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Research Article

Treatment of compost as a source of organic material for bacterial consortium in the removal of sulfate and heavy metal lead (Pb) from acid mine drainage

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Abstract

Acid mine drainage can pollute the environment because it is acidic and contains toxic heavy metals. The purpose of this research was the application of a bacterial consortium to remove sulfate and reduce heavy metal lead (Pb) in acid mine drainage. The application was done in the bioreactor for acid mine drainage treatment that was treated with compost. Observations were made every five days and included observation of total bacterial growth using the Standard Plate Count (SPC) method, determination of sulfate content by gravimetry, determination of pH by use of pH meter, and determination of the concentration of heavy metal Pb using the AAS method. As a result, it was obtained that the treatment of non-sterile compost in acid mine drainage was able to reduce the initial heavy metal concentration of Pb of 84% and reduce the sulfate content by 72%, along with increasing pH and an increase in total bacterial growth. Meanwhile, sterile compost treatment was only able to reduce the Pb content by 63% and sulfate by 54%. This result indicates that the addition of compost is more effective than the treatment of sterile compost.

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Introduction

Indonesia's mining industry is developing at a very rapid because it is one of the mainstay sectors for the national and regional economy (Abfertiawan et al., 2016; Fahruddin et al., 2020). However, the increasing number of mining activities creates a problem, which is pollution by mining waste in the aquatic environment. Types of mining waste are mine water, the overburden, processing residual solutions, tailings, ore processing residue, and sludge (Luptakova and Kusnierova, 2005; Simate and Ndlovu, 2014).

One of the mining activities in Indonesia is the coal mining activity in Maserengpulu, Lamuru, Bone Regency (Figure 1), which has been taking place since 2008. This mining activity impacts the agricultural land of residents around the mining area due to the waste generated in the mining process, especially acid mine drainage (Widodo et al., 2016; Fahruddin et al., 2020). The characterization of acid mine drainage from the Lamuru Mining Site, Bone Regency found that the acid mine drainage contained Cd, Pb, Fe, Mn, Ti, and Nb, and 6.2 ppm sulfate with pH 3.7 (Putri et al., 2017; Fahruddin et al., 2020). Based on an environmental study in 2019 by the local Environmental Office, people began to complain about the impacts, such as the disruption of paddy fields. In addition, from the results of the study, it is known that acid mine drainage from the mining activity has not been processed. If this is left unchecked, the impact will be more severe and can result in public health problems and environmental damage (Hartaman et al., 2021). One of the biggest problems of the mining industry is the presence of liquid waste called acid mine drainage. Acid mine drainage is acidic and has a low pH of around 1.5-3.5 and contains a number of toxic heavy metals, such as Cd, Pb, Hg, Fe, Al, and Mn. Metal content in acid mine drainage depends on the type of mining (Fahruddin et al., 2018). Acid mine drainage is generated from the oxidation of sulfur, which is in the form of sulfate ions, with oxygen, water, or carbon dioxide to become sulfuric acid. The high sulfuric acid content stimulates the formation of reactive metal ions in acid mine drainage (Badley et al., 2011; Meier et al., 2012). If this acid mine drainage enters water bodies, it will disrupt aquatic biota's life, and if it seeps into the soil, it will disrupt the lives of many living organisms on land, particularly plants (Kushkevych et al., 2017). In addition, because acid mine drainage also dissolves heavy metals, it will cause heavy metal pollution in the aquatic environment, which can consequently be harmful to humans (Meier et al., 2012). Acid mine drainage is difficult to control if it gets into the water. The acid environment triggers the development of the bacteria *Thiobacillus ferrooxidans* that will catalyze the pyrite oxidation reaction (Patel, 2010; Fahruddin et al., 2018). Therefore, acid mine drainage needs to be handled so that it will not be A problem if it is discharged into the aquatic environment.

