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Use of sulfate-reducing bacteria and different organic fertilizer for bioremediation of ex-nickel mining soils

Key words: organic matter, pH, sulfate, nickel

Introduction

Indonesia plays a very important role in the world coal and mineral industry. In 2019, Indonesia was ranked first as a nickel exporting country with a total share of around 37%. While mining and mineral extraction contributed significantly to the development and national economies, they also caused serious impact on environmental degradation. Mineral extraction and its resultant need for the disposal of wastes, slurry, and water can result in a negative impact on the environmental (Jain, Cui & Domen, 2016). The increasing number of businesses in the mining sector led to the problem of post-mining critical lands. Mining often causes exposure to sulfur-containing minerals such as pyrite, pyrotite, chalkopyrite, arsenopyrite, and cobaltite. Ex-mining land often exhibits a low pH character. Decreasing pH will increase the solubility of minerals in soil and water. The high content of sulfates and the solubility of heavy metals in the soil are the main limiting factors for plants grown on soils that exhibit low or acidic pH (Sánchez-Andrea, Sanz, Bymans & Stams, 2014; Pistelli et al., 2017).

One of the nickel mining areas in Indonesia is located in the Sorowako area, South Sulawesi. The nickel mining area in this area is approximately 118,300 ha; the area that has been managed only reached 10,000 ha. Nickel reserves amounted to 107.7 million t of ore, with an average grade of 1.83% Ni. With a production capacity of 200 million t of nickel per year, these reserves will be mined for 18.9 years. The high concentration of heavy metal (nickel) in the ex-mining area can cause high levels of poisoning, which can endanger all aspects of life. Another study reported that in the coastal waters of the nickel mining location in Pomala, Kolaka Regency, Southeast Sulawesi, heavy metal pollution occurs (Fe, Cr, Ni, Pb, and Zn), which exceeded the threshold for assimilation capacity waters (Syahrir, Yaqin, Landu & Tambaru, 2019).

Industrial development has led to generation of large volumes of wastewater containing heavy metals, which need to be removed before the wastewater is released into the environment. This is of great environmental concern as some heavy metals are highly toxic. Chemical and electrochemical methods are traditionally applied to treat this type of wastewater. These conventional methods have several shortcomings, such as secondary pollution and cost (Xu & Chen, 2020). Efforts to improve the quality of ex-mining soil can be carried out by using a bioremediation process using micro-organisms. Within a number of possibilities, biological treatment applying SRB is an attractive option to treat acid mine drainage and to recover metals. The SRB may reduce heavy metal in soil and water such as sulphate, cadmium copper, nickel, ferro, plumbum, zinc, and arsenic (Kiran, Pakshirajan & Das, 2016; Kiran, Pakshirajan & Das, 2017; Serrano & Eduardo, 2017; Zhang, He, Zhao, Kou & Huang, 2020). One type of microorganism that is commonly used is SRB (Li et al., 2018). These bacteria can be used for treating mining wastewaters and recovering metals in several bioreactor configurations (Papirio, Villa-Gomez, Esposito, Pirozzi & Lens, 2013). In the process of reducing the sulfate ion, in addition to producing hydrogen sulfide (H_2S), a hydroxyl ion (OH^-) is also released. The more sulfate ions that are reduced, the more hydroxyl ions are produced, so that the pH increases. The resulting sulfides will react with dissolved metals to form metal sulfides which precipitate, so that their toxicity is reduced. SRB that grow in anoxic sediments or the bottom of the marine sediment, in this case the pond bottom soil, may exhibit different characteristics and characteristics from SRB that grow in normal environments. Thus, these bacteria exhibit the potential to be utilized in overcoming unfavorable conditions in ex-mining soil, be it for the management of ex-mining soil in relation to plants or in relation to other organisms, namely by reducing the solubility of sulfate ions, hydrogen ions, and metal ions (Gavrilescu, 2004).

