Estuary Sediment Treatment for Reducing Sulfate in Acid Mine Water

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ABSTRACT

Acid mine water causes environmental problems due to its high acidity caused by its high sulfate content which can disturb the life of organisms. This problem can be resolved by utilizing sulfate-reducing bacteria (SRB) abundantly found in sediments. The purpose of this research is to study the use of estuary sediments as a source of SRB inoculums to reduce sulfate in acid mine water. The bioremediation treatment of acid mine water is carried out in a column bioreactor, with treatment T1 comprising of sediment and compost, and then comparing it to treatments T2 of sediment, T3 of compost, and T4 as the control treatment of only acid mine water. Results show that treatment T1 was able to reduce sulfate concentrations by 78%, compared to T2 by 56%, T3 by 21% and T4 by 5%. The reduction of sulfate was followed by increases in pH where T1 reached a pH value of 7.1, compared to treatments T2 and T3 which had pH values less than 5.5, whereas treatment T4 had a pH of 2.2. The reduced sulfate and increased pH was also followed by an increase of SRB growth, especially in T1. Estuary sediments as a source of SRB inoculums can be used in the bioremediation of acid mine water by adding compost to maximize the process of sulfate reduction and pH increase.

1. INTRODUCTION

The mining industry in Indonesia has undergone rapid development due to it being one of the main industries in the national and regional economies (Widyati, 2007; Abfertiawan et al., 2016). However, problems have also emerged along with the increase of mining activities, especially in aquatic environments. These include types of mining waste such as mine water, overburden, residues from the mining process, tailings, residual ore and sludge (Gaikwad et al., 2011; Simate and Ndlovu, 2014).

The Lamuru coal mine is one of the mines operating in Indonesia. There have been studies conducted on the coal produced from this mine. Microscopic petrographic analysis results have shown that the coal contains 11.86% sulphur which may be categorized under super high sulphur (Widodo et al., 2016). Additional X-ray diffraction (XRD) analysis of the coal has also found a type of framboidal pyrite of very fine crystals (Widodo et al., 2019). This type of spirit is very sensitive and rapidly reacts with air and is categorized as PAF (Potentially Acid Forming) that will trigger the formation of acid mine water, causing environmental problems at mining locations (Dai et al., 2008). This is also the concern of the Lamuru Mine, Bone Regency, South Sulawesi, Indonesia and therefore must be prevented.

Acid mine water is harmful due to its low pH value of around 1.5-3.5 and presence of a number of toxic heavy metals such as Hg, Cd, Pb, Fe, Al, U and Mn. The type of metal depends on the type of mine (Meier et al., 2012; Simate and Ndlovu, 2014; Fahruddin et al., 2018). Acid mine water is formed through the oxidation of sulfur with oxygen, water or carbon dioxide in the form of sulfate ions forming into sulfuric acid. The high sulfuric acid content causes acid mine water to have a low pH that triggers the formation of metal ions - reactive metals (Matshusa-Masithi et al., 2009; Burgos et al., 2012).

Acid mine water will disrupt water biota if it enters a water source and will also disrupt life on land if it penetrates the soil, particularly vegetation

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(Afriyie-Debrah et al., 2010). Moreover, it can also dissolve heavy metals that can cause pollution in aquatic environments which can be indirectly harmful to humans (Saviour, 2012; Hedrich and Johnson, 2012). Acid mine water is difficult to treat if it has entered a waterway. The acidic environment triggers the growth of *Thiobacillus ferroxidans* bacteria that will catalyze the pyrite oxidation process (Mahmoud et al., 2005; Patel, 2010). Therefore, acid mine water needs to be well managed so that it is not harmful if it enters water environments.

To date, acid mine water has been treated through the use of chemical compounds by adding lime treatments to it; another method is the physical method of storing the acid mine water in a large hole and then tightly covering it; however these two methods have proven to be very inefficient, non-eco-friendly and very costly (Johnson and Hallberg, 2005).