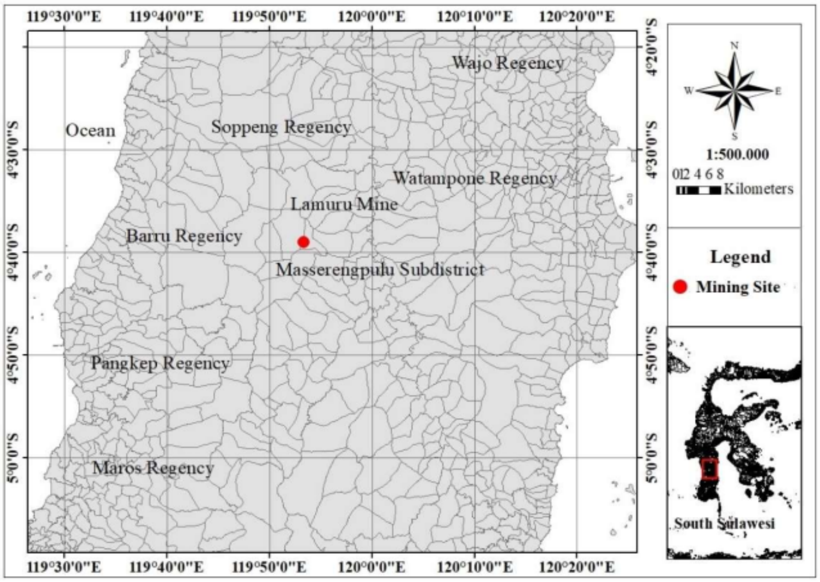

Figure 1. Map of the mining site on the Lamuru Mine, Masserengpulu Subdistrict, Bone Regency, South Sulawesi, Indonesia (Source: Fahruddin et al., 2020).

So far, acid mine drainage has been controlled by adding a chemical compound, namely, lime, to acid mine drainage. Another method is by immersing acid mine drainage in a large pit that is then covered tightly. However, both of these mechanisms are very inefficient, not environmentally friendly, and very expensive (Hard et al., 2004). Bioremediation is a biological method, which is a safe and effective alternative to treat acid wastewater by utilizing microorganisms to reduce sulfates contained in acid mine drainage (Widyati, 2007). The microorganisms that are used to reduce sulfates in acid mine drainage bioremediation are a group of sulfate-reducing bacteria. These bacteria, in doing sulfate reduction, produce hydrogen sulfide (H_2S) and hydroxyl ions (OH^-) and thus, stimulate an increase in pH (Meier et al., 2012; Wu et al., 2017). Sulfate-reducing bacteria mostly live in wetland substrates such as swamp sediments. This is the reason why sediment can be applied directly in the bioreactor for acid mine drainage treatment without having to culture bacterial isolates in the laboratory. Thus, there is no need to inoculate microbial cultures and add nutrients because naturally, there are a large number and types of sulfate-reducing bacteria that live in the wetland sediments (Zhao et al., 2010; Kushkevych et al., 2017; Fahruddin et al., 2020).

Several previous research results stated that the application of swamp sediment to acid mine drainage could change the pH, reduce sulfate content, and affect the growth of bacteria so they can be used to control pollution due to acid mine drainage (Fukui and Takii, 1996; Whitehead and Prior, 2005; Pester et al., 2012). Both sulfate reduction and heavy metal reduction activities by sulfate-reducing bacteria do not work effectively if there is no simpler carbon source that is easily digested by bacteria. On a laboratory scale, the carbon source given to sulfate-reducing bacteria is sodium lactate, which is beneficial for bacterial growth but is ineffective and expensive if used on a large scale. Therefore, compost is used as a good alternative that naturally acts as a simple carbon source for electron donors for sulfate-reducing bacteria (Zhang and Wang, 2014).