The application of SRB plays an important role in reducing pollutant content in the environment characterized by changes in pH and C-organic. The SRB is able to neutralize the acidity of water bodies close to neutral (pH 6–7) and reduce the content of dissolved heavy metals in the waters. Other studies showed that this option is effective for the precipitation of the dissolved metals (copper and iron), for the reduction and removal of sulfates, and even for the alkalizing of the waters. The SRB's ability to remove up to 9,000 ppm of sulfate ion efficiently, to grow in the presence of up to 100 ppm of copper and 30 ppm of iron, and alkalize the medium makes it a potential bioremediation agent (Garciá, Moreno, Ballaster, Blázquez & González, 2001). The SRB facilitate the conversion of sulfate to sulfide with the sulfides reacting with heavy metals to precipitate toxic metals as metal sulfide. These metal sulfides are stable and can easily be removed from acid mine tailings waste (Cohen, 2006). This process facilitates the removal of toxic metals from tailing waste by SRB. Research conducted in ex-coal mining showed that SRB was able to reduce 84.25% sulfate content in 20 days. In consequence, the soil pH was increased from 4.15 to 6.66 during the process. However, no research exists on the application of the SRB carried out by the former nickel mining land. The purpose of this study was to examine the ability of SRB in several organic matters to reduce sulfate and nickel ions, and to increase pH of soil from nickel in mining areas.

Material and methods

This study used the bacteria consortium collection of the Soil Laboratory of the Faculty of Agriculture, Universitas Muslim Indonesia. Those were previously isolated from two cultivating pond of milkfish in the Kuri area of Maros Regency, South Sulawesi, Indonesia. The soil samples were collected from ex-mining areas of the Vale Indonesia Enterprise in Sorowako, South Sulawesi, Indonesia. Those were mixed with organic fertilizer, generated from sugarcane sludge, cow manure and Quickstick leaves. These three types of fertilizers were selected because of their high nutrient content for the soil remediation process (Juradi, Tando & Saida, 2020). Other materials used are chemicals used in the propagation of SRB isolates, analysis of sulfate levels, and heavy metal analysis of nickel.

Propagation of sulfate-reducing bacteria

The SRB isolates were cultured on liquid media, namely Postgate B media. The composition of the per one-liter media is sodium lactate 3.5 g, $MgSO_4 \cdot 7H_2O$ 2.0 g, NH_4Cl 0.2 g, KH_2PO_4 0.5 g, $CaSO_4$ 0.2 g, $FeSO_4 \cdot 7H_2O$ 0.5 g, yeast extract 1, 0 g, 0.1 g ascorbic acid, 0.1N NaOH, and 0.1N HCl to determine the pH of the media. The screw tube contains a liquid medium, inoculated with SRB. The media were incubated in an incubator at 35°C. The multiplication of the SRB isolates was successful when the media forming a black color. This black color is an indicator of sulfate reduction in the media.

Preparation of planting media

Examining soil samples obtained were air-dried and then cleaned of plant debris, stones, and gravel; then, they were sieved with a sieve hole diameter of 2 mm. After that, the soil sample was autoclaved at a temperature of 121°C, 1 atm pressure for 20 min. Furthermore, the 5 kg soil samples were put into a pot and mixed evenly with organic fertilizers. The doses of the organic fertilizer were 50 and 100 g per pot. The mixtures were stagnated with the water as high as 10 cm. Then, the SRB isolate solution was poured in the treatment mixture.

Sulfate content analysis

Sulfate contents were measured on 0, 10, 20, and 30 days after treatment (DAT). The mixture of soil, organic fertilizer, and SRB were extracted and filtered. The 5 ml results of the filtering were taken and then put into a test tube. After that, 1 ml of tween and $BaCl_2$ was added. Next, it was homogenized by shaking and let stand for 15 min. After that, the sample was measured with a spectrophotometer at a wavelength of 494 nm. The absorbance measurement results of the sample were adjusted to the standard curve for sulfate. From the standard curve, the sulfate ion concentration contained in the sample is obtained. Each solution in the test tube was stirred slowly before measuring.

Analysis of nickel heavy metal content in the planting medium was carried out before application of sulfate-reducing bacteria and after application. Then, an average of 5 g of the sample was taken and stored in a tube with a capacity of 100 and 20 ml of 1M HCl was added and shaken until blended. Furthermore, the samples were stored at room temperature for 24 h.