Bioremediation is a good and environmentally friendly alternative for acid mine water waste treatment by utilizing microbes to reduce the sulfate in acid mine water (Luptakova and Kusnierova, 2005). Nowadays, bioremediation has continued to develop in its usage to treat acid mine water in the mining industry (Costa and Duarte, 2005). The microbes utilized in the bioremediation of acid mine water are the group of sulfate-reducing bacteria. In reducing sulfate, this type of bacteria produces hydrogen sulfide (H_2S) and hydroxyl ions (OH^-), thus an increase of pH occurs (Pester et al., 2012), then sulphide reacts with metal cations and forming metal sulfides that have a role as an electron donor and reduce metal cation into metal sulphide in acid mine water (Fahruddin et al., 2018).

Sulfate-reducing bacteria are abundantly found in muddy substrate such as wetland sediments. This is the reason why the sediments can be directly applied to the acid mine water treatment bioreactor without having to perform a bacteria isolate culture in the laboratory (Whitehead and Prior, 2005). It is not necessary to inoculate microbe culture and add nutrients because wetland sediments naturally contain an abundant number and many types of sulfate-reducing bacteria (Pester et al., 2012; Fahruddin et al., 2017). Therefore, research has been conducted regarding the application of wetland sediment as a source of sulfate-reducing bacterial inoculums in reducing sulfate in acid mine water.

Previous research results by Fahruddin and Abdullah (2015) have shown that the application of wetland sediment from mangroves and swamps on acid mine water is able to increase the pH of acid mine water, decrease sulfate levels and increase the growth of sulfate-reducing bacteria, which can be used to treat environmental pollution caused by acid mine water. In several studies, it was found that many types of groups of sulfate-reducing bacteria exist in estuary sediments, the distribution and amount of sulfate-reducing bacteria varies at each location depending on the organic content of sediment (Compeau and Bartha, 1985; Purdy et al., 2002; Colin et al., 2017). Based on these studies, estuary sediment was the wetland sediment chosen as the bacterial inoculum source to reduce sulfate in the acid mine water treatment for application in the bioreactor.

2. METHODOLOGY

2.1 Sampling

The acid mine water sample were obtained from the Lamuru Mine located at Masserengpulu Subdistrict, Bone Regency (Figure 1). The estuary sediment was gathered from the Tallo estuary, Makassar (Figure 2), and the compost was farmyard manure obtained from commercial plant sellers in Panaikang, Makassar. The characteristics of this compost was a decomposed mixture of dung and urine of farm animals and urine along with litter and leftover organic material from domestic waste.

2.2 Acid mine water and sediment characterization

The characterization of estuary sediments involves the measurement of total organic carbon using the TOC method, total nitrogen using the Micro Kjehdahl method, as well as total phosphorous with the Stannous Chloride method (Greenberg et al., 1992). The characterization of acid mine water included measuring sulfate content using a spectrophotometer with a length wave of 420 nm, while pH was measured using a pH meter (Greenberg et al., 1992).

