Heavy metal removal and acid mine drainage neutralization with bioremediation approach

M S M Sihotang¹, A Rinanti²*, M F Fachrul³
¹,²,³Environmental Engineering Department, Faculty of Landscape Architecture and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia
astririnanti@trisakti.ac.id

Abstract. Mining Industry can improve the national economic situation; however, it also can damage the environment, mainly because of its wastewater that contains heavy metal and acidic solid compounds. When exposed to free air, sulfide minerals can be naturally oxidized and create acid mine drainage (AMD), a highly acidic waste that can mobilize heavy metals towards the environment. This literature study will discuss practical and sustainable biological processing to remove AAT. Sulfate Reducing Bacteria (SRB) were isolated from AMD polluted soil and grown inside an AMD-containing batch reactor. The environmental conditions (temperature, AMD concentration, SRB concentration, and contact time) were controlled during this research. The implementation of pH sampling was conducted every day, and the heavy metal final result was measured with an Inductive Coupled Plasma Optical Spectrophotometry or ICP-OES. SRB produced Hbiogenic-S that reacts with heavy metal and creates metal sulfide sediment. The remediation process by SRB will create biogenic alkalinity as an SRB side product that plays a significant role in neutralizing acidic water. Remediation is also influenced by organic compounds such as animal waste, rice, hay, or coconut husks. In this research, SRB plays a significant role as biosorbent that utilizes organic compounds as electron sources. The iron removal efficiency in AMD reached 96% and occurred on a contact time of 144 hours. To reach similar efficiency removal on a pilot scale, we planned AMD bioremediation on a tube-shaped reactor with 7.3 m³ with 3.5 m height and 0.88 of each reactor radiuses. This bioremediation study has provided an alternative solution for environmental management quality due to AAT pollution in water and groundwater around mining areas.

1. Introduction
The mining industry is one of the essential activities to improve the economic situation in a country. However, on the other hand, it can also disrupt the environmental balance, mainly because of its waste rich with heavy metal contents and its highly acidic nature known as acid mine drainage (AMD). Rain that pours down over mining areas will distribute heavy metals and other compounds and create reactivity in soil, which leads to erosion and leaching. Leaching would cause land fertility problems due to lowered pH levels in soil [1, 2].

Factors that influence AAT formation are acidity, alkalinity, morphology, pyrite formation, paleoenvironments, water and oxygen, and bacteria [3]. Minerals such as pyrite, chalcopyrite, pyrrhotite, and arsenopyrite can be naturally oxidized when exposed to air and create acidic waste that dissolves and mobilize toxic heavy metal compounds into the environment [4]. Based on that, mining activity that produces AMD rich in sulfate will lower pH levels due to high metal compounds [5, 6, 7, 8]. AMD
processing can be conducted with the chemical method through soil neutralization by utilizing chemical compounds such as lime (CaCO₃), hydrated lime (Ca(OH)₂), soda-ash (Na₂CO₃), or caustic soda (NaOH). Chemical processing can be accompanied by physical processes such as coagulation, flocculation, and sedimentation [5, 8]. AAT neutralization by utilizing alkali compounds such as lime, limestone, chemical compound, and industrial waste is easy, simple, and requires a relatively short time to remediate AMD pollutants in the environment. However, physical and chemical AMD processing has the potential to create a large amount of secondary mud that requires further processing [7, 9, 10].

Biological processing of AMD pollutants in the environment is a promising alternative. It has several advantages because it comes with a relatively low cost and is environmentally friendly [6, 8, 10]. The bioremediation method is considered environmentally friendly because it utilizes microbes naturally living in soil and water and does not cause any threat to other living things, including humans. Passive processing alternative by utilizing indigenous microbes metabolism on polluted location is generally conducted in situ or around it. BPS consortium is highly effective in optimizing the bioremediation process in AMD polluted environments [5, 10]. Control parameter that supports the growth rate of bacteria community is dissolved oxygen, temperature, pH level [12], Hydraulic Retention Time (HRT), and carbon sources [13, 14]. In AMD bioremediation process utilizes organic compounds as electron donors to support the BPS growth rate [13, 15]. Inside the reactor, environmental enrichment is implemented to accommodate bacteria needs to grow decently. If necessary, exogenous bacteria that are synergistic with indigenous bacteria can improve the insecticide degradation rate. This process is known as bioaugmentation [13, 14, 15].

