Acid mine drainage removal by mixed bacteria culture of 
*Pseudomonas aeruginosa* and *Brevibacterium sp.*

A N Putri, R Ratnaningsih and A Rinanti*

Environmental Engineering Department, Faculty of Landscape Architecture and Environmental Technology, Universitas Trisakti, Jakarta, Indonesia

*astririnanti@trisakti.ac.id*

**Abstract.** Acid mine drainage is a problem faced by the mining industry in Indonesia has a negative impact on the environment because it is very acidic and contains heavy metals. This bioremediation research to determine the ability of a mixed bacteria culture of *Pseudomonas aeruginosa* and *Brevibacterium sp.* in removal acid mine water. The study began with an allowance test in liquid media using artificial growth media Stone Mineral Salt solution (SMSs) with the tested parameters namely contact time (hours) and concentration of acid mine water pollutants (% v/v). Parameter variation consists of contact time (hours) 24 to 240 and concentration of acid mine pollutant water (% v/v) 10 to 25. Mixed culture are resistant to the presence of acid mine water as a substrate that is xenobiotic because no inhibition zones are formed around paper discs that have been exposed to acid mine pollutant water and can grow on Nutrient Agar (NA) media added with acid mine pollutants. The pH of the liquid media increased from 2.14 to 5.87 with optimum contact time 144 hours with a temperature of 30 °C and optimum pollutant concentration 10%.

1. **Introduction**

The high development of Indonesian mining industry in the last few decades is facing a problem of environmental pollution, especially in mining area soil and also in ex-mining land areas with heave degraded by acid mine drainage. Acid mine drainage is shaped by open mining activity will produce sulphide mineral oxidation, and release sulphate acid that would drastically lower pH. The nature of acid mine drainage is highly acidic and contains high dissolved metal [1]. The most dangerous negative impact of acid mine drainage is the escalation of metals accumulation in the soil that causes land productivity decrease. The other negative impact is that acid mine drainage can pollute surface water and groundwater and endanger peoples’ lives that utilize surface and groundwater as their drinking water sources [2].

In reality, the environment has the ability to degrade pollutant compounds through biological and chemical process. However, the situation shows that the pollution level is higher than the ability of the environment to naturally degrade pollutants. Because of that, to degrade the accumulated pollutants, human effort is needed to overcome the pollution by utilizing existed technology [3]. Acid mine drainage processing can be conducted with active and passive system. Active acid mine drainage processing technology can be effectively conducted but with more expensive cost of tools, chemical compounds, and human resource utilizations. Meanwhile a passive acid mine drainage processing can be conducted with a modification intended for special goals such as to increase pH and lower iron concentration [4].
Method utilized in passive system is known as remediation method, which biologically utilize organism physiology, especially microbes, bacteria, fungi, and micro algae. This method is considered as an innovative method to detoxify the environment, it is more environmentally friendly, efficient, and has the ability to reduce pollutant permanently without producing secondary pollutant [5,6].

Of all the microbes utilized in bioremediation, bacteria are the most potential one to be utilized in a toxic environment and are able to reduce heavy metals [7]. Bacteria biomass productivity to produce secondary metabolite to repair polluted environment is depended on nutrition source and several different factors such as pH, temperature, salinity, and contaminant concentration in the environment [8]. Bacteria spectrum and archaic in a shape of consortium with wet soil plants has been utilized to lower heavy metal concentration on acid mine drainage and increase pH [9]. From the previous research, Pseudomonas aeruginosa is able to grow in a toxic environment and remove 80% of cadmium metal contained in electroplating industry waste [10]. Brevibacterium sp. is able to grow in wastewater with high metal dissolve and proven effective to remove copper, zinc, and manganese metals as much as 77%, 63%, and 55% [11]. Because of that, this research was conducted to test acid mine drainage removal by mixed bacteria culture of Pseudomonas aeruginosa and Brevibacterium sp. as bioremediation agents with a passive acid mine drainage processing technology.

