RESEARCH REPORT
DIPA BIOTROP 2021

MANAGEMENT OF ACID MINE DRAINAGE BY *FLOATING TREATMENT WETLAND* AND SULFATE-REDUCING BACTERIA ENRICHMENT

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2021

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5. Research budget :

Bogor, November 29th, 2021

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1. INTRODUCTION

1.1. Background

The mining industry has been regarded as a pioneer industry due to infrastructure development that also opens an area from geographical isolation. One type of mining that is widely operating in Indonesia is coal mining. Open-pit coal mining could trigger the formation of acid mine drainage (AMD). Acid mine drainage is formed due to the oxidation process of pyrite (FeS2) and other sulfide mineral materials. Acid mine drainage will have interrelated impacts, i.e., having low pH and increasing the solubility of microelements, generally heavy metals, that could affect environmental and human health (Gautama 2007).

Changes in the landscape that occur due to mining activities are the emergence of ex-mining pits or voids. Voids are often surrounded by potentially acid-forming (PAF) rocks which cause the voids to be filled by AMD. The overflow of the AMD from the voids into public water would degrade the quality of the water and aquatic ecosystem. Therefore, the government of Indonesia strictly demands the company to treat the water before discharging it to the public water. The most common practice to neutralize the water is by application of lime to the water. However, who will be responsible for liming when the company has completed the operation, while the AMD is still being produced in many more years. The development of passive treatment for AMD treatment in Indonesia is urgently needed. Wetland system has long been applied for managing AMD in other countries, such as UK and USA. Constructed swamp forest has been successfully neutralized AMF passively (Yusmur et al. 2019 and Rahmatia et al. 2019). However, there has no passive technology to neutralize AMD in the deep void. The use of floating rafts loaded with organic matters combined with planting hyperaccumulator plants (referred to as floating treatment wetlands) need to be tested for neutralizing AMD in voids.

In recent years, the application of floating treatment wetlands for domestic and industrial wastewater has received significant attention because it offers an environmentally friendly approach (Solanki et al. 2017). The use of a floating treatment wetland to purify wastewater is an in-situ ecological restoration method, as well as physical (attachment of pollutants to the root surface), chemical (degradation of contaminants to a less toxic form), and biology (involving microbes). The mechanism for removing pollutants in a floating treatment wetland consists of an interaction between bacterial metabolism, plant uptake and accumulation of pollutants in its biomass (Osem et al. 2007). The bacteria that play important roles in improving the quality of AMD are sulfate-reducing bacteria (SRB). These bacteria are able to use sulfate, sulfite, or thiosulfate ions as electron acceptors to get energy in their metabolic process. After accepting the electrons, the ions will be reduced to sulfides so that the accumulation of sulfates in AMD decreases (Yusron 2009). Phyo et al. (2020) showed that the addition of organic matter significantly increased the growth of SRB and decreased the population of autotrophic bioleaching bacteria, such as iron and sulfur-oxidizing bacteria which reduce the pH of the water. This is in line with the concept of floating treatment wetland, where the addition of organic matter is also needed as a growing media for floating plants.

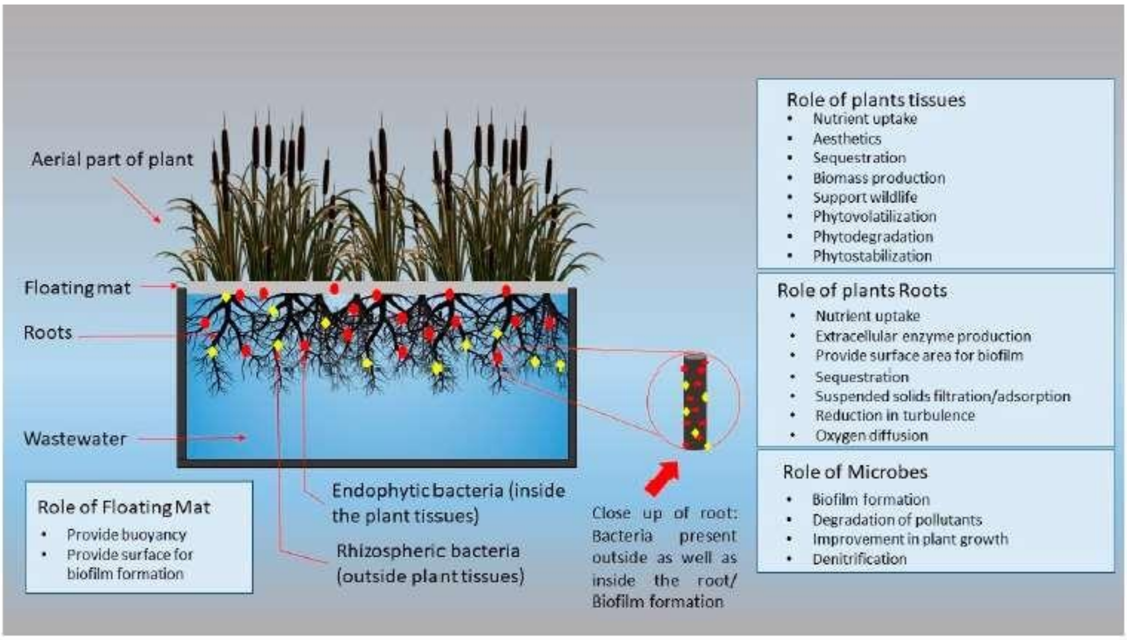


Figure 1 Representation of floating treatment wetland and pollutant removal process (Source: Shahid et al. 2020)

The success of AMD remediation with a wetland is determined by the selection of organic matter, plants, and the application of SRB. This study will examine the use of floating treatment wetlands enriched with SRB for AMD remediation.

1.2. Objectives

The efficiency of the floating treatment wetlands concept depends on several components in it, such as the selection of plants, planting media, floating media, and the application of floating techniques (Solanki *et al.* 2017; Headley and Tanner 2006). Therefore this study aims to:

a. Evaluate the effect of organic matter on various pond depths in neutralizing AMD.
b. The right type and amount of organic matter to increase the population of SRB as well as a growing medium for floating plants.
c. Obtaining potential plant species as AMD phytoremediation agents.
d. Analyzing the increase in SRB population from AMD pond sediments.
e. Measuring the effectiveness of processing floating wetlands with SRB enrichment in AMD management

1.3 Expected Output

The results of this study are expected to provide new technology in the in-situ remediation of AMD in void. The floating treatment wetland technology with SRB enrichment will be useful to water quality in voids left by mining operation.

2. BENEFIT AND IMPORTANCE OF RESEARCH

Many researchers have developed passive treatment of acid mine drainage management techniques, where the costs are relatively low and do not require much maintenance. However, passive treatment of acid mine drainage management is currently only limited to shallow water; there is no technology to manage acid mine drainage in deep voids (pit lakes). In a few years to come, many coal mining companies in Indonesia will complete their operation and will leave many voids containing acid mine drainage. These voids could not be used due to the quality of the water; therefore, this research is important to find develop technology to neutralize and improve the water quality of voids. The results of this research are expected could be applied in void easily, practically, eco-friendly, cheaper, and sustainably, and later the void could be used productively.