2. Methodology

2.1. Acid Mine Drainage (AMD) preparation and Sulphate Reducing Bacteria (SRB) Cultivation

This research refers to [16] and [17] as primary literature sources. AMD was obtained from Sangão River, in Santa Carina State Carbon Basin (28°45'38.7''S 49°25'58.1''W). The samples were gathered on unsterilized polypropylene bottles with no headspace and stored at 4°C temperature. This research utilizes (three) carbon sources: rice husk, coconut husk, and pig farm wastewater treatment sludge. Organic carbon sources were washed on running water, dried, and cut into preferred sizes. Carbon sources were dried and ground into fine powder as organic substrate. 1 gram of organic substrate was mixed with 10 mL of nitrate acid (HNO₃) and heated at 120-140°C temperature.

2.2. Experiment

The reactor filled with a mixture of three carbon sources, as shown in Table 1, reached 3L and occurred at a constant temperature of 30°C.

| Reactor Number | PWTS (L; % v/v) | RH (L; % v/v) | CHC (L; % v/v) | Total Volume (L) |
|----------------|-----------------|---------------|----------------|------------------|
| 1              | 1.0; 33.3       | 1.0; 33.3     | 1.0; 33.3      | 3                |
| 2              | 1.8; 60         | 0.6; 20       | 0.6; 20        | 3                |
| 3              | 0.6; 20         | 1.8; 60       | 0.6; 20        | 3                |
| 4              | 0.6; 20         | 0.6; 20       | 1.8; 60        | 3                |

PWTS = Pig Farm Wastewater Treatment Sludge; RH = Rice Husk; CHC = Coconut Husk Chips.

2.3. Sample Analysis

Heavy metal, pH, and sulfate concentration in the sample were gradually analyzed. The acidity rate was measured on a pH meter—bacteria growth analysis was conducted with the Total Plate Count (TPC) method by utilizing the spread plate technique. Sulfate removal as a BPS performance indicator is
measured on a spectrophotometer turbidimetry. Heavy metal compounds (Fe and Mn) were measured on a Perkin Elmer Inductive Coupled Plasma (ICP) optic spectrophotometry or ICP-OES.

3. Result and discussion
The influence of the carbon source factor on AMD bioremediation, as explained, is shown in Figure 1 [17]. Figure 1. shows significant pH improvement that almost reaches neutral with an average value of 7.31 from the initial pH of 4.16. The best result occurred in reactor 2 (Table 1), on observed 30°C temperature for 200 days. Carbon sources composition on reactor 3 shows the lowest pH level at only 6.82. Figure 1. b shows the highest Fe metal removal occurred on reactor 4 (Table 1) that reached a removal efficiency of 95% than any other reactors (Figure 1. c). The Lowest Mn removal occurred on reactors 1, 3, 4. Reactor 2 and 3 show the highest and the lowest removal rates. Reactor 2 shows the best Fe and Mn removal efficiency at more than 95% up to 99%.

![Figure 1](image)

Figure 1. The influence of carbon source towards AMD bioremediation [17].

According to [19], the biostimulation process with the addition of carbon sources such as sheep waste, compost, and hay through batch experiment was able to remove 98% of Fe heavy metal. Similar results were also shown in research [17] that removed 99% Fe heavy metal. In 2020, research by [19] on local beach sand as inoculum shows the highest level of bioremediation with pH level increased from 2.85 to 6.80 and Fe$_{2}^{3+}$ removal efficiency higher than 82% by utilizing Sulphate Reducer Bio Reactors (SRBRs) on 30°C temperature in an anaerobic condition to stimulate BPS growth rate.