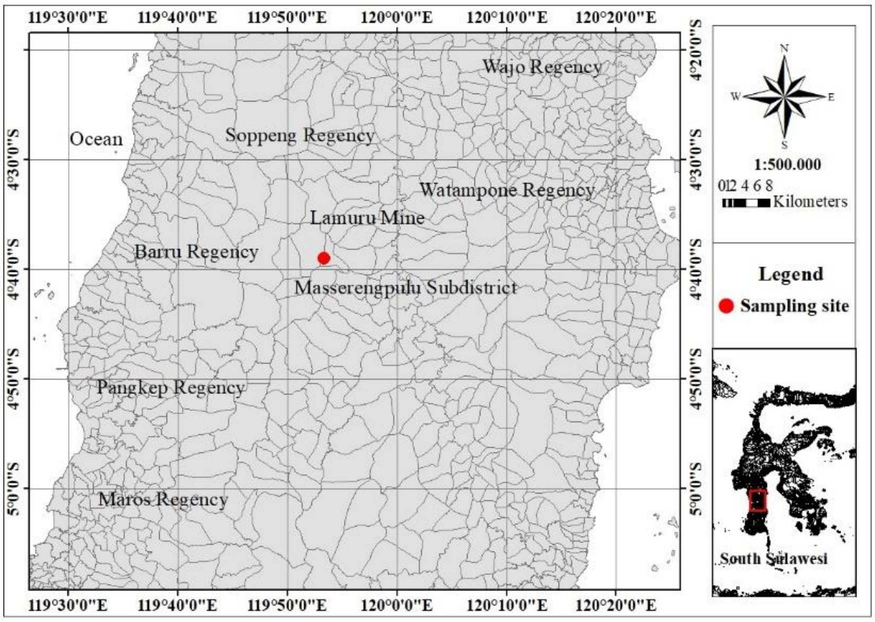

Figure 1. Map of the sampling site of acid mine water on the Lamuru Mine, Masserengpulu Subdistrict, Bone Regency, South Sulawesi, Indonesia.

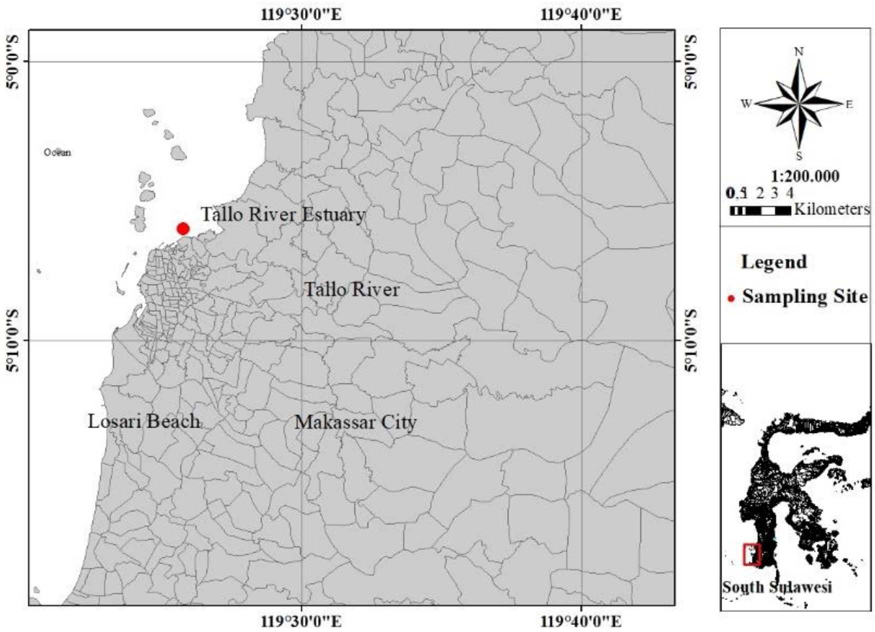

Figure 2. Map of the sampling site of the estuary sediment on the Tallo estuary, Makassar, South Sulawesi, Indonesia

2.3 Treatments and experiments

The treatments were made in a bioreactor column made from a modified container with a laboratory-scale anaerobic chamber with an inner diameter of 15 cm and length of 35 cm (Figure 3). The bioreactor column is filled with 800 mL of acid mine water along with 10% sediment and 5% compost, the best ratio based on previous research by Fahruddin and Abdullah (2015) in various sized volumes. In this study, the sediment is the source of sulfate-reducing bacteria, while the compost is rich with nutrients and becomes the simple carbon source for bacterial growth. All treatments were performed in duplicate and incubated for 30 days at room temperature (27 ^{o}C). The four treatments were as follows:

- Treatment T1 was 10% sediment and 5% compost
- Treatment T2 was 10% sediment
- Treatment T3 was 5% compost
- Treatment T4 was the control treatment without any added sediment and compost

The treatments were left alone for 30 days and sampled on days 0, 5, 10, 15, 20, 25 and 30 to observe sulfate concentration, change in pH and the amount of sulfate-reducing bacteria.