Samples were filtered using Whatman filter paper 42. The filtered sample is taken as much as 5 ml and then put into a 50 ml volumetric tube, and then 45 ml of distilled water was added. Next, 2 ml was taken and dissolved in 18 ml 0.1M HCl in a 20 ml volumetric tube. Then, the sample was analyzed to determine the concentration of heavy metal Ni, using a Perkin Elmer Analyst 300 Atomic Absorption Spectrometer. The nickel concentration was measured merely on the 30 DAT.

Experiment design and data analysis

This research was arranged based on block design. It consists of three factors, treatment of SRB inoculum, the type, and dose of organic matter. The first factor of the SRB inoculum consists of no SRB inoculum (control) and the SRB inoculum (treatment). The two factors of organic matter were sugarcane sludge, manure, and Quickstick leaves, each with doses of 50 and 100 g. The combination of these three factors obtained 12 treatments. Each treatment was repeated three times. By adopting a general linear model repeated measures analysis of variance (ANOVA), we tested the sulfate concentration and pH level differences among the treatments over time, with SRB treatment, fertilizer type, and fertilizer doses as between-subject factors and measurement time as a within-subject factor and with three replicates. Meanwhile, the concentration of nickel was analyzed by multivariate ANOVA. The tests were performed using SPSS® version 25 software (SPSS Inc. Chicago, IL, US), and the results of the F-statistic test were considered significant when $p < 0.05$.

Results and discussion

This study indicated that the effect of SRB treatment was significant on the decrease in the concentration of sulfate ($F = 438.3$, $p < 0.001$), nickel ($F = 1026.6$, $p < 0.001$), and change in pH level ($F = 4.7$, $p < 0.05$). The effect of fertilizer type was also significant for the three dependent variables. The fertilizer dose resulted in a significant effect on reducing sulfate (F = 38.7, p < 0.001) and nickel (F = 32.1, p < 0.001) but not a significant change in the pH level (p > 0.05). Sulfate concentrations and pH differed significantly between test times, indicating a change over time. The interaction between factors did not significantly affect the dependent variables, except of interaction between SRB treatment and fertilizer dose for nickel remediation (Table 1).

TABLE 1. Summary of F-statistic followed by probability of the effect of time, the SRB inundation, fertilizer type, and fertilizer dose

Parameter	Sulfate	pH	Nickel
Time (t)	438.3***	58.6***	not performed
SRB treatment (b)	102.8***	4.7*	1 026.6***
Fertilizer type (f)	44.4***	38.7***	3.8*
Fertilizer doses (c)	38.7***	4.1^{ns}	32.1***
Interaction (b × f)	1.3^{ns}	0.2^{ns}	84.2***
Interaction (b × c)	1.2^{ns}	0.4^{ns}	3.6^{ns}
Interaction (f × c)	1.7^{ns}	1.6^{ns}	0.0^{ns}
Interaction (b × f × c)	2.7^{ns}	0.9^{ns}	0.7^{ns}

Note: ns = not significant; *p < 0.05; ***p < 0.001; data of sulfate and pH means were analyzed by repeated measures ANOVA; while nickel was measured by multivariate ANOVA

In general, soils treated with organic matter and SRB contained lower levels of sulfate than those treated with organic matter without BPS. The effect of SRB treatment was significant in reducing sulfate levels in all organic fertilizer treatments. Among the three types of organic fertilizers used, manure exhibited a more effective reduction rate. At 10 DAT, the sulfate concentration decreased from 2,530 ppm to 1,443 ppm in treatment of SRB and manure with dose of 50 g and 1,363 ppm with that of 100 g. At the end of the observation (30 DAT), those were decreased to 1,217 ppm in the treatment of SRB and manure with dose of 50 g and 1,167 ppm with that of 100 g. Meanwhile, the lowest effect of the SRB treatment was found in that with sugarcane sludge fertilizer application. At 10 DAT, the sulfate concentration decreased from 2,530 ppm to 1,553 ppm in treatment of SRB and manure with dose of 50 g and 1,510 ppm with that of 100 g. At the end of the observation (30 DAT), those were decreased to 1,273 ppm in treatment of SRB and manure with either 50 or 100 g doses (Fig. 1).