Compost can accumulate heavy metals and contains dissolved organic compounds as an important carbon source for BPS growth [17]. Organic husks such as coconut, rice, and hay, contain a lower rate of dissolved organic carbon than compost. Because of that, compost utilization as an organic carbon source produced a higher removal efficiency rate than organic husks made from plants. Organic carbon improves reduction process, removes Fe$_{3}^{3+}$ and increase pH level up until it reaches neutral [1], [13], [15].
Figure 2 explains that BPS possesses a specific growth rate ($\mu$) at around 0.014 to 0.083 hour$^{-1}$, with specific substrate utilization ($q$) rate of from 0.226 to 4.697 hour$^{-1}$. The relationships between $\mu$ and $q$ can be seen in Figure 3. The relationships between $q$ and $\mu$ values result in a total growth value ($Y_T$) of 0.016 and $K_d$ of 0.0123 on Fe metal of 64.46.

**Figure 2.** The relationships between specific growth rate ($\mu$) and specific substrate utilization rate ($q$).

**Table 2.** Bacteria-specific growth rate ($\mu$) and specific substrate utilization rate ($q$) measurement results.

| Time (hour) | Removal % | Fe (mg/L) | Amount of Biomass (gr/L) | q (/hour) | $\mu$ (/hour) |
|------------|------------|-----------|--------------------------|-----------|---------------|
| 0          | 0          | 100       | 0                        | 0         | 0             |
| 24         | 62         | 38        | 1.1                      | 4.697     | 0.083         |
| 48         | 75         | 25        | 1.9                      | 1.645     | 0.042         |
| 72         | 88         | 12        | 2.8                      | 0.873     | 0.028         |
| 96         | 90         | 10        | 3.2                      | 0.586     | 0.021         |
| 120        | 94         | 6         | 4.1                      | 0.382     | 0.017         |
| 144        | 96         | 4         | 5.9                      | 0.226     | 0.014         |

**Figure 3.** The relationships between substrate limitation and bacteria growth rate.

Table 2 shows that the highest iron metal removal occurred on 144 hours of contact time with a removal percentage of 96% with an initial dissolved iron amount of 100 mg/L into just 4 mg/L, by
optimum effective bacteria growth at 5.9 gr/L. The lowest removal efficiency at 62% occurred on 24 hours of contact time, with an initial dissolved iron concentration of 100 mg/L into 38 mg/L. This condition occurred because 24 hours of contact time is not considered as optimum contact time. After all, the effective bacteria lag phase generally occurs on the first 24 hours of contact time [13], [15].

Figure 3 shows the relationship between bacteria growth numbers in limited culture ($X_m$) and specific substrate utilization ($S$). Relationship graphic between $X_m$ and $S$ will create a linear line with Yobs slope of 0.1135 hour$^{-1}$. The maximum substrate utilization rate value ($q_{max}$) is at 4.697 with a maximum substrate utilization rate value ($q_{max}/2$) of 2.348 (Figure 4), which leads to reaction order determination in this research. Based on the graphic between $\mu$ and substrate, we obtained $K_s$ value of 25.05 (Figure 5). The kinetic measurement of dissolved iron concentration removal in AMD can be seen in Table 3.

![Figure 4](image-url)  
**Figure 4.** The relationships between substrate utilization rate and iron concentration.

![Figure 5](image-url)  
**Figure 5.** The relationships between specific growth rate ($\mu$) and substrate.

| Table 3. Kinetic calculation recapitulation of dissolved iron concentration removal in AAT. |
|---------------------------------|----------------|
| **Parameters**                  | **Calculation Results** |
| $Y_T$ (hour$^{-1}$)             | 0.016            |
| $K_d$ (hour$^{-1}$)             | 0.0123           |
Yobs (hour$^{-1}$) & 0.1135 \\
Ks & 25.05 \\

| Reaction Order Equations | 0 | 1 | 2 |
|--------------------------|---|---|---|
| Slope (a)                | 0.0694 | 0.0052 | 0.0006 |
| Intercept (b)            | 72.792 | 1.2805 | 0.0205 |
| Regression (R$^2$)       | 0.646 | 0.8746 | 0.9931 |

Table 4. Pilot-scale implementation calculation.

| Parameter Design   | Units | Values  |
|--------------------|-------|---------|
| Reactor volume     | m$^3$ | 7.3     |
| Reactor height     | m     | 3       |
| Base diameter      | m     | 1.76    |
| Base radius        | m     | 0.88    |
| Reactor height     | m     | 3.5     |

Figure 6. Tube-shaped pilot-scale reactor design.