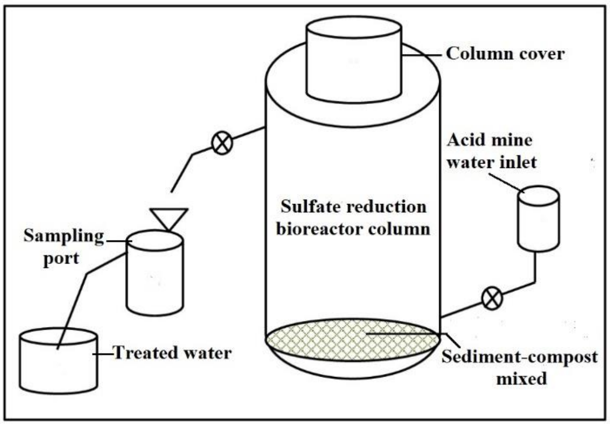

Figure 3. Schematic of the bioreactor for sulfate reduction

2.4 Measurement of sulfate concentration

The measurement of the sulfate concentration of the acid mine water treatments was performed by using a spectronic-20 spectrophotometer, where a sulfate concentration calibration curve was made beforehand. $BaCl_2$ crystals and buffer acid salt were added to the acid mine water samples to form a colloid suspension through the presence of turbidity, then absorbency was measured using a spectrophotometer with a wave length of 420 nm and the measurements recorded (Greenberg et al., 1992).

2.5 Measurement of pH

pH was measured using a pH meter that had been calibrated using a buffer of pH 4 and pH 7 with a stabilization time of 15 min. The electrodes were rinsed with distilled water and dried, and then dipped into the acid mine water treatment solutions (Greenberg et al., 1992).

2.6 Enumeration of the sulfate-reduction bacteria

The suspensions from the acid mine water treatment samples were made into serial dilutions by taking 1 mL from each sample, inoculating it in a reaction tube filled with 9 mL of sterile physiological salt solution (0.85%), and then homogenized using a vortex mixer. Then, 1 mL of suspension was placed into a petri dish containing Postgate medium and incubated at 37 °C for 48 h. Sodium lactate was used as a carbon source in the anaerobic chamber. Sulfate-reduction bacteria growth is marked by the formation of a dark brown or blackish colony due to the formation of iron sulfides. The Post gate standard medium contained per L: KH_2PO_4 0.5 g; NH_4Cl 1.0 g; $CaSO_4 \cdot 2H_2O$ 1.26 g; $MgSO_4 \cdot 7H_2O$ 2.0 g; Sodium lactate 3.5; Yeast extract 1.0 g; Ascorbic acid 0.1; Thioglycolic acid 0.1; $FeSO_4 \cdot 7H_2O$ 0.5 g (Atlas, 1993).

3. RESULTS AND DISCUSSION

3.1 The characterization of estuary sediment and acid mine water

The chemical characterization of estuary sediment and compost comprised of analyses of organic carbon, total phosphorus and total nitrogen; while the characterization of acid mine water comprised of sulfate concentration and pH analyses (Table 1). Results from X-ray Fluorescence (XRF) analysis of the mine water showed there was not much organic carbon, nitrogen and phosphorus, but had particularly higher concentrations of Fe, Mn, K, and sulfate. Therefore, mine water bioremediation required compost as a source of organic carbon. According to Hu et al. (2018), the availability of carbon sources is the critical limiting factor for sulfate-reducing bacteria reaction. In acid mine water, the carbon source is limited and requires additional or external carbon sources for successful treatment.