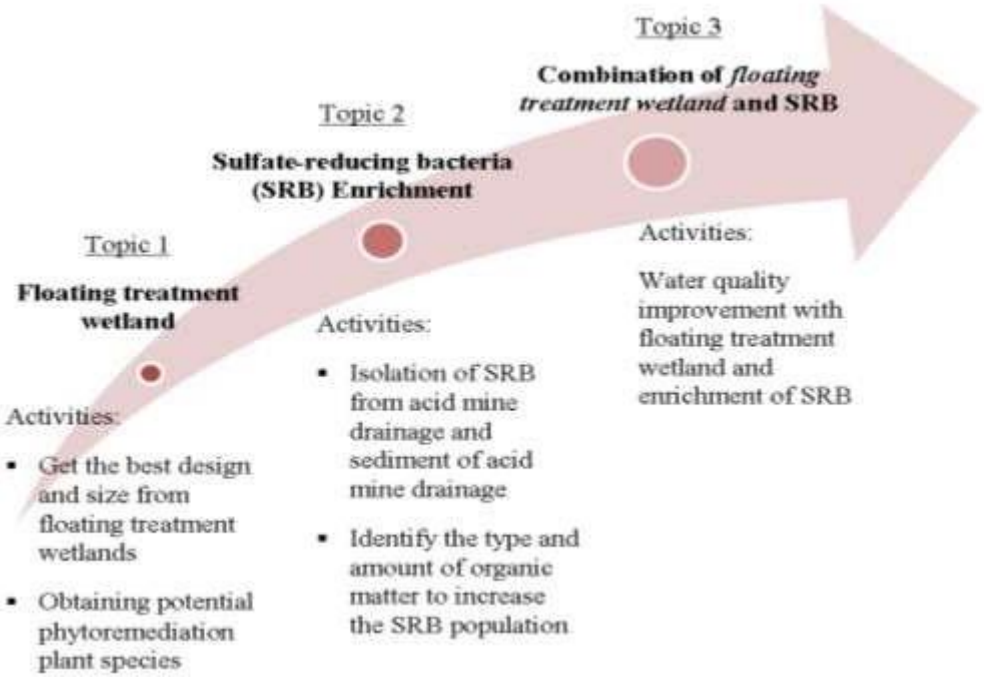


Figure 2 Research road map

3. METODOLOGY

3.1 Site Study

This research was conducted in the greenhouse, and Laboratory of Biotechnology and Landscape Management of SEAMEO BIOTROP from February to November 2021.

3.2 Method

3.2.1 Depth Test of Neutralization of Acid Mine Water

The research in the greenhouse was carried out using an 8-inch diameter paralon pipe with various depth variations (Figure 3). The treatment tested was adding organic material as thick as 15 cm with a weight of ± 900 grams, namely a mixture of oil palm empty fruit bunches (OPEFB) and cow manure in a ratio of 2:1 in each pipe. Paralon pipe was cut with a height of 50 cm, 100 cm, and 150 cm. The design used in this study was a completely randomized design (CRD) with four levels of treatment, namely control treatment (without organic matter), pipe depth of 50 cm, 100 cm, and 150 cm; each treatment was repeated three times so that a total of 12 experimental units. The pH measurement was carried out every 20 cm height. This was done to determine the effectiveness of the organic matter that was floated in neutralizing the pH of acid mine drainage seen from the depth of the water. Besides pH, heavy metal analyses such as Fe, Mn, Cu, and Zn, sulfate, and sulfide levels, Total Suspended Solid (TSS), and Biochemical Oxygen Demand (BOD) were also analyzed before and after treatment.

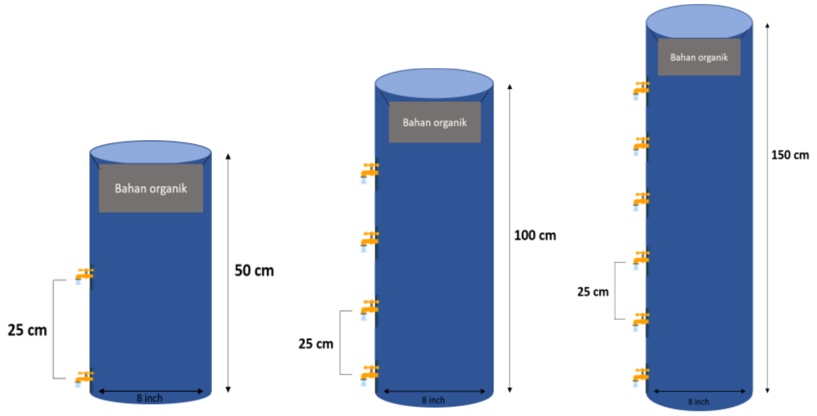


Figure 3 Pipe model for depth test against AMD neutralization

3.2.2 Inoculation of Sulfate-reducing bacteria (SRB)

Inoculation of sulfate-reducing bacteria (SRB) was taken from AMD sediment using Postgate B media as much as 3 L. Isolation of SRB was carried out to obtain isolates that would be used for research. Observations were made for 14 days. The presence of growing SRB is indicated by a change in the color of the media to black. After that, the number of growing SRB was calculated using the most probable number (MPN) technique with incubation at 33 oC observed for 14 days.

3.2.3 Propagation of Sulfate-reducing bacteria

Sulfate-reducing bacteria that have been isolated are propagated by growing in organic matter that is flooded with water. The organic material used was single empty oil palm fruit bunches (OPEFB) and mixed with milk factory sludge, cow manure, and chicken manure in a ratio of 1:1. Before being inoculated with SRB, the organic matter was first dried to reduce its moisture content, then sterilized by irradiating Cobalt-60 at a dose of 60 kGy, which was put into bags made of jute sacks. The organic matter was also measured for its water content, pH, and C/N ratio. The amount of SRB inoculated was 1% of the total volume of organic matter. Every week, the number of SRB growth is checked until it reaches a minimum of 108 MPN-unit/soil dry weight (dry weight).

Figure 4 OPEFB that has been dried

Figure 5 Pockets of organic material used for SRB propagation

3.2.4 Test the Effect of SRB for Acid Mine Water Remediation

Sulfate-reducing bacteria that have been propagated are put into synthetic AMD with the composition as shown in the table. The number of samples tested was 12 plus three samples as controls, so that 15 samples were observed. The test was carried out using a 5-inch paralon pipe with a height of 150 cm. At each increase in the size of 25 cm, a water faucet is installed as a point to take samples whose pH will be measured until it exceeds six according to the Regulation of the Minister of the Environment No. 113 of 2003. At the end of the observation, the levels of heavy metals Fe, Mn, Zn, Cu, and Al were measured to determine the decrease in dissolved metal levels.

