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# Acid Mine Drainage Treatment with Organic Waste in Constructed Wetlands – Effluent Recirculation

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#### ABSTRACT

Vertical subsurface flow constructed wetland (VSSF-CW) was evaluated to neutralise acid mine drainage (AMD) using organic waste and planted with mangroves. The type and composition of media, as well as the improper assembly and operation of the system, are among the reasons why the wetland system has not been effective and efficient so far. The main objective of this research is to develop a method to neutralise AMD using organic waste (oil palm empty fruit bunches and eucalyptus leaf waste). To achieve this research's main goal, the steps were to analyse the characteristics of AMD at the research site, analyse the type and composition of organic waste, determine the retention time, and analyse the concentration of contaminants in the water after the treatment process. To implement the stages, sample preparation, plant acclimatisation, organic material selection, primary characterisation of samples, assembly of the CW reactor, and operation of the system were carried out. After the study, it was found that the system maximally increased the pH from 3.32 to 7.34 in the 12-day retention time oil palm empty fruit bunches reactor, and maximally removed total suspended solids (TSS) and manganese (Mn) with efficiencies of 97.52% (from 444 to 11 mg/L); and 95.97% (from 4.47 to 0.18 mg/L) in the 12-day retention time eucalyptus leaf waste reactor, respectively. Rhizophora sp. showed bioaccumulation ability > 1 (accumulator) and translocation < 1 (phytostabiliser). The media type and composition, as well as the assembly and operation of the system in this study successfully neutralised AMD with good efficiency and a relatively short time. In addition, the addition of mangrove plants and fly ash-bottom ash (FABA) bricks, also contributed to the good results of AMD treatment and also became an innovation in AMD treatment.

**Keywords:** retention time, oil palm empty fruit bunches, eucalyptus leaf waste, *Rhizophora* sp., fly ash-bottom ash (FABA) bricks.

#### INTRODUCTION

Human-induced pollution of natural resources or natural contamination is a growing problem [Tony and Lin, 2020]. Acid mine drainage, resulting from surface coal mining activities, is currently one of the primary environmental challenges confronting the mining industry [Hengen et al., 2014]. AMD or acid metalliferous drainage is wastewater formed as a result of mining activities. AMD is a global issue and causes harmful environmental impacts. AMD is considered a pollutant because of its high acidity (pH < 4), high concentrations of metalloids or toxic metal ions (Fe, Zn, Cd, Al, Cu, Pb, etc.), contain dissolved anions (sulfate, nitrate, chloride, arsenate, etc.), and suspended solids (SS), making it toxic to plants, animals and humans [Dhir, 2018; Du et al., 2022]. The formation of AMD is a natural phenomenon and cannot be stopped. So it is necessary to plan for proper treatment [Patel et al., 2018]. Traditional AMD treatment has long been widely applied. However, there are some limitations such as low efficiency and secondary

contamination, so the method is replaced by other bio-based methods [Du et al., 2022]. Biological techniques using microbes are a competitive alternative to recovering dissolved heavy metals in AMD [Ayangbenro et al., 2018]. Sulfate dissimilation reduction (DSR)-based techniques mediated by sulfate-reducing bacteria (SRB) have been widely used in sulfate-containing wastewater treatment systems, particularly for AMD, groundwater, sewage, and industrial wastewater remediation [Qian et al., 2019]. Sediments can be used as a source of inoculum for sulfate-reducing bacteria [Fahruddin et al., 2014]. Fahruddin et al. [2021] reported that sulfate-reducing bacterial isolates from swamp sediments and the provision of organic materials such as compost were able to reduce sulfate levels in AMD.

Many methods to neutralise AMD have poor performance, high cost, use of hazardous chemicals, depletion of natural resources, and generate new waste. So because of these limitations and this need will continue, it is necessary to use waste materials or by-products from other industries for AMD remediation [Moodley et al., 2017]. SRB require a carbon source to support their growth which is used as an electron donor to reduce sulfate in AMD. Biomass solid waste has good prospects as a carbon source for biological wastewater treatment [Nielsen et al., 2019; Chen et al., 2022]. Organic waste from agro-industrial activities that can be utilised in AMD treatment is oil palm empty fruit bunches (EFB). In addition to EFB, another organic waste that can be utilised is eucalyptus leaf distillation waste (ELW). So this research seeks to employ both wastes to neutralise AMD.

Another waste that can be utilised in AMD treatment is fly ash-bottom ash (FABA). Win et al. [2020] reported that mixing FABA and organic matter is effective in neutralising AMD by consuming oxygen in the upper layer and reducing dissolved metal concentrations. The alkalinity content of lime (CaO) and dolomite contained in FABA will produce hydroxide ions that will consume acidity and increase pH. Mangroves have been widely researched as plants capable of absorbing and accumulating heavy metals in their tissues [Wilda et al., 2020]. Rhizophora mucronate mangroves are metal-tolerant and able to accumulate large amounts of metals in their root tissues [Nualla-ong et al., 2020]. Also reported mangrove Rhizophora mucronate was able to accumulate Cu, Fe, Mg, Zn, Al, Co, Mn, and Cr metals [Mullai et al., 2014].