2. Research methodology

2.1. Stone Mineral Salt solution (SMSs) media preparation
SMs media is a growing media utilized in this research. A liter of SMSs media contains 0.5 grams of CaCO₃; 2.5 grams of NH₄NO₃; 1 gram of Na₂HPO₄.7H₂O; 0.5 grams of KH₂PO₄; 0.5 grams of MgSO₄.7H₂O; and 0.2 grams of MnCl₂.7H₂O.

2.2. Acid mine drainage preparation as pollutant source
The utilized acid mine drainage used as pollutant source was obtained from PT Bukit Asam, Palembang mine pit.

2.3. Pseudomonas aeruginosa and Brevibacterium sp. bacteria cultivation
Pseudomonas aeruginosa and Brevibacterium sp. bacteria was obtained from Environment Microbiology Laboratory Universitas Trisakti, Jakarta Collection. Pseudomonas aeruginosa and Brevibacterium sp. was grown in SMSs media with pH of 7.

2.4. Acid mine removal test in liquid media
This research aims to improve acid mine drainage pH by using mixed bacteria culture of Pseudomonas aeruginosa and Brevibacterium sp. was conducted with an Erlenmeyer flask filled with SMSs media with solution composition (v/v) of 50% SMSs media, 20% mixed bacteria culture, 20% of molasses as carbon source and 10% of acid mine drainage pollutant. Erlenmeyer flask was inserted into an incubator shaker with the speed of 150 rpm, under temperature of 30°C as an optimum temperature to grow mixed bacteria culture of Pseudomonas aeruginosa and Brevibacterium sp. [12]. Observation and sampling was conducted on hour 48, 96, 144, 192 and 240 to obtain optimum contact time that is able to increase acid pH and alter it into neutral pH.

The research was continued to obtain acid mine drainage concentration (% v/v) with pH that can be raised by mixed bacteria culture. To achieve this result, the research was conducted by controlling acid mine drainage concentration variations of 10, 15, 20 and 25 (% v/v), in optimum contact time obtained from the previous research step.

There are two control treatments used, which are 1) bacteria culture was grown in SMSs media enriched with molasses as carbon source but no acid mine drainage was added; 2) acid mine drainage was added into SMSs media without adding mixed bacteria culture. After conducting incubation with a temperature of 30°C on optimum contact time, the observed parameters are pH increase measurement with pH meter and total bacteria number using haemocytometer.
3. Results and discussion

3.1. *Pseudomonas Aeruginosa* and *Brevibacterium* sp. sensitivity against acid mine drainage

Sensitivity test was conducted to measure growth ability of mixed bacteria culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. in acid mine drainage containing environment, which is a xenobiotic compound; a foreign compound in biological system because of its unknown molecular structure for microbes [13,14]. Mixed bacteria culture sensitivity test was conducted on Gel Nutrient media that contains mixed bacteria culture of *Pseudomonas aeruginosa* and *Brevibacterium* sp. with acid mine drainage addition. Figure 1 shows that there is no obstruction zone formation during 48 hours’ observation in around disc paper that contains acid mine drainage. This finding shows that *Pseudomonas aeruginosa* and *Brevibacterium* sp. mixed bacteria culture is not sensitive or resistant against acid mine drainage as a toxic xenobiotic compound but it seems to be able to act as a suitable substrate as mixed culture bacteria growth. With this finding, this research can be further continued by utilizing *Pseudomonas aeruginosa* and *Brevibacterium* sp. bacteria.

![0 Hour](image1.png) ![24 Hour](image2.png) ![48 Hour](image3.png)

**Figure 1.** *Pseudomonas aeruginosa* and *Brevibacterium* sp. bacteria sensitivity test against acid mine drainage existence.