This is evidenced in research by Matshusa-Masithi et al. (2009), which discovered that the use of compost reduced sulfate by 38% with an average ratio of chemical oxygen demand and sulfate (COD/SO_4) of 0.56 mg/L in the reactor. This is also supported by research by Cheong et al. (2010), which found that the addition of compost in bioremediation of acid mine drainage in a bioreactor using sulfate-reducing bacteria was able to reduce the content of heavy metals such as Fe, Cu, and Mn using an average ratio between the effluent and influent of 0.12 and has succeeded in increasing the pH to 7 and reducing acidity by 1 mg/L. The addition of compost and sediment to the treatment of acid mine drainage was able to increase the pH to 6.3, from the initial pH of 3.0, while the treatment without the addition of compost only succeeded in increasing the pH to 3.4 (Fahruddin and Abdullah, 2015). Furthermore, treatment of acid mine drainage with the addition of compost was able to reduce sulfate content by 78% and reduce cadmium by 87% (Fahruddin et al., 2017). In another research, the addition of compost to acid mine drainage successfully increased the pH of acid waste from 2.5 to 6.2-6.7 with lead, zinc and cadmium reduction efficiency reaching 86-99% (Suyasa et al., 2019). Based on these findings, bioremediation of acid mine drainage with the addition of compost is a cost-effective method to remove sulfates and heavy metals before mining waste is discharged into the environment. Based on this explanation, the swamp sediment was selected as an inoculum source of a sulfate-reducing bacterial consortium to be applied to a bioreactor for acid mine wastewater treatment.

Materials and Methods

Sampling and media

Swamp sediment was obtained from the Nipah - Nipah Swamp, Makassar, and put into a plastic container. Acid mine drainage samples were taken from coal mining in Bone Regency and placed in sample bottles for analysis and treatment. Nutrient Agar (NA) was used to grow bacterial isolates with a composition of 5 g of peptone, 2 g of yeast extract, 5 g of sodium chloride, 15 g of agar, 1 g of beef extract, and 1000 mL of demineralized water. Tryptone Soy Broth (TSB) medium, with the composition of 17 g tryptone, 3 g soytone, 2.5 g dextrose, 5 g NaCl, 42.5 g K_2HPO, 15 g agar, and 1000 mL demineralized water, was used to grow bacterial consortium.

Characterization of acid mine drainage and swamp sediment

Swamp sediment characterization included measurement of total organic carbon by using the total organic carbon (TOC) analyzers, measurement of total nitrogen content using the micro-Kjeldahl method and measurement of total phosphorus content by the use of the gravimetric method. Characterization of acid mine drainage included measurement of sulfate content by titration method and pH measurement by using a pH meter. The results are shown in Table 1.

Table 1. Chemical characterization of swamp sediment, compost, and acid mine drainage.

Swamp sediments	**Value**
Organic carbon	291.000 mg/L
Nitrogen	12.520 mg/L
Phosphorus	1.330 mg/L
Compost	**Value**
Organic carbon	321.000 mg/L
Nitrogen	29.230 mg/L
Phosphorus	16.000 mg/L
Acid mine drainage	**Value**
Sulfate	2.70 mg/L
pH	3.52

Characterization of the sample was conducted to determine the initial conditions for the bioremediation treatment of acid mine drainage. The addition of 5% compost to acid mine drainage treatment was made because compost contains simple carbon compounds that bacteria require for growth and development during the acid mine drainage treatment reduction process (Pester et al., 2012; Fahruddin and Abdullah, 2015). Hydrogen from composted organic compounds is an electron donor in reducing sulfate to hydrogen sulfide (Costa and Duarte, 2005; Fahruddin and Abdullah, 2015).

Isolation of bacteria from swamp sediments

Swamp sediment was weighed as much as 10 g and then put in 90 mL of distilled water. Serial dilution was made, then 1 mL was taken, and the Nutrient Agar medium was inoculated by using the pouring method. After that, it was incubated for 24 hours at 37 ºC. The type of isolated bacteria was determined based on the morphological differences of the growing bacterial colonies.

Preparation of the bacterial consortium inoculum

One inoculating loop of each type of bacterial isolate was taken to be grown into Tryptone Soya Broth (TSB) medium and then incubated for 24 hours at room temperature. The cultures were centrifuged to obtain the biomass of the bacterial cell, which was then suspended with sterile distilled water. The absorbance value was measured at 25% T. After that, each cell suspension was mixed with the same ratio to be used as the inoculum of bacterial consortium.