The results of the observations indicated that the pH concentration of ex-mining soil increased from the initial pH of 5.52. The increase in pH occurred in all treatments, both those treat with SRB and control. Ex-mining soil pH increased toward neutral condition. In addition, at 10 DAT, the average pH in all treatments exceeds 7 and then decreases toward an average approaching 7. Among the three types of organic fertilizers used, Quick-stick exhibits the more effective reduction rate. At 10 DAT, pH increased in SRB treatment to 7.06 at a concentration of 50 g and 7.01 at a concentration of 50 g. At the end of the observation (30 DAT), the pH became 6.67 at a concentration of 50 g and 6.82 at a concentration of 50 g. Meanwhile, the organic fertilizer application that generates the lowest pH is sugarcane sludge. At 10 DAT, pH in

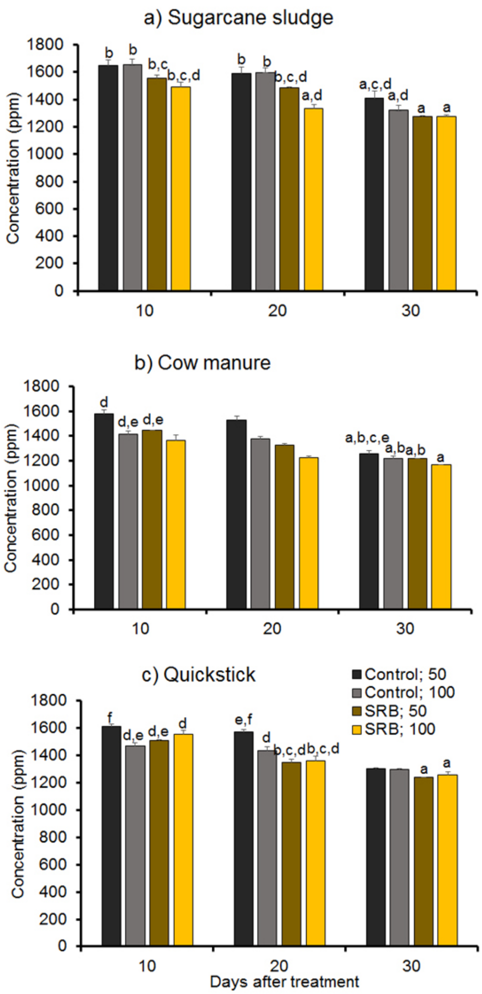

FIGURE 1. Changes in sulfate concentration in ex-nickel mining soil treated with SRB with the addition of sugarcane sludge (a), cow manure (b) and Quickstick fertilizers (c) from 10 DAT to 30 DAT. Error bars followed by different alphabet mean that the averages are significantly different

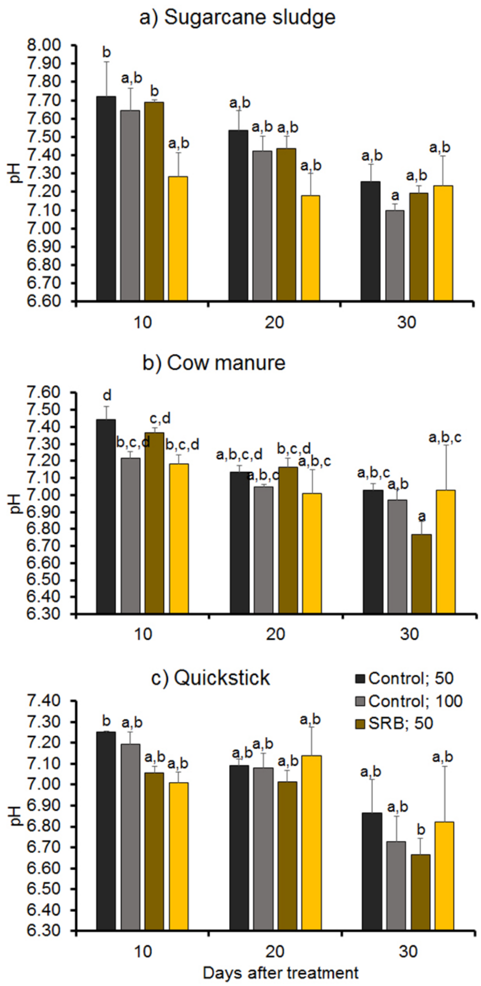

FIGURE 2. Changes in pH level in ex-nickel mining soil treated with SRB with the addition of sugarcane sludge (a), cow manure (b) and Quickstick fertilizers (c) from 10 to 30 DAT. Error bars followed by different alphabet mean that the averages are significantly different