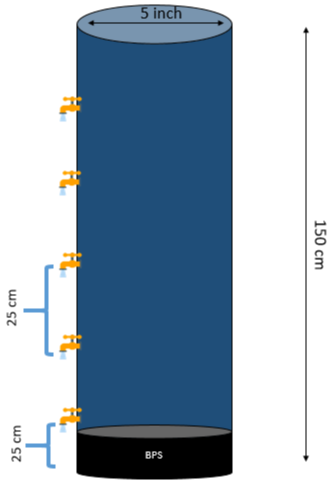


Figure 6 Pipe building used for SRB test

3.2.5 Combination of Floating Treatment Wetland and SRB Enrichment

This research was conducted by combining the research in the previous stage, namely the manufacture of floating treatment wetlands and SRB enrichment. At the bottom of the pond was given potential acid-forming (PAF) mud with a thickness of 10 cm as a place for SRB to grow. Sludge enrichment is done by adding organic matter, as in previous studies. The number of samples tested was 12 samples plus three samples as controls, so that a total of 15 samples were observed. The control consisted of AMD.

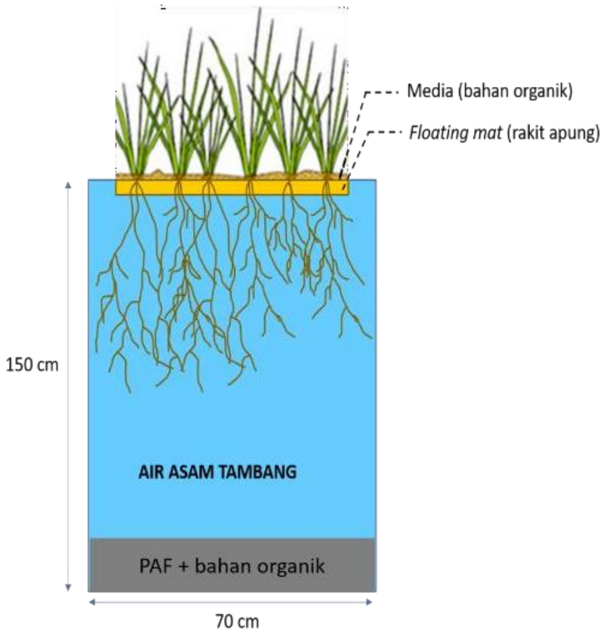


Figure 7 Combination of floating treatment wetland and SRB

3.3 Data analysis

Data analysis used analysis of variance (ANOVA) at a level of 5% and if there was a significant difference, it was continued with the Duncan Multiple Range Test (DMRT) with a level of 5%. The correlation test of C/N ratio data with pH was analyzed using SPSS v16 software.

4. RESULTS AND DISCUSSION

4.1 Depth Test of Neutralization of Acid Mine Water

Prior to the start of the study, an AMD quality analysis was conducted to determine the difference in the quality of acid water before and after treatment. The results of the analysis are shown in Table 1. From the analysis of the quality of AMD, it was found that AMD has a very low pH ranging from 2.85 to 3.22, and the solubility of heavy metals such as Fe, Mn, Cu, and Zn is high, respectively. of 58.67; 8.33; 3.17; and 3 mg/l. Likewise, the BOD value is 6.14 mg/l. If viewed from the quality standard of Minister of Environment Decree No. 113 of 2003 concerning Wastewater Quality Standards for Coal Mining Businesses and or Activities, the pH parameters and Fe and Mn metals have not met these quality standards. Based on PP No. 82 of 2001 concerning Water Quality Management and Water Pollution Control, the parameters for Cu and Zn metals, as well as BOD, also have not met the quality standards set for both drinking water and agricultural irrigation water criteria. Therefore, it is important to manage AMD before it is distributed to public water bodies, one of which is the addition of organic matter.

Table 1 Results of acid mine water quality analysis (AMD)

Parameter	**AMD***	**Quality Standard****
pH	2,85 – 3,22	6-9
Fe (mg/l)	58,67	7
Mn (mg/l)	8,33	4
Cu (mg/l)	3,17	0,02 – 0,2
Zn (mg/l)	3	0,05 – 2
SO_4 (mg/l)	394	-
H_2S (mg/l)	<0,01	-
BOD (mg/l)	6,14	2 – 12
TSS (mg/l)	30,93	400

*: *Services Laboratory* SEAMEO BIOTROP

**: Decree of the State Minister of Environment Number 113 of 2003 concerning Wastewater Quality Standards for Coal Mining Businesses and or Activities and Government Regulation Number 82 of 2001 concerning Water Quality Management and Water Pollution Control

In this study, we tested the ability of organic matter to increase pH at various depths. The results showed that the addition of floating organic matter was able to increase the pH up to >6 at both 50 cm and 150 cm depths (Figure 8).

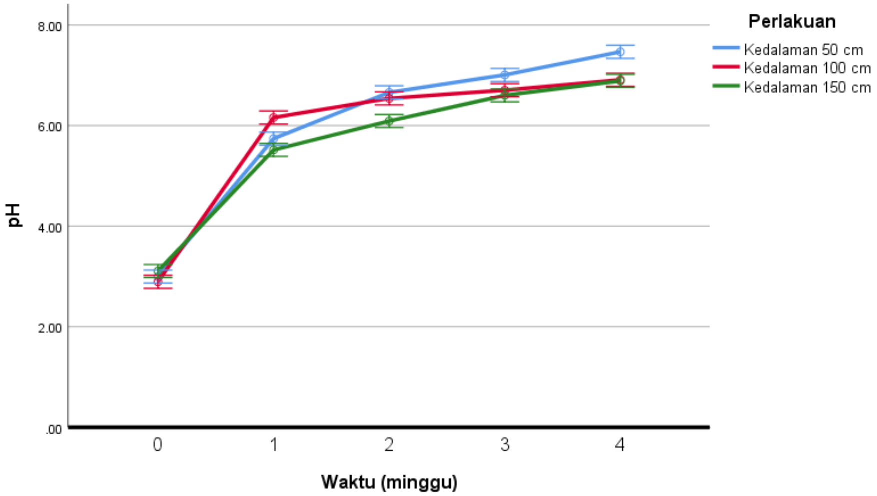
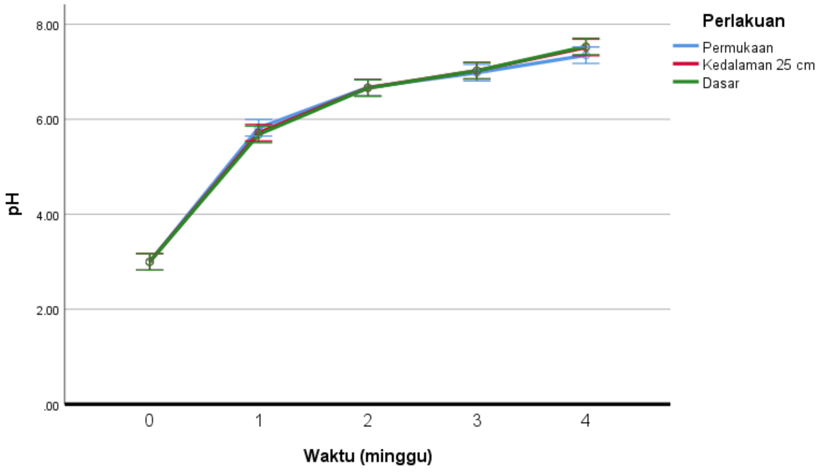


