Treatment of SRB				Average
	I_0	I_1	I_2	I_3	
	Ni content of Rice Crop Plants (ppm)				
B_1	287.11^a_x	213.69^a_x	110.12^a_x	$55,271^a_x$	166.55
B_2	237.19^a_x	151.32^a_x	51.43^a_x	98.04^a_x	134.50
B_3	477.09^a_x	58.78^a_y	55.76^a_y	156.07^a_{xy}	186.92
B_4	357.38^a_x	203.57^a_x	122.42^a_x	247.10^a_x	232.62
Average	339.69	156.84	84.93	139.12	

Information: The numbers followed by the same letter (a, b) on the line and (x, y) in the same column are not significantly different based on the 5% LSD test.

Table II shows the results of Ni soil content analysis. The result of LSD test of 5% showed that the highest B_4I_0 treatment was 3666,46 ppm and the lowest was B_3I_2 treatment with Ni 1810,69 ppm. At the SRB treatment showed that the treatment of the lowest Ni content of 2330.57 ppm was not different from I_1 and I_3 but significantly different from the Io treatment of 3314.54 ppm Ni.

The result of LSD test of 5% level in Table III shows that the Ni content in the inundation water for the highest B_4I_3 treatment was 1.41 ppm and the lowest was B_1I_1 treatment with Ni content of 1.21 ppm. In the treatment of the type of organic material showed that the treatment of B_1 of the lowest Ni content of 1.22 ppm was not different with B2 and B3 but was significantly different with the treatment of B_4 which was 1.37 ppm Ni highest.

TABLE III. AVERAGE NI CONTENT IN POST-MINING SOILS AFTER BIOREMEDIATION TREATMENT

Type of Organic Material	Treatment of SRB				Average
	I_0	I_1	I_2	I_3	
	Ni Content of soil (ppm)				
B_1	3649.62	2600.26	2373.98	2657.80	2820.41
B_2	3197.97	2538.68	2483.27	2509.57	2682.37
B_3	2744.11	2022.11	1810.69	2219.30	2199.05
B_4	3666.46	3216.18	2654.33	2608.34	3036.33
Average	3314.54^{a}	2594.30^{ab}	2330.57^{b}	2498.75^{ab}	

TABLE IV. THE AVERAGE CONTENT OF NI IN PUDDLE WATER AFTER BIOREMEDIATION TREATMENT

Type of Organic Material	Treatment of SRB				Average
	I_0	I_1	I_2	I_3	
	Ni content of puddle water (ppm)				
B_1	1.22	1.21	1.22	1.24	1.22_{y}
B_2	1.28	1.28	1.27	1.30	1.28_{xy}
B_3	1.30	1.31	1.33	1.33	1.32_{xy}
B_4	1.35	1.35	1.38	1.41	1.37_{x}
Average	1.29	1.29	1.30	1.32	

Information: The numbers followed by the same letter (a, b) on the line and (x, y) in the same column are not significantly different based on the 5% LSD test.

The result of LSD test of 5% level in Table IV shows that the Ni content in puddle water for the highest B_4I_3 treatment was 1.41 ppm and the lowest was B_1I_1 treatment with Ni content of 1.21 ppm. In the treatment of the type of organic material showed that the treatment of B_1 of the lowest Ni content of 1.22 ppm was not different from B_2 and B_3 but was significantly different with the treatment of B_4 which was 1.37 ppm Ni highest.

TABLE V. AVERAGE SULFATE ION LEVELS IN POST-MINE SOILS AFTER BIOREMEDIATION TREATMENT

Type of Organic Material	Treatment of SRB				Average
	I_0	I_1	I_2	I_3	
	Soil Sulphate Content (ppm)				
B_1	47.67^{d}_{w}	46.04^{c}_{x}	46.71^{c}_{y}	45.00^{c}_{z}	46.36
B_2	48.34^{c}_{w}	44.26^{d}_{x}	45.60^{d}_{y}	43.22^{d}_{z}	45.36
B_3	50.27^{b}_{w}	49.16^{b}_{x}	47.90^{b}_{y}	46.12^{b}_{z}	48.36
B_4	62.82^{a}_{w}	54.73^{a}_{x}	59.11^{a}_{y}	51.02^{a}_{z}	56.92
Average	52.28	48.55	49.83	46.34	

Information: The numbers followed by the same letter (a, b) on the line and (x, y) in the same column are not significantly different based on the 5% LSD test.

Data of sulfate content in soil after treatment application in Table V showed that the highest B_4I_0 treatment of sulfate content of 62.82 ppm was significantly different from B_4I_1, B_4I_2 and B_4I_3. And the lowest sulfate content is B_2I_3 treatment that is 43, 22 ppm. The SRB treatment showed that Io treatment had the highest sulfate content of 52, 28 ppm and the lowest at I_3 treatment was 46, 34 ppm.