Table 1. Chemical characterization of estuary sediment, compost, and acid mine water

Estuary sediment	Value
Organic carbon	327,000 mg/L
Nitrogen	19,200 mg/L
Phosphor	8300 mg/L
Compost	Value
Organic carbon	386,000 mg/L
Nitrogen	32,300 mg/L
Phosphor	18,000 mg/L
Acid mine water	Value
Sulfate	1.18 mg/L
pH	3.2

Characterization of the sediment, compost and acid mine water was conducted to determine the initial condition of all acid mine water bioremediation treatment samples. The addition of 5% compost to the acid mine water bioremediation treatment was done due to the compost having a simple carbon content. The compost serves as nutrients for microbes for their growth and development during the reduction process in the acid mine water treatment. The donor electron is a molecular hydrogen sourced from organic compounds in compost and is required to enhance microbial activity for reduction of sulfate to hydrogen sulfide (Fukui and Takii, 1996; Zhao et al., 2010; Pester et al., 2012), while the reduction occurs by the presence of organic carbon as an electron donor (Sánchez-Andrea et al., 2014). The sulfur-reducing bacteria obtain energy through the reaction of organic compounds oxidation from compost to the reduction of sulfate or other sulfur compounds to sulphide (Matshusa-Masithi et al., 2009).

3.2 Sulfate concentration

The sulfate concentration measurement results of the acid mine water bioremediation treatments for treatment T1 containing estuary sediment and compost showed the treatment was able to reduce the sulfate concentration to 0.39 ppm on the 30th day, from an initial concentration of 0.92 ppm. Treatment T2 which contained sediment experienced a slight gradual decrease up to the 30th day with a sulfate concentration of 0.58, compared to the initial concentration of 1 ppm. Treatment T3 which only contained compost showed a small reduction in sulfate concentration on the 30th day at 0.67 ppm from an initial concentration of 0.92 ppm. Meanwhile, treatment T4, as the control, experienced the lowest reduction of sulfate concentration on the 30th day at 0.79 ppm compared to the initial concentration of 1.03 ppm (Figure 4).

The reduction of sulfate content in the treatments was caused by the activities of the sulfate-reducing bacteria from the estuary sediment. This caused a large reduction of sulfate concentration in treatment T1 and was also the case in T2 although not as large as the T1 treatment with compost. The reason for this is because the two treatments comprised of sediments act as an inoculating source of sulfate-reducing bacteria.

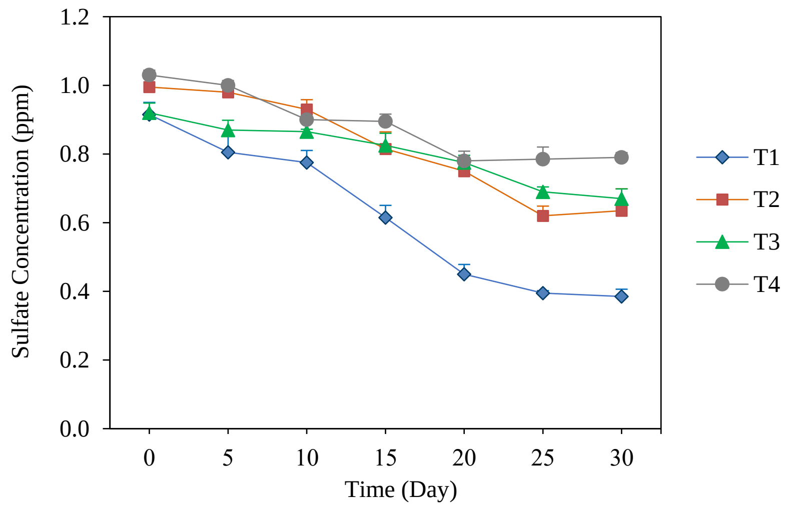

Figure 4. Sulfate concentration in acid mine water with treatments: 10% sediment and 5% compost (T1), 10% sediment (T2), 5% compost (T3) and without added sediment and compost (T4)