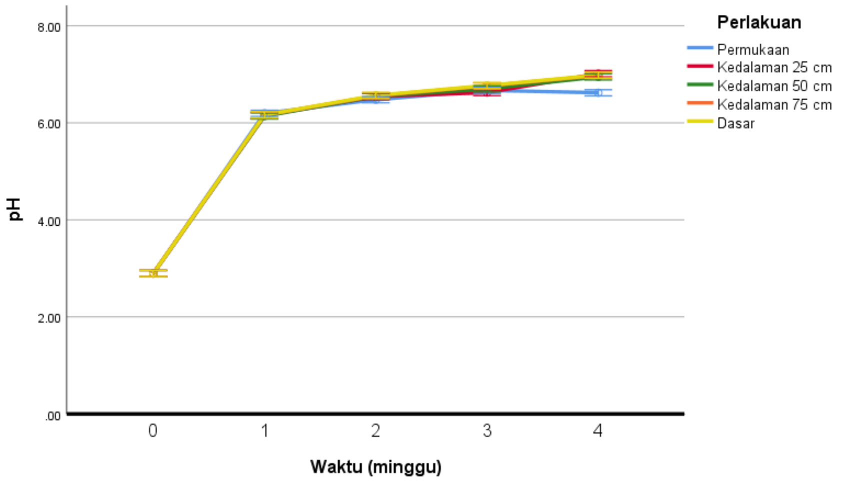
Figure 8 Effect of organic matter at various depths on the pH of AMD

The addition of organic matter in AMD proved to be able to increase the pH of AMD. Based on the statistical analysis performed, the pH values at a depth of 50 cm, 100 cm, and 150 cm showed significantly different results ($P<0.05$). The fastest increase in pH occurred at the 100 cm depth treatment, which was able to increase the pH to > 6 with a residence time of 5 days, while at a depth of 50 cm, treatment required a residence time of 7 days. However, the longer the observation period, the increase in pH at a depth of 50 cm increased, while at a depth of 100 cm, the increase in pH was more stagnant.

To see the effect of depth on the same medium, pH measurements were also carried out at every 25 cm increase in height, the results of which are presented in Figure 9.



(a)



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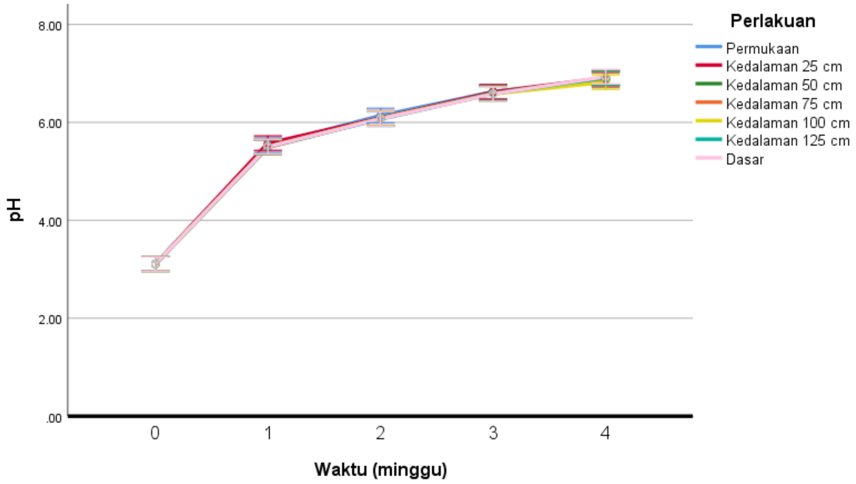


(c)

Figure 9 Differences in pH values at various depths: (a) 50 cm depth; (b) depth of 100 cm; (c) depth 150 cm

Based on statistical analysis, the pH values from the surface to the bottom at a depth of 50 cm, 100 cm, and 150 cm did not give significantly different results ($P>0.05$). This shows that organic matter floating on the surface is not only able to increase the pH at the surface but can also reach the bottom. It is seen that the increase in pH at the surface and bottom shows uniform results. From these results, it can be concluded that one interesting thing is that when the floating treatment wetland is applied in the field, the pH measurement on the surface is able to represent the pH value at the bottom of the pond.

The increase in the pH value was contributed by the presence of organic matter added to the system. The organic material used in this study was a combination of oil palm empty fruit bunches (OPEFB) and cow manure with a ratio of 2:1 (Sekarjannah 2021). The analysis was carried out to determine the characteristics of the two organic materials, which are presented in Table 2.

Table 2 Results of the analysis of organic matter

Parameter	**OPEFB**	**Cow manure**
pH	9,1	8,8
C-organik (%)	54,43	41,72
N total (%)	1,19	1,54
C/N	46	27
P total (%)	0,06	1,79
K total (%)	3,58	7,17
Ca total (%)	0,6	3,23
Mg total (%)	0,47	1,02
KTK ($cmol_c/kg$)	29,36	63,22

Source: Sekarjannah (2021)

Oil palm empty fruit bunches (OPEFB) and cow manure had high pH and cation content of Ca, Mg, and K, so they could increase the pH of AMD (Table 2). Munawar and Riwandi (2010) stated that organic materials with high pH and alkaline cations (Ca, Mg, K, and Na) have the potential to be a source of alkalinity for AMD, which is consistently able to increase AMD pH> 7 (Gibert et al. 2004). In addition, OPEFB and cow manure also have high cation exchange capacity (CEC) values, namely 29.36 and 63.22 cmolc/kg (Table 2), where a high CEC indicates that the material is capable of absorbing or exchanging cations. It is able to remove dissolved metal cations in AMD (Munawar and Riwandi 2010). Provision of OPEFB and cow manure is also a source of energy needed by sulfate-reducing bacteria (SRB), where SRB will play a role in the process of reducing sulfate content along with a decrease in heavy metal content through the formation of metal sulfides (Hards and Higgins 2004). Analysis of the content of heavy metals and sulfates, and sulfides was also carried out to see the effect of the application of organic matter on the quality of AMD (Table 3).