3.2. Acid mine drainage removal test in liquid media with contact time variations

Acid mine drainage test result removal by *Pseudomonas aeruginosa* and *Brevibacterium* sp. mixed culture bacteria with contact time variation of 240 hours, conducted with sampling activities on hour 48, 96, 144, 192 and 240 as shown in Figure 2. The figure shows that there is a constant pH increase until hour 240. The value of pH on hour 0 shows 2.14, which means that the nature is acidic, and constantly increase until it reaches 5.87 pH at the end of the incubation period of 240 hours. This finding never occurred in control treatment. In control graphic, starting from hour 0 until hour 240, pH value is
constantly showing 2.3 without pH increase. The increase of pH on treatment shows enzyme activity as metabolite produced by *Pseudomonas aeruginosa* and *Brevibacterium* sp. mixed bacteria culture that is able to neutralize acidic compound as substrate.

![Figure 2](image)

**Figure 2.** The change of pH on contact time variation test.

This mixed culture bacteria activity increase is also similar with bacteria density escalation because of the environmental condition that supports mixed culture bacteria growth. This situation is shown by a more concentrated media color on hour 240 if compared with early observation. Figure 3 describes bacteria density measurement result by using haemocytometer, which are in hour 0 is $1.8 \times 10^5$ cells/ml and constantly increased until at hour 144 reaches $12 \times 10^5$ cells/ml. On hour 192 and 240 bacteria cell density is constantly decreasing until it reaches $10.5 \times 10^5$ cells/ml and $9.7 \times 10^5$ cells/ml.

![Figure 3](image)

**Figure 3.** Bacteria growth on contact time variation test.

The decrease of bacteria density number was caused by a change of environmental factors such as pH, oxygen availability, and lower nutrient source either macro or micro nutrients that are able to alter each bacteria nature from synergic into antagonist. In control treatment, bacteria cell density of hour 0 is $1.7 \times 10^5$ cells/ml and constantly decreased until on hour 240 it reaches $0.23 \times 10^5$ cells/ml. From the graphic, we can see that optimum contact time is hour 144, when bacteria cells reach highest density of $12 \times 10^5$ cells/ml, which produce highest pH decrease into 5.29 from the previous pH of 3.23 on hour 96. This research shows that both bacteria are acidophilic bacteria groups that physiologically able to lower pH from highly acidic into neutral pH.
3.3. Acid mine drainage removal test in liquid media with pollutant concentration variation
This research was continued to acknowledge *Pseudomonas aeruginosa* and *Brevibacterium* sp. mixed culture bacteria maximum ability to live and grow in various acid mine drainage concentration as pollutant (% v/v) such as 10, 15, and 25, which are incubated with 144 hours of contact time.

![Figure 4](image)

**Figure 4.** The change of pH on acid mine drainage concentration variation test as pollutant.

Figure 4 shows that even though there is no significant difference, the higher the pollutant concentration, the lower the pH level. Mixed bacteria culture grown in 10% acid mine drainage polluted environment can increase pH to the level of 5.52 meanwhile mixed culture bacteria grown in 25% acid mine drainage polluted environment is only able to increase pH to the level of 4.31.

Next, in Figure 5, a higher mixed culture bacteria density level shows no significant pH increase. In various acid mine drainage concentration variations as pollutant shows no significant bacteria density differences, which is between $11.8 \times 10^5$ cells/ml to $12.4 \times 10^5$ cells/ml in every treatment variations starting from 10% to 25% acid mine drainage pollutant concentration. Even though that there is no significant different, this research has strengthened the fact that *Pseudomonas aeruginosa* and *Brevibacterium* sp. are classified as acidophilic group bacteria which possess the ability to grow on an environment with pH level less than 4 [15]. The higher acid mine drainage concentration, the more acidic the liquid media pH, but the number of bacteria is increased for 144 hours.

![Figure 5](image)

**Figure 5.** Bacteria number on acid mine drainage concentration variations test as pollutant.

It seems that acidic pH condition is a suitable environment to grow *Pseudomonas aeruginosa* and *Brevibacterium* sp. bacteria by utilizing acidic compound on acid mine drainage that would finally produce neutral environment pH.