The estuary sediment is a wetland sediment with anaerobic environmental conditions that support the growth of anaerobic bacteria (Colin et al., 2017). Therefore, the sediment is rich in sulfate-reducing bacterial species (Fahruddin et al., 2018). However, the growth of these bacteria requires simple carbon and nutrients that can be obtained from compost that is rich with organic matter. The compost becomes the carbon sources for triggering sulfate-reducing bacteria to reduce sulfate producing sulfide and bicarbonate that affects the rise in pH (Dong et al., 2019). When only using sediment in the treatment, the sulfate-reducing bacteria do not get the energy of the oxidation of organic compounds as an electron donor to generate hydrogen sulfide under anaerobic conditions (Hu et al., 2018). Conversely, if only compost alone is present, there is no sulfate-reducing bacteria to reduce sulfate in acid mine water (Zhang and Wang, 2014).

Meanwhile, treatment T3 encountered a low reduction of sulfate-reducing bacteria from the compost. In the case of treatment T4 as the control, according to Fahruddin and Abdullah (2015), the lowest reduction of sulfate concentration was due to loss caused by the abiotic loss factor.

Wetland sediment contains a large amount of sulfate-reducing bacteria due to its high organic content that provides an ideal environment for its population (Fukui and Takii, 1996; Fichtel et al., 2012; Sánchez-Andrea et al., 2012). A simpler carbon source with a low molecule weight can be naturally found in the sediment which can act as an electron donor for the sulfate-reducing bacteria (Whitehead and Prior, 2005). This bacteria utilizes the sulfate contained in the acid mine water for its metabolic activities by transferring hydrogen to sulfate as an electron acceptor under anaerobic conditions using organic matter contained in sediments or compost as the electron source (Elliott et al., 1998). Therefore, sulfate is reduced to hydrogen sulfide, so the sulfate concentration will be reduced in the acid mine water. The molecular H_2S, formed from sulfate reduction, dissolves in acid mine water, as shown by the following reaction:

$$SO_4^{2-} + \text{organic matter} \xrightarrow[\text{anaerobic}]{\text{sulfate-reducing bacteria}} S^{2-} + H_2O + CO_2 \quad (1)$$

$$S^{2-} + 2H^+ \longrightarrow H_2S \quad (2)$$

The sulfate reduction process occurs during an anaerobic condition which is similar to respiration that uses oxygen as an electron acceptor on the aerobic condition, hence is called sulfate respiration or disimilatory sulfate reduction (Jansen et al., 1985; Bradley et al., 2011; Qian et al., 2018).

3.3 Change in pH values

The pH measurement results of the acid mine water bioremediation treatments are: Treatment T1 which contained estuary sediment and added compost showed a significant change in pH, from an initial value of pH 3.7 to pH 7.1 on the 30th day of observation. Treatment T2 which contained sediment showed a small change of pH value, from pH 3 to pH 5.4 on the 30th day of observation. Treatment T3 containing compost showed a very slight increase in pH, from pH 3.2 to pH 4.2 on the 30th day, while T4 as the control treatment did not see much of a change in pH value (Figure 5).

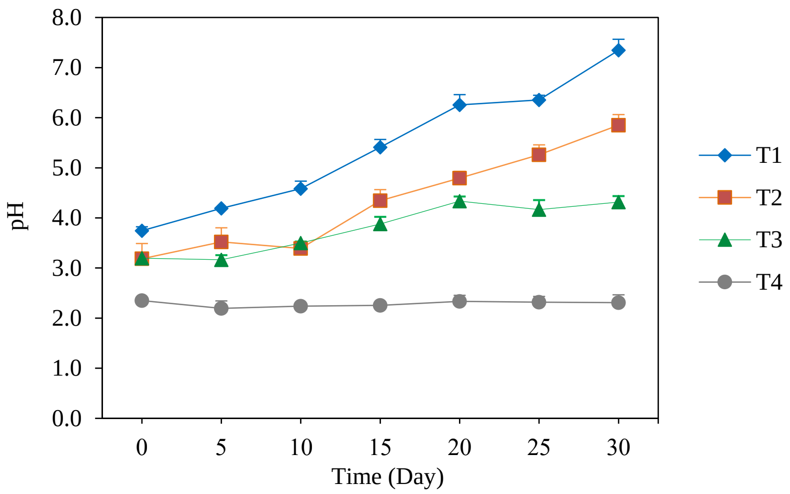

Figure 5. Changes of pH in acid mine water with treatments: 10% sediment and 5% compost (T1), 10% sediment (T2), 5% compost (T3) and without added sediment and compost (T4)