Table 3 Results of AMD quality analysis before and after treatment

Perlakuan	Waktu	Fe	Mn	Cu	Zn	sulfida	sulfat	BOD	TSS
Air asam tambang	Awal	58,67	8,33	3,17	3,00	0,01	531,00	6,14	30,93
Kedalaman 50cm	2 minggu	5,00 a	5,93 a	0,27 a	0,5 a	0,01 a	608 c	275 c	41,47 b
	4 minggu	0,4 ab	3,33 a	0,13 a	0,23 a	0,037 a	513 a	238,33 b	40,13 a
Kedalaman 100cm	2 minggu	20,00 b	8,33 ab	0,43 a	0,5 a	0,012 a	548 b	82,6 c	31,6 a
	4 minggu	1,47 b	7,33 b	0,23 a	0,27 a	0,13 a	552,67 a	69,03 a	42,4 a
Kedalaman 150cm	2 minggu	4,33 a	9,67 b	0,83 b	1,00 b	0,014 a	529,3 a	122,67 b	33,2 ab
	4 minggu	0,23 a	7,67 b	0,57 b	0,87 b	0,01 a	519,33 a	111,33 a	60,4 a

Sumber: *Services Laboratory* SEAMEO BIOTROP

Based on statistical analysis, there was a decrease in dissolved metal content in both the second and fourth weeks. From Table 3, it can be seen that at a depth of 50 – 150 cm, the metal content of Fe, Zn, and TSS values have met the environmental quality standards set. As for the metal content of Mn and Cu, only treatment at a depth of 50 cm has met environmental quality standards. When viewed as a whole, the value of the heavy metal and sulfate levels in water is influenced by the depth of the water. At relatively shallow depths, the metal and sulfate removal process will be faster than at deeper depths. The removal of heavy metals can occur due to adsorption by organic matter and also due to the interaction between sulfide (S2-) produced in the sulfate reduction process with 2-valent metals (such as Mn2+, Cu2+, and Zn2+) to form metal sulfide deposits which at the same time reduce metal and sulfate content. In water (Hards and Higgins 2004). This is evidenced by the increasing value of sulfide in water (Table 3).

All treatments showed an increase in the value of BOD (Biochemical Oxygen Demand) at a depth of 50 cm. The resulting BOD was significantly ($P<0.05$) higher than at a depth of 100 cm and 150 cm. BOD is a description of organic matter levels, namely the amount of oxygen needed by aerobic microbes to oxidize organic matter into carbon dioxide and water. In other words, BOD indicates the amount of oxygen consumed by the aerobic microbial respiration process (Effendi 2003). At first, the BOD of water was only around 6.14 mg/l but increased after adding organic matter to the water. This is because the presence of organic matter in water increases the amount of oxygen needed by aerobic microbes to decompose the organic matter. However, the BOD value in the fourth week is lower than the BOD value in the second week, which indicates that the source of organic matter that needs to be decomposed will gradually decrease so that the BOD value also decreases.

4.2 Isolation of Sulfate-reducing Bacteria

Table 4 SRB population isolated from AMD after 21 days

Inoculum source	**MPN-unit/ dry weight**
AMD sediment	7.9×10^5

Figure 10 Reducing bacteria isolated from AMD

Isolation of sulfate-reducing bacteria (SRB) was carried out to determine the presence of SRB that grows in extreme environments such as AMD. According to Postgate (1984), SRB can be found in almost all environments on earth, ranging from soil, freshwater, seawater and brackish water, hot springs, geothermal areas of oil and gas wells, sulfur reserves, silt deposits, sewers, rusty iron, goat rumina, and insect intestines. The things that affect the diversity of SRB are environmental conditions, environmental acidity, sediment depth, energy availability from organic matter, and sulfate content (Yusron 2009). The presence of organic C in the sediment helps SRB to grow. Table 4 shows the number of SRB that can be isolated from AMD sediment. As much as 7.9×10^5 MPN-Unit/dry weight will be used for SRB propagation. The use of the MPN-Unit/dry weight unit is carried out because SRB is isolated from mud which has solid particles.

Figure 11 Isolation of SRB from AMD

4.3 Propagation of Sulfate-reducing bacteria

Table 5 Measurement results of pH, C and N of organic materials

Organic matter	Moisture content (%)	pH	Organic carbon (%)	Total N (%)	C/N ratio
Oil palm empty compost (OPEFB)	17.85	8.68	53.12	0.71	74.82
Mill sludge mixed with oil palm empty fruit bunch compost (PRTK)	20.15	7.60	39.575	1.2	32.98
Cow manure mixed with oil palm empty sign compost (SBTK)	23.55	8.15	44.275	1.73	25.59
Chicken manure mixed with oil palm empty sign compost (SITK)	18.71	8.56	40.14	1.62	24.78

Sulfate-reducing bacteria are heterotrophic anaerobic bacteria whose growth depends on the availability of organic substrates. The pH value, organic carbon, and nitrogen are the main requirements for microbes. Analysis of pH, C-organic, and N-total on the organic matter used was carried out to determine the correct pH value and C/N ratio for SRB growth. Table 3 shows that oil palm empty fruit bunches (OPEFB) have the highest C/N ratio of 74.82, with a C content of 53.12% and N of 0.71%. The high C/N ratio is influenced by the low N content and the high C in OPEFB, which contains high lignin and cellulose. The lower the C/N ratio (< 40) in organic matter indicates that the organic matter has been decomposed by microbes (Tanimu et al., 2014). The pH value of organic matter shows a high number with a value range of 7.60 – 8.68. Phyo et al. (2020) stated that SRB generally favors pH >5.5, which means all types of organic matter are suitable for SRB propagation.

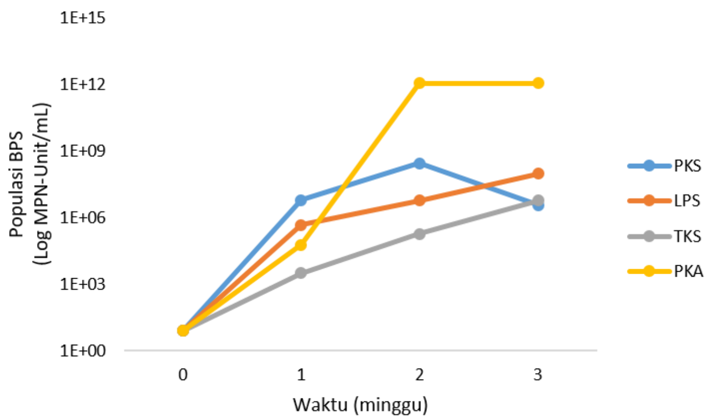


Figure 12 SRB multiplication results

SRB incubation for three weeks showed that all selected organic materials were able to be utilized by SRB to reproduce themselves. The mixture of oil palm empty fruit bunches with chicken manure (PKA) is the best carrier compared to other organic materials. Carrier PKA was able to increase the population of SRB up to 1.0 x 1012 MPN-Unit/mL compared to a mixture of oil palm empty fruit bunches with dairy factory waste (LPS), a mixture of oil palm empty fruit bunches with cow manure (PCS), or with oil palm empty fruit bunches (TKS) only had SRB growth in the range of 1.0 x 106 – 1.0 x 107 MPN-Unit/mL. This is because the mixture of oil palm empty fruit bunches with chicken manure has complete nutrition for the growth of SRB. According to Hartatik and Widowati (2006), broiler chicken manure has a relatively higher P nutrient content than other manure and decomposes relatively quickly, and has a higher nutrient content than other manure.

4.4 Effect of SRB Inoculation on AMD Characteristics

SRB inoculation in acid mine drainage was tested using several depths to determine the effect of SRB activity and population on improving AMD characteristics. The results of Duncan's Multiple Range Test (DMRT) on the final pH value showed that the administration of SRB incubated in dairy factory waste had the highest value (Table 4). In Figure 13, it can be seen that SRB inoculation was able to increase AMD pH beyond six, which is the AMD quality standard value. The Minister of Environment Regulation Number 13 of 2003 states that the permissible pH value of AMD is 6-9, which means that the pH of AMD that has been treated meets the quality standard. The AMD control contained in this study also experienced an increase in pH, presumably due to the presence of dissolved oxygen in the AMD, so that the pH increased.