creased in SRB treatment by 7.69 at a concentration of 50 g and 7.28 at a concentration of 50 g. At the end of the observation (30 DAT), the pH became 7.19 at a concentration of 50 g and 7.23 at a concentration of 50 g (Fig. 2).

Treatment with SRB exhibits a very significant effect on nickel concentration. The initial nickel concentration of 4,720 ppm decreased to an average of below 3,000 ppm in the treatment of all types of fertilizers added by SRB. The best decrease occurred in the treatment of SRB with manure fertilizer. The nickel concentration decreased from origin concentration to 1,950 ppm in the treatment of SRB and manure with dose of 50 g and 1,690 ppm with that of 100 g. The lowest reduction was found in the treatment of SRB with Quickstick fertilizer. The nickel concentration decreased from the origin concentration to 2,560 ppm in treatment of SRB and manure with dose of 50 g and 2,370 ppm with that of 100 g (Fig. 3).

The ex-mining soil is characterized by its high acidity and lower fertility. Degradation of chemical properties such as acid soil and high nickel content are the factors that limit the level of soil fertility as a planting medium. The addition of fertilizers in the bioremediation process of ex-mining soil plays an important role in increasing the input of organic matter. Organic matter increases the activity of microorganisms for nitrogen fixation and transfer of certain nutrients such as nitrogen, phosphor and sulphure. The role of organic matter on soil chemical properties is to increase cation exchange capacity so that it may affect nutrient uptake by plants. Cow manure"s ability to reduce sulfate and nickel may be due to the high levels of nitrogen and carbon in the material (Halifah, Soelistyono & Santoso, 2014). The availability of nitrogen and carbon is very supportive to increase the activity of microorganisms including SRB in reducing sulfate and nickel. However, in increasing the pH, the ability of sugarcane sludge fertilizer was better than the other two fertilizers. This is probably because sugarcane sludge has high levels of phosphorus and calcium, so it has a more neutral pH. In addition, the process of decomposition of organic matter in soils made of high organic matter, such as with cow manure, is always found in acidic soils with low pH, this is due to the decomposition process of organic matter which in the process will expel and remove elements of calcium from the soil (Palupi, 2015).

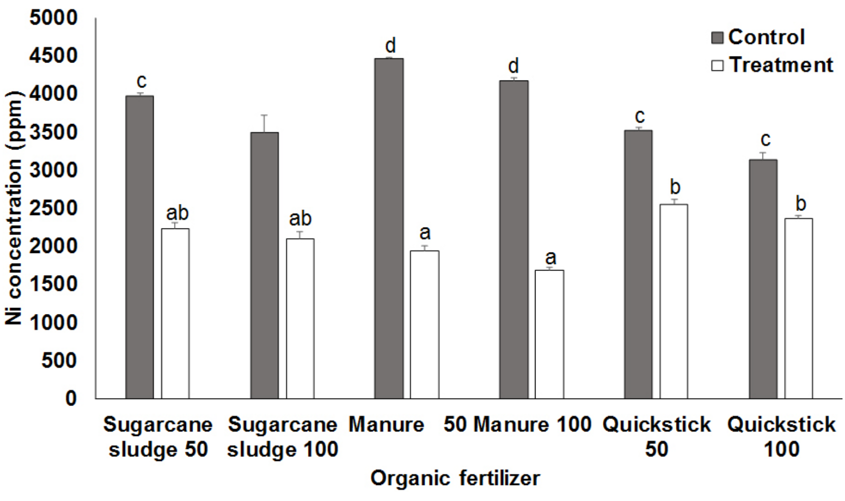

FIGURE 3. Effect of fertilizer application and the addition of SRB on nickel concentration. Error bars followed by different alphabet mean that the averages are significantly different