4. Conclusion
Based on the preliminary research in this study, *Pseudomonas aeruginosa* and *Brevibacterium* sp. mixed culture bacteria is able to increase acid mine drainage pH in a synergic way from 2.14 to 5.87 with
optimum contact time of 144 hours and optimum pollutant concentration (v/v) 10% in SMSs liquid growth media. The result of this research shows that *Pseudomonas aeruginosa* and *Brevibacterium* sp. mixed culture bacteria has the potential as bioremediation agent to remove acid mine drainage that polluted Indonesian mining land.

References

[1] Li Y, Li W, Xiao Q, Song S, Liu Y and Naidu R 2018 Acid mine drainage remediation strategies: A review on migration and source control *Minerals & Metallurgical Processing* 35(3) 148-158

[2] Chen T, Tan B, Lei C and Xiao X 2014 Pollution control and metal resource recovery for acid mine drainage *Hydrometallurgy* 147 112-119

[3] Mahardika G, Rinanti A and Fachrul M F 2018 Phytoremediation of heavy metal copper (Cu²⁺) by sunflower (*Helianthus annuus l.*) *IOP Conference Series: Earth and Environmental Science* 106(1)

[4] Said N I 2014 Coal Acid mine Drainage Processing Technology: Alternative Technology Choosing *JAI* 7(2) 119 – 139

[5] Dixit R 2015 Bioremediation of Heavy Metals from Soil and Aquatic Environment: An Overview of Principles and Criteria of Fundamental Processes. *Sustainability* 7(2) 2189-2212

[6] Garcia J D, Sanchez Thomas R and Moreno Sanchez R 2016 Biorecovery of non-essential heavy metals by intracellular and extracellular mechanisms in free living microorganism *Biotechnology Advances* 34(5) 859-873

[7] Garbisu C 2017 Plasmid-Mediated Bioaugmentation for the Bioremediation of Contaminated Soils *Frontiers in Microbiology* 8

[8] Arslan M, Imran A, Khan Q M and Afzal M 2015 Plant-bacteria partnerships for the remediation of persistent organic pollutants *Environment Science and Pollution Research* 24(5) 4322-4336

[9] Chockalingam E, Subramanian S and Braun J J 2010 Bioremediation of acid mine water utilising red mud and Desulfotomaculum nigrificans *Mineral Processing and Extrative Metallurgy* 119(3) 153–162

[10] Muneer B, Iqlbal M J, Shakoori F R and Shakoori A R 2016 Isolation, Identification and Cadmium Processing of *Pseudomonas aeruginosa* (EP – Cd1) Isolated from Soil Contaminated with Electroplating Industrial Wastewater *Pakistan J. Zool* 48 1495-1501

[11] Ojoawo S O, Rao C V and Goveas L C 2015 Bioremediation of Zn, Cu, Mg and Pb in Fresh Domestic Sewage by *Brevibacterium* sp. *Int. J. Environ. R* 10 139-148

[12] Permatasari R, Rinanti A and Ratnaningsih R 2018 Treating domestic effluent wastewater treatment by aerobic biofilter with bioballs medium by *IOP Conference Series: Earth and Environmental Science* 106(1) 012048

[13] Vernans B A K R, Iswanto B and Rinanti A 2019 Bioremediation of soil polluted with copper (Cu²⁺) by mixed culture bacteria *Thiobacillus* sp. and *Clostridium* sp *International Journal of Scientific and Technology Research* 8(12) 3915-3919

[14] Godheja J, Shekar S, Siddiqui S and Modi D 2016 Xenobiotic Compounds Present in Soil and Water: A Review on Remediation Strategies *Journal of Environmental & Analytical Toxicology* 6

[15] Sunaryo T, Widyatmoko H and Rinanti A 2018 Chlorpyrifos removal by *Thiobacillus* sp. and *Clostridium* sp. in liquid medium *MATEC Web of Conferences* 197 13012