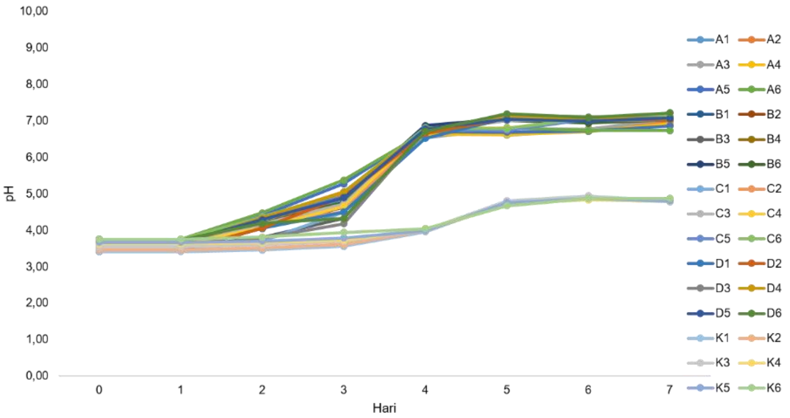


Figure 13 Changes in AMD pH given SRB at multiple depths: A = PKA; B = PKS; C = TKS; D= LPS; K = AMD

Table 6 Comparison of SRB inoculated AMD with control

Treatment	pH	Treatment	pH	Treatment	pH
LPS6	7.21a	TKS5	7.05abc	PKA2	6.97abc
TKS6	7.12ab	TKS4	7.05abc	PKA4	6.92bcd
PKS3	7.09abc	TKS3	7.04abc	PKA5	6.85cd
PKS5	7.08abc	TKS1	7.04abc	PKA6	6.73
LPS3	7.07abc	PKA3	7.04abc	AMD6	4.87e
LPS5	7.07abc	LPS2	7.02abc	AMD2	4.81e
PKS4	7.07abc	TKS2	7.02abc	AMD5	4.80e
PKS1	7.06abc	PKS6	7.02abc	AMD3	4.79e
LPS4	7.06abc	LPS1	7.02abc	AMD4	4.79e
PKS2	7.05abc	PKA1	6.99abc	AMD1	4.77e
LPS6	7.21a	TKS5	7.05abc	AMD2	6.97abc

Note: TKS = Oil palm empty fruit bunches (OPEFB); PKA= Chicken manure + OPEFB; PKS = Cow manure + OPEFB; LPS = Dairy factory waste + OPEFB; AMD = Acid mine drainage (control). The number after the treatment code indicates the depth of the sample taken: 1 = 25 cm; 2 = 50 cm; 3 = 75 cm; 4 = 100 cm; 5 = 125 cm, 6 = 150 cm. The letters after the results show the difference in treatment at the 95% level

Table 7 Characteristics of treated and control AMD

Parameter	**Treatments**				
	PKA	**PKS**	**LPS**	**TKS**	**AMD**
TSS (mg/L)	183.67a	140.67b	58.00c	30.07c	57.73c
Fe (mg/L)	3.30a	3.33a	2.67a	3.60a	0.06a
Mn (mg/L)	1.00a	0.67a	0.80a	0.83a	0.93a
Cu (mg/L)	0.34a	0.04b	0.06b	0.09b	0.07b
Zn (mg/L)	0.67ab	0.21c	0.33bc	0.17c	0.80a
H_2S (mg/L)	1.18a	0.15a	0.11a	0.20a	<0.01a
SO_4 (mg/L)	9.27c	187.67b	254.00a	163.00b	264.67a
BOD (mg/L)	533.67a	386.67b	347.67b	407.33b	11.70c

Note: TKS = Oil palm empty fruit bunches (OPEFB); PKA= Chicken manure + OPEFB; PKS = Cow manure + OPEFB; LPS = Dairy factory waste + OPEFB; AMD = Acid mine drainage (control). The letters after the results show the difference in treatment at the 95% level

Based on table 5, it is known that dissolved metals Fe and Mn are at the threshold set in the Regulation of the Minister of the Environment Number 13 of 2003. Dissolved Cu and Zn are also at low levels. Iron (Fe) and Manganese (Mn) are included in the heavy metal category. According to Kamarati et al. (2018), Heavy metals are one of the most common types of environmental pollutant substances found in waters, so that they can have a bad impact on aquatic organisms and on humans who use the water. The content of Fe and Mn in AMD is the result of leaching from PAF rock after coal mining. Engwa et al. (2019) explained that Fe is a heavy metal that is useful in the human body because it is a constituent of certain biological molecules such as hemoglobin and is involved in various physiological activities. However, in its free state, iron is one of the heavy metals which is generally known to produce hydroxyl radicals (OH•) as follows:

$$Fe^{3+} + O^{2-} \rightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\bullet + OH^-$$

The hydroxyl radical (OH•) is the most common free radical produced by the oxidation of iron. OH• is able to react with biological molecules such as proteins, lipids and DNA.

The formation of H_2S gas in the treatment given by SRB occurred due to the activity of SRB. Table 6 shows higher H_2S than control. The activity of SRB is also proven by the change in the color of AMD given by SRB to black this is due to the bond between Fe^{2+} and H_2S gas which then forms black and insoluble FeS. The increase in biological oxygen demand (BOD) indicated that the AMD treated was in anoxic condition which supported the growth of SRB.

4.5 Combination of Floating Treatment Wetland and SRB Enrichment

This research was conducted by combining a floating treatment wetland planted with Melaleuca, Nauclea, and Vetiver, then enriched with the presence of sulfate-reducing bacteria at the bottom of the pond. The results showed that the given treatment was able to increase the pH from 2.2 to > 6 (Figure 14).

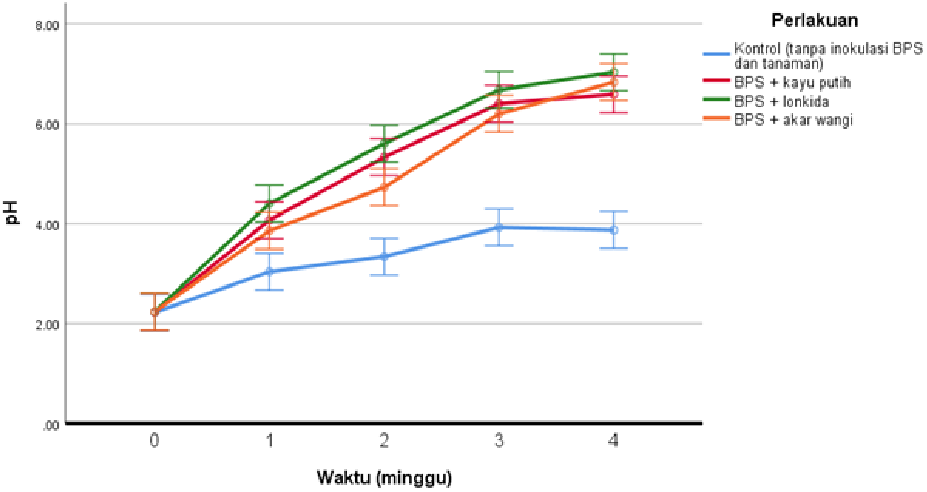


Figure 14 Effect of SRB inoculation on the increase in pH

Table 8 Characteristics of AMD before treatment

Parameter	TSS (mg/L)	Fe (mg/L)	Mn (mg/L)	Cu (mg/L)	Zn (mg/L)	H_2S (mg/L)	SO_4 (mg/L)	BOD (mg/L)
Test results	13.8	7	26	16	10.2	0.028	234	14.3