Fe metal removal in AMD utilized two orders. The regression value closest to 1 was shown by an R$^2$ value of 0.9931 with the Y = 0.0006 x – 0.0205. AMD processing implementation calculation on the pilot scale can be seen in Table 4, and the reactor design can be seen in Figure 6.

4. Conclusion

*Indigenous Desulfovibrio, Desulfotomaculum,* and *Pseudomonas* bacteria in AMD are SRB that we can neutralize pH and remove heavy metals. BPS can survive on highly acidic conditions, with a temperature range of 25 to 35°C. The organic compound as electron source or electron donors such as animal waste, rice, hay, or coconut husks can assist BPS to reduce sulfate by neutralizing AMD pH levels up to 6-7. Fe metal kinetic removal on AMD follows reaction order 2 with the equation of Y = 0.0006 x – 0.0205 that resulted in an R$^2$ value of 0.9931. On pilot-scale implementation, a tube-shaped reactor unit with a volume of 7.3 m$^3$ with 3.5 m of height and 0.88 m radius can remove up to 96% of iron metal.

References

[1] Villegas-Plazas M, Janeth S, Howard J 2019 *Journal of Environmental Management* **251** 109581
[2] Hille R v, Nechal M, Tynan M, Nikhil N, Geoff M, Susan H, Ritva M 2016 *Mining Meets Water – Conflicts and Solutions.*
[3] Acharya B S and Gehendra K 2020 *Journal of Hydrology* 125061
[4] Bwapwa J K 2018 *Environmental Management and Sustainable Development* **7**(1)
[5] Acharya B S and Gehendra K 2020 *Journal of Hydrology* 125061
[6] Sheoran A S, V Sheoran R P, Choudhary 2010 Minerals Engineering 23(14) 1073–1100
[7] Sinharoy A, Kannan P, Piet N L Lens 2020 Current Pollution Reports
[8] Sánchez-Andrea I, Jose L S, Martijn F M, Bijmans, Alfons J M, Stams 2014 Journal of Hazardous Materials 269 98–109
[9] Yildiza M, Tulay Y, Cemile S A, Adem Y, Anna H K, Deniz U 2019 Minerals Engineering 133 52–59
[10] Hussain A, Ali H, Arshad J, Javed I Q 2016 3 Biotech 6(2)
[11] Ayangbenro A S, Oluwaseyi S O and Olubukola O B 2018 Frontiers in Microbiology 9
[12] Laroche E, Corinne C, Lidia F-R, Angélique D, Vincent T, Odile B, Fabienne B-B, Catherine J and Marina H 2018 Front. Microbiol. 9 3169
[13] Putri A N, R Ratnaningsih, A Rinanti IOP Conference Series: Materials Science and Engineering 1098 (5) 052072
[14] Zhang M and Haixia W 2014 Minerals Engineering 69 81–90
[15] Imaniar K R, T Tazkiaturrizki, A Rinanti IOP Conference Series: Materials Science and Engineering 1098 (5) 052071
[16] Alvarado L C R 2016 Optimization of the electron donor supply to sulphate reducing bioreactors treating inorganic wastewater (Degree of Doctor in Environmental Technology. Erasmus Joint Doctorate Programme in Environmental Technology for Contaminated Solids, Soils and Sediments (ETeCoS3))
[17] Kijjanapanich P 2013 Sulfate reduction for remediation of gypsiferous soils and solid wastes. Earth Sciences (Université Paris-Est)
[18] Ruehl M D and Sage R H 2020 Minerals Engineering 157 106554
[19] Oporto C, Baya G and Vandecasteele C 2019 Environmental Technology 1–33