A change in pH is related to the decrease of sulfate concentration, which proves that the estuary sediment contains bacteria that are able to reduce sulfate into sulfide in acid mine water treatments (Luptakova and Kusnierova, 2005). This was the case for treatments T1 and T2; while there was not a significant change in pH for treatment T3 at only pH 4.2 and was similar to T4 because neither contained sediment as an inoculum source of sulfate-reducing bacteria (Whitehead and Prior, 2005).

The sulfate reduction process by the group of sulfate-reducing bacteria produces sulfide and bicarbonate that causes an increase in pH, the sulfide will react with the dissolved metal ions to create insoluble metal sulfides (Wu et al., 2017).

The reaction of sulfide minerals and water releases hydrogen ions that cause the pH value to decrease in acid mine water and is a conducive environment for the growth of *Thiobacillus ferooxidans* bacteria. This bacteria will accelerate the pyrite oxidation rate which will then form sulfuric acid. On the other hand, sulfate-reducing bacteria can increase pH or restore it to neutral pH through the reduction of sulfate into sulfide (H_2S) and releasing hydroxyl ions (OH^-) (Patel, 2010; Fahruddin and Abdullah, 2015).

3.4 Number of sulfate-reducing bacteria

The number of sulfate-reducing bacteria in the acid mine water bioremediation treatment was determined by the Standard Plate Count (SPC) method by using Postgate medium. The growth of blackish-brown colored bacterial colonies is the indicator of the presence of sulfate-reducing bacteria. The results showed that treatment T1 containing estuary sediment and added compost increased in the number of sulfate-reducing bacteria from initially 16×10^4 CFU/mL to 53×10^4 CFU/mL after 30 days. Treatment T2 containing sediment shows a reduction in the number of sulfate-reducing bacteria from 12×10^4 CFU/mL at the beginning to 11×10^4 CFU/mL on day 10, however their numbers increased to 53×10^4 CFU/mL on the 30th day. Treatment T3 which only comprised of compost showed a decrease in the number of sulfate-reducing bacteria until the 20th day, from initially 6×10^4 CFU/mL to 3×10^4 CFU/mL. However, their numbers slightly increased on the 30th day of observation to 8×10^4 CFU/mL. Meanwhile treatment T4 as the control shows the existence of sulfate-reducing bacteria in pure acid mine water which experienced a decrease from 5.7×10^4 CFU/mL at the beginning to 2×10^4 CFU/mL on the 30th day of observation (Figure 6).

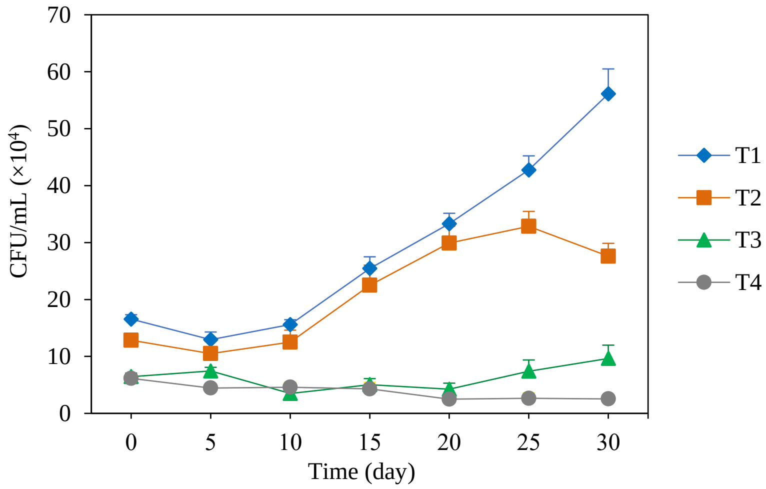

Figure 6. Number of sulfate-reducing bacteria in acid mine water with treatments: 10% sediment and 5% compost (T1), 10% sediment (T2), 5% compost (T3) and without added sediment and compost (T4).