There has been a lot of research on neutralising AMD with constructed wetlands (CW) that utilise organic matter, but it still results in low efficiency and long treatment times. The type and composition of media, as well as improper assembly and operation of the system, can be one of the reasons why the CW system is still not effective and efficient. As a form of solving these problems, we conducted an AMD treatment experiment with CW which was preceded by the identification and analysis of waste around the mining site, then calculated the type and composition of media, wastewater retention time, reactor dimensions, and hydraulic loading rate. Wastewater in the research location is very acidic, so materials are needed that can neutralise the pH of the water. The material used in this study is organic waste, namely eucalyptus ELW and oil palm EFB. This waste has advantages over other organic wastes, namely having a neutral pH (pH 9,1 for EFB and pH 7.5 for ELW) so that it can increase pH quickly, besides that, the amount is also abundant in tropical countries. However, the disadvantage that may occur is that the use of organic materials can cause unpleasant odours if the wastewater is in the reactor for a long time, so it is necessary to consider a residence time that is not too long. This research not only exploits the potential of organic materials in neutralising AMD, but also tries to exploit the potential of FABA (fly ash-bottom ash), which is also a coal processing industry waste that can be a substitute for limestone which is commonly used as an alkaline material for AMD. To the best of the author's knowledge, the combination of organic waste, FABA bricks, wetland sediments, and mangrove plants in a CW system in AMD neutralisation has never been done.

#### MATERIAL AND METHODS

AMD, wetland sediments, ELW, oil palm EFB, *Rhizophora* sp. mangrove plants, filter paper, aluminium foil, cotton, tissue paper, distilled water, buffer solution, acetylene gas  $(C_2H_2)$ , Mn metal mother liquor, working solution with 3 different levels, concentrated nitric acid (HNO<sub>3</sub>) p.a, 0.05 M HNO<sub>3</sub> diluent solution, 5% (v/v) HNO<sub>3</sub> wash solution, and compressed air, microbial growth media, 5 mm glass, and glass glue.

Madia	Retention time (RT)		
wedia	T1	T2	
K0 (control)	K0T1	K0T2	
M1	M1T1	M1T2	
M2	M2T1	M2T2	

 Table 1. Research matrix

**Notes:** K0 = control, without organic matter; M1 = eucalyptus leaf waste (ELW); M2 = oil palm empty fruit bunches (EFB); T1 = 12 – day retention time; T2 = 6 – day retention time.

#### **Research matrix**

Table 1 shows the research matrix used. There are 6 types of treatment variations in this study. Based on this matrix, the treatment application in the CW wetlands for AMD treatment in this study is as follows:

- K0T1 = AMD + FABA bricks + sediment + plants (control) (12-day retention time)
- K0T2 = AMD + FABA bricks + sediment + plants (control) (6-day retention time)
- M1T1 = AMD + FABA bricks + sediment + ELW + plants (12 - day retention time)
- M2T1 = AMD + FABA bricks + sediment + EFB + plants (12-day retention time)
- M1T2 = AMD + FABA bricks + sediment + ELW + plants (6-day retention time)
- M2T2 = AMD + FABA bricks + sediment + EFB + plants (6-day retention time)

#### Laboratory-scale CW configuration

The mini-constructed wetlands were designed on a laboratory scale with a length of 80 cm, width of 40 cm, and height of 50 cm. The CW reactors in this study were designed as many as six pieces. The type of water flow in this reactor is a vertical subsurface flow (VSSF), water flows from top to bottom vertically and exits through the tap at the bottom of the reactor. The reactor consists of an AMD basin (inlet basin/reservoir), a reaction basin or treatment basin (reactor), and an AMD basin that has gone through the reactor (outlet basin). The reactor was filled with 10 kg each of EFB and ELW, sediment (± 20 kg), and 10 pieces of FABA bricks, then each mangrove was planted in the sediment. The Rhizophora sp. mangroves used were 6 months old and had the same average weight and height. Figure 1 illustrates the design and size of the reactor in this study and Figure 2 illustrates the material composition in CW.

#### Sample preparation and plant acclimatisation

The AMD that has been obtained is put into each inlet tub as much as 100 L. The manufacture of FABA bricks refers to the research of Saputra et al. [2021] and has been modified to adjust to the amount of wastewater and the conditions of this study. The FABA mixture used was 500



Figure 1. Illustrates the design and size of the reactor in this study



Figure 2. Material composition in CW

g then 250 g of cement, was moulded, and dried. This is the composition of FABA bricks for each reactor. All organic wastes that had been obtained at the research site were sun-dried and reduced in size to expand the surface area. The mangrove plants were acclimatised for two weeks by being planted in sediment and treated with AMD.