According to Ansari and Malik (2010), the high heavy metal content in soil like zinc, copper, nickel, lead, chromium, and cadmium, low pH, and low nutrients are factors that limit plant growth. During extreme conditions, mining activities destruct soil quality such as poor nutrients, toxic due to heavy metal, physical properties alteration. Therefore, to improve the quality, new technology is required such as bioreactor (Dikinya & Areola, 2010; Papirio et al., 2013), bed reactor (Liu, Liu, Zhou & He, 2017), or semi-continuous stirred tank reactors (Kieu, Müller & Horn, 2011).

Soil pH value

In this study, the initial pH of ex-mining soil was acidic; however, the level of acidity increased after being inundated with water and organic matter. Inundation with water caused the release of hydroxyl ions which bind H^+ ions. In addition, the increase in pH to neutral occurs because organic matter has a buffering capacity so that it can increase or decrease the pH of its environment. The activity of SRB greatly increases the effluent pH. Even at an influent pH of 3.0, 60.8% of sulfate, 41.3% of COD and 91.2% of heavy metals could be removed, and the effluent quality can meet the national discharge standard (Liu et al., 2017). In the natural environment, the pH of the soil has an enormous influence on soil biogeochemical processes. Soil pH is, therefore, described as the "master soil variable" that influences myriads of soil biological, chemical, and physical properties and processes that affect plant growth and biomass yield (Neina, 2019).

An increase in pH may occur due to the presence and activity of SRB. Therefore, SRB is often used as bioremediation of ex-acid mining land because it exhibits the ability to increase soil pH (Winch, Mills, Kostka, Fortin & Lean, 2009). The results of research by (Sandrawati, Suryatmana, Putra & Kamaluddin, 2019) showed an increase in pH after inundation, provision of organic matter and SRB. The initial pH, which ranges from 3.6–5.2, increased to 6.6–8.1. SRB utilize organic material as a source of electron donors in reducing sulfate to sulfide and produce bicarbonate so that the soil pH increased from 4.15 to 6.66 during the process (Widyati, 2007). Bioremediation was rapid once the initial pH increased to > 4.5, as SRB's are known to perform better in more neutral environments (Koschorreck, 2008).

Sulfate concentration

The sulfate content in the ex-mining area is reduced after 30 days of inundation. Decreasing sulfate content occurred in relation with increasing soil pH levels. Next, the sulfate reduction process produced metal sulfide deposits and increased the alkalinity. The increase in pH occurred because SRB used sulfate as an electron acceptor and carbon of organic matter as an electron donor by producing hydrogen sulfide. Meanwhile, SRB uses an electron donor H_2 and a C (CO_2) source, which can be obtained from organic materials. Hydrogen sulfide immediately binds with metal to form metal sulfide, which is insoluble so that metal availability decreases (Widyati, 2007). The type of organic material affects the rate of sulfate reduction because each organic material exhibits a different amount of carbon (Sandrawati et al., 2019). The 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 (Cao et al., 2020).

The sulfate content of the soil soaked with organic matter without giving bacteria showed higher sulfate levels (0.141–0.122) than the organic matter added with SRB (0.127–0.117). This is in accordance with previous study whom stated that the addition of SRB can increase the rate of reduction of sulfate to hydrogen sulfide (Sandrawati et al., 2019). The more the amount of SRB in the soil, the lower the sulfate concentration, even reducing heavy metals such as Fe in the soil (Reyes et al., 2017). The problems faced by ex-nickel mining areas are the acidic soil pH concentration, high Ni content, and low phosphate availability. If ex-mining land is developed for agricultural activities, it becomes a limiting factor and possibly an obstacle in the production process. Furthermore, plants that live in ex-mining areas are deficient in nutrients such as K, Ca, Fe, Cu, and Mn. In addition, nickel mining soils are formed from basic or ultra-alkaline igneous rock base materials which contain heavy metals that reach toxic levels in plants, including Ni and Cr. Meanwhile, Pb and Cd metals are in relatively safe concentrations (Singh, Upadhyay, Pathak & Gupta, 2011). The nickel content of ex-mining soil, which was given organic matter and SRB accompanied by water inundation for 30 days, showed a lower Ni content than the application of organic matter to the ex-mining soil without being given bacteria. Providing organic materials such as compost and manure is an alternative solution for the supporting of life in the soil to reduce nickel levels in the soil. The nickel content was provided organic matter and soaked for 30 days and was still high, namely 3.14–4.47%, while the nickel content treated with organic matter and the addition of SRB ranged from 1.69–2.56%. Land with a nickel content between 3–5% are unable to be used as a medium for growing plants. The level of nickel content in ex-mining land became lower after treatment with SBR and manure. This occurred because it was suspected that the amount of C-organic from manure was very high so the sulfate reduction process carried out by SRB was more optimal. In addition, the rate of nickel reduction carried out by sulfate bacteria occurs due to water immersing. When inundation is carried out, O_2 deficiency occurs so that the SRB population increases thousands of times in approximately two weeks.