The presence of enrichment/inoculation of SRB into the system had a significant effect ($P<0.05$) on the increase in water pH. It can be seen in Figure 14 that the control treatment (without SRB inoculation and without plants) increased the pH much more slowly than other treatments inoculated with SRB. This shows that SRB has a significant role in increasing the pH value. The viability of SRB is also determined by the surrounding environmental conditions, one of which is the redox conditions in the waters. Redox potential (Eh) plays an important role in creating anaerobic conditions in accordance with the needs of sulfate-reducing bacteria, where SRB requires anaerobic and anoxic conditions with redox potentials ranging from -300 mV (Prasad et al. 1999).

There is a very close relationship between the potential redox value (Eh) and the pH value. The main cause of the increase in pH is from the addition of organic matter, which is able to create reduction conditions due to the decomposition process of organic matter (Headley and Tanner 2006). This condition causes the iron ion, which was originally in the form of ferric (Fe3+), to be reduced to ferrous ion (Fe2+) and releases one molecule of OH- where OH- plays a role in increasing the pH of the water.

$$Fe(OH)_3 + e^- \longleftrightarrow Fe(OH)_2 + OH^-$$

This reduction reaction can be evidenced by a significant decrease in the value of the redox potential of AMD over time which is presented in Figure 15.

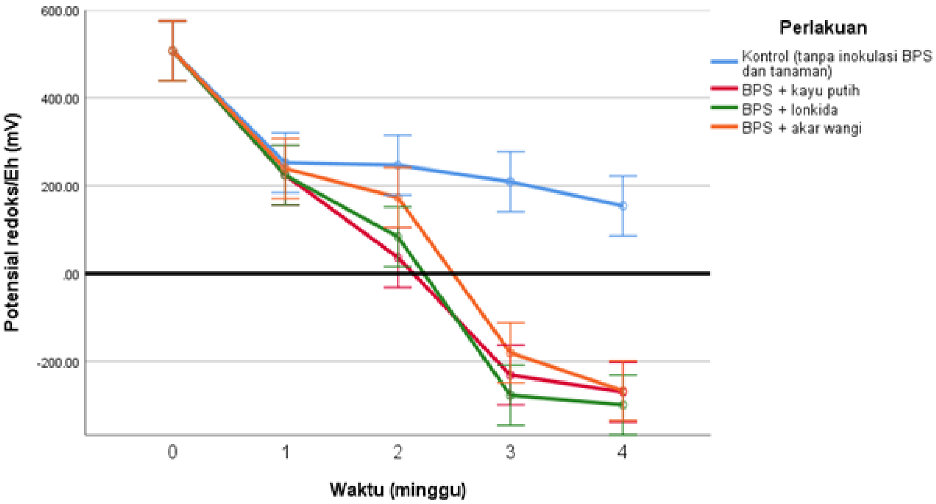


Figure 15 Effect of SRB inoculation on reduction of redox potential (Eh)

Figure 16 Floating treatment wetland

In this study, AMD initially had an Eh value of 507 mV which means it was in an oxidation state, and then the Eh value decreased to between +154 mV in the control treatment and up to -270 mV in the other treatment. The control treatment has not reached the reduction condition, and this has an impact on the pH increase, which tends to be slower. The slower increase in pH is also caused by the presence of SRB. The control treatment was not inoculated with SRB, so that the population was much smaller than other treatments inoculated with SRB. It can be seen from Figure 17, which shows the population of SRB.

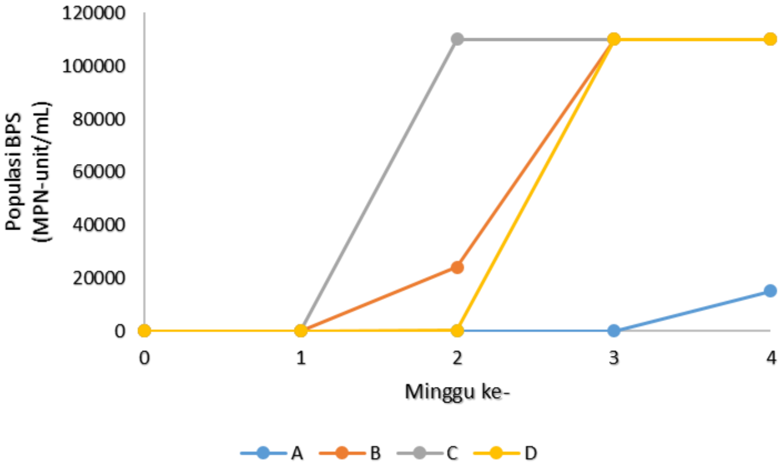


Figure 17 SRB population growth during incubation: A = OPEFB; B = SRB + Melaleuca; C = SRB + Nauclea; D = SRB + Vetiver

Figure 18 Measurement of Eh

Sulfate-reducing bacteria (SRB) isolated from the combination of floating treatment and SRB showed that the population of SRB increased in all treatments. The SRB treatment plus longkida showed the highest SRB growth. This is supported by the low redox potential. The low Eh indicates the growing environmental conditions of SRB have low dissolved oxygen. Krekeler et al. (1998) explained that oxygen inactivates various enzymes of sulfate-reducing bacteria, and exposure to oxygen for hours or days reduces their viability. Dolla et al. (2006) added, some SRB has a tolerance to oxygen, but not for a long time. Desulfovibrio desulfuricans NCIB8301 was able to grow in an environment exposed to oxygen for the first 24 hours but decreased drastically when it was longer. This is supported by the low growth of SRB on control treatment (without SRB inoculation and without plants) (Figure 19).