Treatments

The treatments were made by adding the inoculum of bacteria consortium to the bioreactor for acid mine drainage (AMD) treatment. Compost, as a simple carbon source for bacteria, was added to the bioreactor (Fahruddin, 2020). The treatments were as follows:

- Treatment P1: AMD plus 10% sterile compost and 20% bacterial inoculum
- Treatment P2: AMD plus 10% non-sterile compost and 20% bacterial inoculum
- Treatment P3: AMD without the addition of both compost and bacterial inoculum as a control

The compost was sterilized by placing it in a plastic container, and then it was sterilized in an autoclave at 121℃ for 2 hours. After cooling, then it was added to the treatment. The treatments were left for 30 days at room temperature, and observations of bacterial growth, sulfate content, pH value, and Pb metal concentrations were conducted on days 5, 10, 15, 20, 25, and 30.

Measurement of sulfate content

A Spectrophotometer 20^{TM} was used to determine the sulfate concentration in the acid mine drainage treatment. A sulfate concentration calibration curve had already been created. $BaCl_2$ crystals and acidic buffer were added to the acid mine drainage sample to form a colloidal suspension, which was indicated by turbidity. Furthermore, the absorbance was measured using a spectrophotometer at a wavelength of 420 nm, and the numbers listed were recorded (Greenberg et al., 1992).

pH measurement

The pH value was calculated using a pH meter that had previously been calibrated at pH 4 using buffer and pH 7 with a 5-minute stabilization time. After being cleaned with distilled water and dried, the electrodes were immersed in the treatment solution. The pH value was as stated on the pH meter.

Analysis of the content of the heavy metal lead (Pb)

The concentration of heavy metal Pb in the sample was measured using the AAS (Atomic Absorption Spectrophotometry) method. Preparation was carried out by adding 65% HNO_3 to the erlenmeyer flask, which was then destructed at 120 ℃. Before measurement, a standard solution was made first, and the regression equation was obtained. The samples were measured at a wavelength of 228.8 nm.

Results and Discussion

Types of bacterial isolate and bacterial growth

From the isolation of bacteria from swamp sediments, five types of bacterial isolates were obtained based on differences in the morphological characteristics of the growing bacterial colonies, which were labeled R1, R2, R3, R4, and R5 isolates. The colony of each isolate showed different colour characteristics, namely, yellow, white, and milky white. The shapes of bacterial colonies were circular, entire, undulate, raised, and flat presented in Table 2.

Table 2. Morphological characteristic of bacterial isolates colonies.

Isolate	**Morphological characteristic of colonies**			
	Color	**Shape**	**Edge**	**Elevation**
R1	Yellow	Circular	Entire	Raised
R2	White	Circular	Undulate	Flat
R3	White	Circular	Entire	Raised
R4	Yellow	Circular	Entire	Flat
R5	Milk white	Circular	Entire	Raised

Observation of bacterial growth in P1 treatment (i.e. acid mine drainage treated with sterile compost) showed that on day 0, the number of bacteria was 5.4 x 10^5 CFU/mL and continued to increase until day 20, but then decreased to 4.3 x 10^5 CFU/mL on day 30. In the P2 treatment (i.e. acid mine drainage treated with non-sterile compost), the observation on day 0 found that the number of bacteria was 6.7 x 10^5 CFU/mL and continued to increase until the 20th day of observation, but decreased on the 25th day and on the 30th day. P3 treatment (i.e. acid mine drainage without the addition of both compost and bacterial inoculum) as a control did not show bacterial growth (Figure 2). From day 0 to day 5, the growth of bacteria was low because the bacteria were still in the lag phase or the adaptation period of bacteria to environmental conditions in order to survive. Bacterial cells that are unable to survive in acidic conditions will die (Fahruddin et al., 2019). The growth of bacterial cells increased until the 20th day, indicating an exponential phase in which the bacteria were able to adapt by utilizing nutrition in the form of organic matter contained in compost. Compost acts as a source of organic material needed by bacteria to grow and develop, such as carbon (C), nitrogen (N), and phosphorus (P) (Matshusa-Masithi et al., 2009). The decrease in the number of bacteria on the 25th to the 30th day showed that the bacterial population in the bioreactor was experiencing a death phase due to the nutrients in the bioreactor starting to run out and the bacteria producing secondary products that can be toxic to the bacteria themselves (Costa and Duarte, 2005; Fahruddin et al., 2021).