There was a reduction in the number of sulfate-reducing bacteria up to the 10th day in all treatments; this was because some sulfate-reducing bacteria from the sediment and compost were unable to survive the very acidic conditions (Costa and Duarte, 2005; Whitehead and Prior, 2005; Pester et al., 2012). This is called the lag phase, where microbes in this condition will try to adapt to survive and those that do not will die. However, on the 15th day of treatments T1 and T2 saw a sharp increase in the number of sulfate-reducing bacteria up to the 30th day. This is called the log phase, where the bacterial cells that are able to survive low pH will increase in number (Kushkevych et al., 2017). On the other hand, the sulfate-reducing bacteria in treatment T3 saw almost no increase; this was also the case with treatment T4 as the control treatment where there was even a reduction in the number of cells. This is called the stationary phase and is followed by the death phase that is caused by an unsupportive environment, such as a very acidic environment, low pH, exhausted nutrients or toxic substances produced by the microbes themselves that can inhibit their growth (Meier et al., 2012; Kushkevych et al., 2017).

If we compare the treatments, we can see that the main source of the sulfate-reducing bacteria came from the sediment. There was more sulfate-reducing bacteria growth in treatments T1 and T2, whereas treatments T3 and T4 contained a very small number of sulfate-reducing bacteria. Hence, an increase in sulfate-inducing bacteria numbers can lower the concentration of sulfate, which follows an increase of pH value. Thus, estuary sediment as an inoculum source of sulfate-reducing bacteria is effective in lowering sulfate levels in acid mine water.

The results of this study were more successful in reducing sulfate of acid mine water using the estuary sediment as a source of sulfate-reducing bacteria inoculums with the addition of compost as source of nutrients, when compared to previous studies. Dong et al. (2019) recently reported that the removal percentages of sulfate in a column were

52.94%, and the effluent pH value was 6.56 with treatment of acid mine water by modified corncob fixed SRB sludge particles in the column test models. Yim et al. (2015) reported that passive treatment systems utilizing mushroom compost indicated a sulfate treatment efficiency of 63% within 120 days with a neutral pH value. Another monitoring study indicated a mushroom compost-based SAPS in Korea had sulfate removal efficiency of 7.8-20.3% (Cheong et al., 2010). Bai et al. (2013) reported that more than 61% of sulfate was removed and the effluent pH was improved from 2.75 to 6.20 during the operation. Furthermore, laboratory scale test with sawdust bioreactor gave removal efficiency of 50.27% on 35th day (Medírcio et al., 2007).

4. CONCLUSION

The use of sediment on the bioremediation of acid mine water has an effect on its sulfate content and pH value. Acid mine water treated with estuary sediments as a bacterial inoculum source has proven successful in reducing sulfate concentration and increasing pH value in acid mine water. This study utilized four types of treatments; the one with the best results was treatment T1 with added sediment and compost that was able to reduce sulfate by 78% as well as increasing pH to 7.1. In the case of treatment T2 with only added sediment, sulfate content decreased by 56% and pH was measured at 5.4, whereas treatment T3 with only added compost saw a reduction of 21% in sulfate content and a pH value of 4.2. It can be concluded that the application of estuary sediment on the treatment of acid mine water will be more effective in reducing sulfate if compost is also added. The sulfur-reducing bacteria obtain energy by oxidation of organic compounds from compost as an electron donor reacting with elemental sulfur in the acid mine water under anaerobic condition that utilizes sulfate as a terminal electron acceptor to generate hydrogen sulfide.

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