Conclusions

The results of this study indicate that the application of fertilizer and the addition of SRB exhibits an effect on reducing levels of sulfate and nickel. Among the three types of organic fertilizers, manure was effective to reduce of sulfate and nickel concentrations, while Quickstick fertilizer was the more effective to stabilize pH level. Fertilizer doses exhibited a significant effect on decreasing sulfate and nickel concentrations, but it exhibited no significant effect on stabilizing pH levels. Thus, the application of manure fertilizer and the addition of SRB is recommended for bioremediation of sulfate and nickel from ex-mining soil.

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Summary

Use of sulfate-reducing bacteria and different organic fertilizer for bioremediation of ex-nickel mining soils. The microbiological activity associated with ex-mining soil remediation can be considered useful to accelerate the contaminant degradation. The use of sulfate-reducing bacteria (SRB) and organic matter exhibits potential in improving ex-nickel mining soil quality. The purpose of this study was to examine the ability of SRB in several organic fertilizers to reduce sulfate and nickel ions, and to increase pH of soil from nickel in mining areas. This study used the bacteria collection of the Soil Laboratory of the Faculty of Agriculture, Universitas Muslim Indonesia. Those were previously isolated from two cultivating pond of milkfish in the Kuri area of Maros Regency, South Sulawesi, Indonesia. The soil samples were collected from ex-mining areas of the Vale Indonesia Enterprise in Soroako, South Sulawesi, Indonesia. Those were mixed with organic fertilizers, generated from sugarcane sludge, manure, and Quickstick (*Gliricidia sepium*) leaves, each with 50 and 100 g doses. The 5 kg soil samples were put into a pot and mixed evenly with organic fertilizers. A general linear model (GLM) repeated measures analysis of variance (ANOVA) was adopted to analyze the data. The results of this study indicate that the application of SRB and fertilizer was effective in reducing concentration of sulfate and nickel. Among the three types of organic fertilizers, manure was effective in reducing sulfate and nickel concentrations, while Quickstick fertilizer was the more effective in stabilizing pH level. Fertilizer doses exhibited a significant effect on decreasing sulfate and nickel concentrations, but it exhibited no significant effect on stabilizing pH levels. At 10 days after treatment (DAT), the sulfate concentration decreased from 2,530 ppm to 1,443 ppm in treatment of SRB and manure with dose of 50 g and 1,363 ppm with that of 100 g. At the end of the observation (30 DAT), those were decreased to 1,217 ppm in treatment of SRB and manure with doses of 50 g and 1,167 ppm with that of 100 g. Among the three types of organic fertilizers used, Quickstick demonstrates the more effective reduction rate. At 10 DAT, pH increased in SRB treatment by 7.06 at a concentration of 50 g and 7.01 at a concentration of 50 g. At the end of the observation (30 DAT), the pH became 6.67 at a concentration of 50 g and 6.82 at a concentration of 50 g. The nickel concentration decreased from an origin concentration to 1,950 ppm in treatment of SRB and manure with doses of 50 g and 1,690 ppm with that of 100 g. Thus, the application of manure fertilizer and the addition of SRB is recommended for bioremediation of sulfate and nickel from ex-mining soil.

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