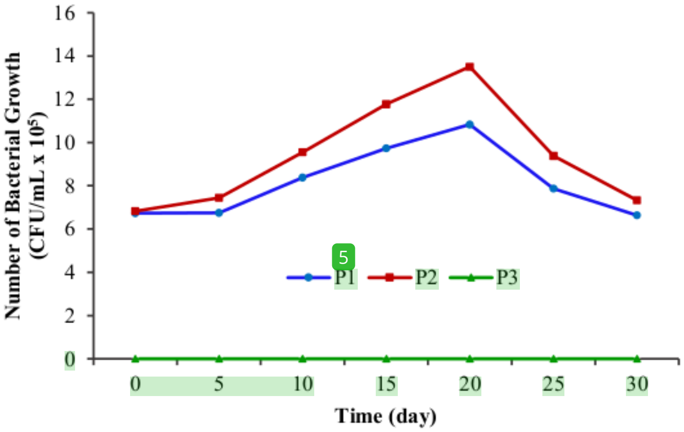

Figure 2. The number of bacterial growth in each treatment of acid mine drainage with treatment: P1: acid mine drainage treated with sterile compost, P2: acid mine drainage treated with non-sterile compost and P3: acid mine drainage without the addition of compost.

Sulfate content

The measurement results of sulfate concentration in the treatments of acid mine drainage showed that in the P1 treatment, the sulfate concentration decreased to 0.42 ppm on the 30th day from the initial concentration of 0.92 ppm (54%). Meanwhile, in the P2 treatment, the sulfate content decreased slowly until the 30th day, with a concentration of 0.28 ppm from the initial concentration of 1 ppm (72%). P3 treatment as control showed a decrease in the sulfate concentration in the least amount on the 30th day, which was 0.79 ppm from the initial sulfate concentration of 1.03 ppm (23.30%). The decrease in sulfate content in the treatment was caused by the presence of active sulfate-reducing bacteria originating from swamp sediment, while in the control treatment, there was a smaller decrease in sulfate levels, which according to Fahruddin et al. (2018), was due to losses caused by abiotic factors (Figure 3).

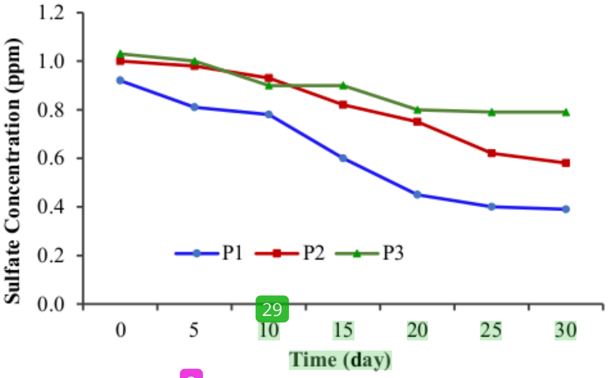

Figure 3. Sulfate concentration in each treatment of acid mine drainage with treatment: P1: acid mine drainage treated with sterile compost, P2: acid mine drainage treated with non-sterile compost and P3: acid mine drainage without the addition of compost.

Reduction of sulfate in the treatment of acid mine drainage by a consortium of sulfate-reducing bacteria occurs through dissimilatory metabolism using sulfate as the only terminal electron acceptor that is then reduced to sulfide and therefore, sulfate can be said to be the limiting factor for sulfate-reducing bacteria activity (Widdel and Pfennig, 1981; Tian et al., 2017). The role of sulfate-reducing bacteria was seen in the treatment of sterilized compost, where there was a significant decrease in sulfate concentration. Similarly, a redox reaction chemically occurred in which organic compost as an electron donor formed a sulfide compound. Apart from being a source of nutrients, compost contains heterotrophic microorganisms that act as providers of organic matter from the decomposition process, which will be a source of short-chain organic materials for activities of mixed culture of bacteria or consortium of sulfate-reducing bacteria (Zhang and Wang, 2014; Fahruddin and Abdullah, 2015). According to Fahruddin et al. (2020), there are many sulfate-reducing bacteria in wetland sediment because its high organic material content provides an ideal environment for the population of sulfate-reducing bacteria. Sediment naturally contains simple, low-molecular-weight carbon source that can be utilized by sulfate-reducing bacteria as electron donors (Meier et al., 2012; Nwankwoala, 2012). These bacteria use the sulfate contained in acid mine drainage for their metabolic activity by transferring hydrogen to sulfate as an electron acceptor (Elliot et al., 1998; Luptakova and Kusnierova, 2005). Then, these sulfate compounds will be reduced to sulfides so that the sulfate concentration in acid mine drainage will decrease, as shown by the following reaction:

$$4H_2 + SO_4 \rightarrow H_2S + 2H_2O + 2OH^-$$

Sulfate reduction is referred to as sulfate respiration or dissimilatory sulfate reduction because it happens in an anaerobic situation similar to that of aerobic respiration using oxygen as an electron acceptor (Pester et al., 2012; Fahruddin et al., 2018).

Change in pH

The results of pH measurements on the treatments for acid mine drainage showed that the P1 treatment, namely, The pH of acid mine drainage that was treated with compost changed significantly from pH 3.7 to pH 7.1. In the P2 treatment, there was a slight change in the pH value, from pH 3 to pH 5.4. In the P3 treatment as control, it can be said that there was almost no change in the pH value (Figure 4). Increasing pH is related to decreasing levels of sulfate, proving that in swamp sediments, there are bacteria capable of reducing sulfate to sulfide in acid mine drainage treatment (Meier et al., 2012). This happened in P1 treatment and P2 treatment, while P3 as control was not supported by sediment as an inoculum source of sulfate-reducing bacteria (Fukui and Takii, 1996). The mechanism of sulfate reduction in acid mine drainage by the sulfate-reducing bacteria produces sulfides and bicarbonates that have an effect on increasing pH; then the sulfides will react with dissolved metal ions to form insoluble metal sulfides (Pester et al., 2012; Wu et al., 2017). Hydrogen ions are released when sulfide minerals react with water and cause a decrease in the pH value in acid mine drainage. This provides an ideal environment for the growth of *Thiobacillus ferrooxidans* bacteria, which will speed up the oxidation of pyrite and produce sulfuric acid. Conversely, sulfate-reducing bacteria can increase pH or neutralize pH by reducing sulfate to sulfide (H_2S) and produce hydroxyl ions (OH^-) (Fahruddin and Abdullah, 2015).

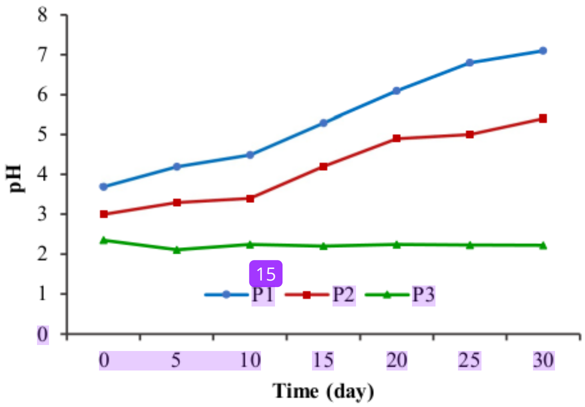

Figure 4. Changes of pH in each treatment of acid mine drainage with treatment: P1: acid mine drainage treated with sterile compost, P2: acid mine drainage treated with non-sterile compost and P3: acid mine drainage without the addition of compost.