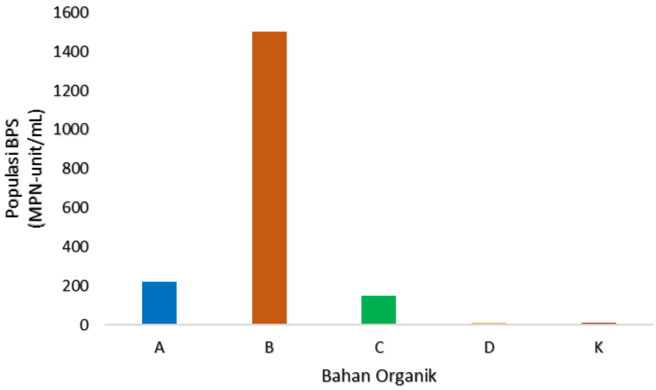
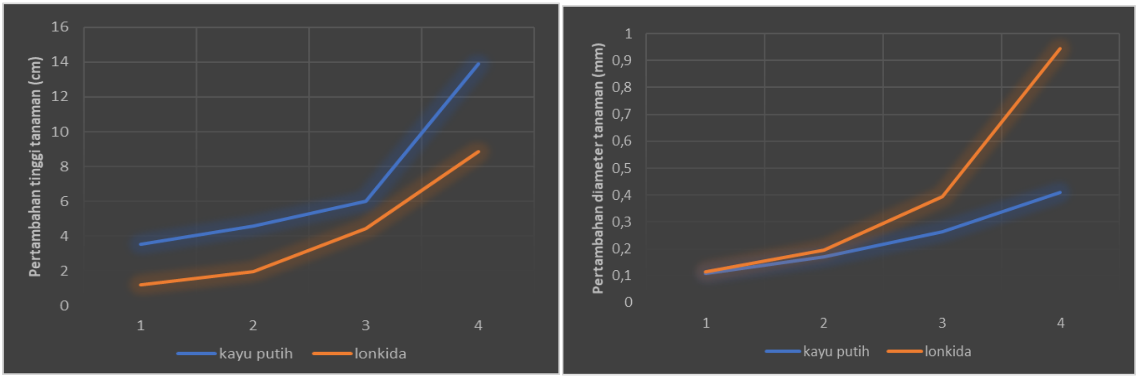


Figure 19 Population of SRB in organic matter

Figure 20 Measurement of plant diameter

In addition to water quality, plant growth was also observed in this study. The response of seedling growth to the effect of AMD can be seen from the increase in diameter and height of seedlings presented in Figure 21.



(a) (b)

Figure 21 Increase in height and diameter of Melaleuca and Nauclea in a floating treatment wetland affected by AMD

The increase in diameter is a secondary growth in plants. Secondary growth is initiated by the cambium, which is present in the vascular tissue. This cambium continuously produces vascular tissue, namely the xylem and phloem. Plant height is a plant size that is often observed, both as an indicator of growth and as a parameter used to measure the effect of the environment or the treatment applied. This is based on the fact that plant height is the most easily visible measure of growth (Sitompul and Guritno 1995). The average height increase of Melaleuca in this study reached 13.9 cm, while Nauclea were only able to reach 8.8 cm during the four weeks of observation. Meanwhile, the average increase in diameter of Nauclea in this study reached 0.95 mm, while for Melaleuca, it was only 0.4 mm during the 4-week observation period. In addition to the increase in plant height and diameter, plant morphology was also observed after being inundated with acid mine drainage, which can be seen in Figure 22.

(a)

(b)

Figure 22 Root forms of plants: (a) Vetiver, Nauclea, and Melaleuca after being flooded with acid water and (b) on normal soil

From Figure 22, it can be seen that there are differences in the morphology of plant roots that grow on normal land and after being flooded with acid water. Plants that are flooded with acid water tend to have more massive roots and have more fibrous roots than plants that live on normal land. This is a form of adaptation of plants that are inundated with water to increase the average survival of tolerant species.

5. CONCLUSION

Based on the research that has been done, it can be concluded that the addition of organic matter floating on the surface can increase the pH and reduce dissolved heavy metals at a depth of 50 cm, 100 cm, and 150 cm. The best organic material that is appropriate to increase the population of SRB is a mixture of oil palm empty fruit bunches with chicken manure, while the best planting medium is oil palm empty bunches. In the application of floating treatment wetland, the addition of SRB inoculation had an effect on the AMD neutralization process, and the population of sulfate-reducing bacteria increased during four weeks of incubation.

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Profile

Lecturer and senior researchers of silviculture and have 26 years experience in ex-mining land reclamation. Passive acid mine drainage management, namely the development of swamp forests is one of the innovations that have been tested in the field but continues to be refined to obtain more stable results.

Educations

PhD, 2000 Department Biosciences, University of Kent at Canterbury, England
MForSc, 1994 School of Forestry University of Canterbury at Christchurch, New Zealand Ir, 1988 Faculty of Forestry IPB University

Work Experiences

2015-2020 Director SEAMEO BIOTROP
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2003-2005 Head of Department of Forest Management, Faculty of Forestry, IPB University 1990-now Lecturer at IPB University

Publication

Kodir A, Hartono DM, Haeruman H, **Mansur I**. 2017. Integrated post mining landscape for sustainable land use: A case study in South Sumatera, Indonesia. *Sustainable Environment Research*. 27: 203-213.

Kristanti R, Kartodihardjo H, Nugroho B, **Mansur I**. 2019. Institutional performance of mining reclamation in forest areas of East Kalimantan. *Jurnal Manajemen Hutan Tropika*. 25(2): 69-81.

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Profile

An expert research engineer mastered on remote sensing for city planning, forestry and mining, grew up in a regional research institute since 2000 and has experience in managing projects from conception to completion.

Educations

MSi, 2017 IPB University, Soil Management and Land Resources Department. Disaster Mitigation and Land Degradation Study Program. Thesis: Aplikasi penginderaan jauh dan SIG dalam mitigasi dan pengelolaan air asam tambang.

S.Si, 2002 Department of Geophysics meteorology, Faculty of Mathematics and Natural Sciences, IPB University

Work Experiences

2018 – … Research Assistant at Biosystem and Landscape Management Lab SEAMEO BIOTROP
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2018. Yusmur A, Ardiansyah M, Mansur I. Identifikasi dan Mitigasi Air Asam Tambang Melalui Hutan Rawa Buatan di Lahan Pasca Tambang.

2011. Pujirahayu Y, AFM Zain, Yusmur A. Identifikasi Karakteristik Ruang Terbuka Hijau 2010. A Anggraeni, HA Adrianto, AFM Zain, A Yusmur. Sistem Informasi Geografi Fasilitas

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2010. Pranita I,AFM Zain, Yusmur A. Identifikasi Karakteristik Ruang Terbuka Hijau di Kota- Kota Pantai Indonesia (Studi Kasus Kota Padang, Denpasar, dan Makassar)

2008. Faikoh, AFM Zain, Yusmur A. Deteksi Perubahan Ruang Terbuka Hijau di Kota Industri Cilegon

2008. Meiliyani, AFM Zain, Yusmur A. Identifikasi Perubahan Penggunaan dan Penutupan Lahan di Kota Palembang dari Zaman Klasik hingga Kemerdekaan (683-2007)

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Profile

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2019. Sekarjannah FA, Wardoyo SS, Ratih YW. Management of mine acid drainage in a constructed wetland using hyacinth plant and addition of organic materials. *Journal of Degraded and Mining Lands Management.*

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Bachelor of forestry from Silviculture Department, Faculty of Forestry, IPB University, that interested to post-mining land reclamations with thesis titled "Respons Pertumbuhan Bibit Kayu Kuku (*Pericopsis Mooniana* THW.) pada Media Tailing yang Diberi Perlakuan Kascing, *Rhizobium*, dan FMA". Currently pursuing a master's degree at the Environmental and Soil Biotechnology study program, Soil Science and Land Resources Department, IPB University focus on acid mine drainage bioremediaton.

Educations

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