The mean pH measurements after the treatment application are listed in Table VI. The 5% LSD test results showed that the highest treatment of B_1I_1, B_2I_2, B_2I_2, B_4I_1, B_4I_3 was significantly different from the other treatments. The lowest average soil pH is the treatment of B_2Io, B_3Io, and B_4Io is 5.00. At the SRB treatment showed that the treatment of I_2 and I_3 had the highest pH of 5.75 and the lowest at Io treatment was 5.21.

TABLE VI. AVERAGE SOIL PH AFTER BIOREMEDIATION TREATMENT

Type of Organic Material	Treatment of SRB				Average
	I_0	I_1	I_2	I_3	
	Soil pH after treatment application				
B_1	5.83^{a}_{xy}	6.00^{a}_{x}	5.67^{b}_{y}	5.67^{b}_{y}	5.79
B_2	5.00^{b}_{y}	5.17^{c}_{y}	6.00^{a}_{x}	6.00^{a}_{x}	5.54
B_3	5.00^{b}_{y}	5.67^{b}_{x}	5.67^{b}_{x}	5.33^{c}_{y}	5.42
B_4	5.00^{b}_{z}	6.00^{a}_{x}	5.67^{a}_{y}	6.00^{a}_{x}	5.67
Average	5.21	5.71	5.75	5.75	

Information: The numbers followed by the same letter (a, b) on the line and (x, y) in the same column are not significantly different based on the 5% BNT test.

B. Discussion

The observation result of all parameters indicates that there is a difference between those given isolate SRB and without given SRB isolate. Rice growth showed better results in treatment given SRB isolate than without SRB isolate. Ni content of rice, soil, and water and soil pH showed higher values with treatment without SRB isolate compared with SRB, whereas soil sulfate content after treatment application showed that without SRB higher than SRB application. Provision of organic matter increases the activity of SRB in reducing sulfate, so that there appears to be an increase in all parameters measured. The difference between treatment given by SRB and without SRB is caused by SRB doing sulfate reduction using sulphate as energy source that is as electron acceptor and using organic material as carbon source (C). Carbon plays a role other than as an electron donor in metabolism is also a constituent of the cell [6]. Meanwhile, according to [7] SRB uses H_2 electron donor and source C (CO_2) which can be obtained from organic material [6].

Sulfate reduction may occur in stagnant or water-saturated soils. Waterlogging causes the soil to become anaerobic which is characterized by redox potential changes (Eh) to negative. The decrease of Eh indicates a change of environmental conditions from aerobic (positive) to anaerobic (negative) because oxygen that fills the soil pores is pushed and replaced by water. In anaerobic conditions organic matter will act as an electron donor [5]. When sulfate accepts electrons from organic matter it will undergo reduction to form sulphide compounds. The decrease of sulfate concentration in the treatment of BPS occurs because in anaerob condition the electron acceptor which in aerobic condition is done by free oxygen will be replaced by another molecule [8], such as nitrates and sulfates [6]. In this study acting as electron acceptor is sulfate which concentration is high enough on nickel mine and sulfate from organic material treatment [8]. The occurrence of sulfate reduction will lead to a decrease in sulfate concentration and increase soil pH. This happens because of several interrelated processes, namely due to inundation, addition of organic matter and BPS activities. In the flooding process as shown by the reaction is released hydroxyl ions which will bind H + ions. Besides, the increase of pH also occurs due to the giving of organic material. Organic materials have a buffering capacity that can increase or decrease the pH of the surrounding environment [6]. According [9] SRB treatment can reduce soil sulphate > 80% so as to increase the pH close to neutral. This suggests that the sulfate reduction reaction catalyzed by SRB is more efficient than chemical reduction due to saturation and addition of organic matter. However, the addition of organic material and saturation is still required because the sulfate reduction reaction by SRB to sulfide can be increased by adding moisture content and addition of soil organic matter [10]. The increase of soil pH resulted in better macro nutrient availability and reduced availability of micro nutrients, especially those classified as heavy metals.

The type of organic matter has a significant effect on the dry weight of the plant. This is due to the difference between the C / N ratio of the organic material. Organic materials with a higher C / N ratio give better effect than organic material with lower C / N ratio. SRB is unable to use complex organic materials [11].

IV. Conclusion

The treatment of SRB inoculum K18 and Gamal gave the best effect to the growth of rice plants, i.e. dry weight of plant 21.48 g. While treatment without inoculum and blotong gave the lowest growth in rice plants. The decrease of Ni metal content in over burdened soil by treatment of SRB K18 and type of the lowest blotong organic material is 1810.69 ppm. The lowest soil sulfate content was 43.22 ppm treated with SRB K 28 with organic manure.

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