Content of lead (Pb)

The measurement of lead (Pb) concentration showed that in the PI treatment, the initial concentration of Pb was 0.86 ppm, which then gradually decreased until the 30th day to 0.31 ppm (84%). In P2 treatment, the initial concentration of heavy metal Pb was 0.92 ppm, which gradually decreased until the 30th day to 0.11 ppm (63%). Meanwhile, the control P3 treatment did not experience a significant reduction in Pb (Figure 5). The decrease in the concentration of heavy metal Pb was caused by the activity of sulfate-reducing bacteria, which reduced sulfate under anaerobic conditions to become sulfides, which then reacted with dissolved metal ions to form insoluble metal sulfides so that the dissolved metal concentration in acid mining water decreased (Bradley et al., 2011). The formation of sulfides by the biological process of the sulfate-reducing bacteria consortium can precipitate Pb metal ions (Costa and Duarte, 2005). In the reduction of lead (Pb) in the treatment of acid mine drainage, compost only acted as a nutrient-supply agent for the sulfate reduction process and reduced lead (Pb) metal cations to metal sulfides. This is evidenced by the treatment with compost that was sterilized by removing the biological effects in the compost, which was able to reduce the concentration of heavy metals such as lead. However, the presence of heterotrophic microorganisms in compost may also contribute to the removal of lead (Pb) in the treatment through absorption mechanisms on the cell membrane of microorganisms and also, chemically, heavy metals will be chelated on the ligand in compost. On the other hand, microorganisms in compost do not survive when they are treated with acid mine drainage that has a low pH (Zhang and Wang, 2014; Ayangbenro et al., 2018; Retnaningrum et al., 2019; Xu and Chen, 2020).

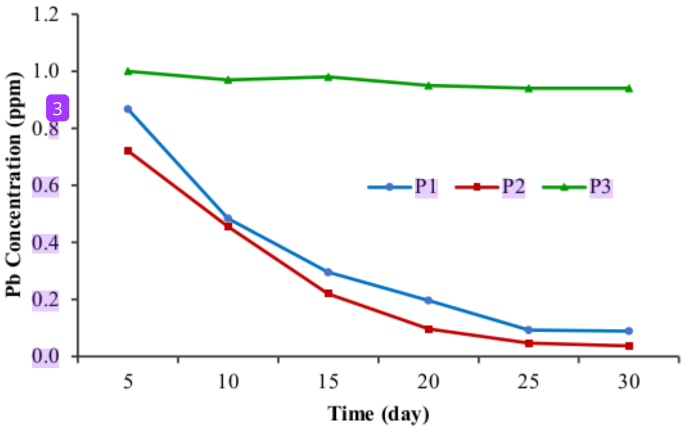

Figure 5. Lead (Pb) concentration in each treatment of acid mine drainage with treatment: P1: acid mine drainage treated with sterile compost, P2: acid mine drainage treated with non-sterile compost and P3: acid mine drainage without the addition of compost.

The reduction of Pb heavy metal ions in their oxidized form by sulfate-reducing bacteria can occur in two ways. The first is the formation of sulfides from the reduction of sulfates that will react with metal cations to form precipitating metal sulfides. The second, the H_2S produced from sulfate reduction by sulfate-reducing bacteria acts as an electron donor and reduces metal cations to metal sulfides (Fahruddin and Abdullah, 2015). The more the population of sulfate-reducing bacteria in the bioreactor increases, the more sulfate ions are reduced to sulfides, which will bind with metal ions to form precipitating metal sulfides (Luptakova and Kusnierova, 2005; Bradley et al., 2011). Sulfate-reducing bacteria use H_2 electron donor and a carbon source which can be obtained from organic matter. The addition of organic matter in the form of compost in treatment I and treatment II can increase the effectiveness of sulfate-reducing bacteria in reducing sulfate to sulfide, which causes a decrease in the concentration of heavy metal Pb in the water column in the bioreactor (Meier et al., 2012; Fahruddin et al., 2017).

Conclusion

Five types of bacterial isolates were obtained from swamp sediment based on macroscopic morphological characteristics of colony growth, then were used as mixed cultures as bacterial consortium isolates. In its application in the acid mine drainage treatment bioreactor, it was known that the bacterial consortium was able to reduce sulfate levels, indicated by an increase in pH and a decrease in the concentration of lead metal (Pb). Based on the indicator of decreasing sulfate concentration and Pb content, the most effective treatment for acid mine drainage was the treatment with non-sterile compost because compost is a source of organic material for sulfate-reducing bacteria in reducing sulfate.

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