#### Screening of organic matter species

The screening process was carried out concerning the research of Othman et al. [2015] which has been modified according to the needs of this study, namely by using 50 grams of each type of organic material to neutralise every 500 mL of AMD, then recording changes in pH. The retention times used were 1 hour, 2 hours, 3 hours, 4 hours, 5 hours, and 24 hours. This experiment was conducted in duplicate. Changes in the pH of AMD in each container containing different organic materials (Fig. 3) show the results of the screening of organic materials that can increase the pH most optimally.

## Characterisation of acid mine drainage, sediment, organic matter, and plant

The AMD obtained was analysed for pH, TSS, and manganese (Mn) as initial characteristics of AMD before treatment by the procedures set out in the Indonesian National Standard Method of Water and Wastewater Examination. Table 2 shows

the characteristics of AMD at the research site. Sediment characterisation includes measurements of C-organic (Walkley and Black method), total N (Kjeldahl method), C/N, P (spectrophotometric method), K (flame photometric method), CEC (NHOAc pH 7.0), Mn (atomic absorption spectrophotometer method), total microorganisms (MPN method) as described in the research of Perala et al. [2020], and sediment porosity. Table 3 shows the results of the initial characterisation of sediments. Organic matter characterisation includes analysis of C-organic (LOI method), N-total (Kjeldahl method), C/N ratio, and moisture content (gravimetry method). Table 4 shows the results of the initial characterisation of organic matter. After completing the experiment, we also conducted metal analyses of sediment and organic matter from each reactor. Metal content analysis was also carried out on the plants before and after the acclimatisation process.

#### **Operation of CW**

The AMD treated in this study is 100 L each which is accommodated in an inlet tub with a capacity of 100 L which serves as a reservoir for the AMD source. This reactor has a surface area of  $0.33 \text{ m}^2$ . The inlet basin was given a tap and the flow rate was set at 16 L/day (5.5 mL/min) for a retention time of 12 days and 8 L/day (11 mL/min) for a retention time of 6 days from 100 L of AMD in each reactor, so that the hydraulic loading rate (HLR) applied to the system was  $0.02 \text{ md}^{-1}$  and

0.05 md<sup>-1</sup>. The calculation of HLR is listed in Eq. (1). The sewage treatment process takes place in a continuous recirculation system until optimum treatment results are obtained. Water will come out of the outlet tap and will be collected in the outlet basin then the recirculation process will occur.

$$Hydraulic loading rate (HLR, md-1) = = \frac{Flow rate m3d-1}{Surface area (m2)}$$
(1)

#### Sample analysis

The analysed water came from the inlet basin and outlet basin. Analysis of treated water consists of pH, TSS, and Mn. pH analysis is carried out using a pH meter, TSS measurement by gravimetric method, and Mn measurement by atomic absorption spectrophotometer (AAS). The calculation of contaminant removal efficiency in water is listed in Eq. (2). Sample testing will be carried out in duplicate from the outlet and inlet. Sediment testing after the treatment process is the analysis of Mn metal content.

$$\frac{RE (\%) =}{\frac{Initial \ concentration - final \ concentration}{Initial \ concentration}} \times 100^{(2)}$$

After 22 days of the experiment, the plants that emerged on the CW were harvested for chemical analysis in the form of Mn content measurement. All plant samples were dried and ground to a fine powder (< 2 mm) for elemental analysis. The plant elemental analysis method was described by Singh and Chakraborty [2020]. Plant height was also recorded before and after the AMD treatment process. In addition, the BCF and TF values of plants were also analysed. The *BCF* describes the phytoaccumulation ability of plants, which is defined as the ratio of total metal content in plant tissues ( $C_p$ , mg/kg) to total metal content in topsoil ( $C_s$ , mg/kg). The calculation of the BCF value of plants is listed in Eq. 3 [Dan et al., 2017]:

$$BCF = \frac{C_p}{C_s} \tag{3}$$

Meanwhile, the translocation factor (TF) was used to calculate the ratio between heavy metal

concentrations in leaves ( $C_a$ , mg/kg) and roots ( $C_s$ , mg/kg). The calculation of the *TF* value of plants is listed in Eq. 4 [Ayujawi and Takarina, 2020]:

$$TF = \frac{c_a}{c_s} \tag{4}$$

This study uses statistical analysis using twoway ANOVA to determine the effect of media variation and detention time on changes in pH, TSS, and Mn of AMD.

#### **RESULTS AND DISCUSSION**

#### Characteristics of acid mine drainage (AMD)

AMD in this study was taken from one of the coal mining industries in South Sumatera, Indonesia. The characteristics of AMD at the site at the time of collection were observed (Table 2). From Table 2, it is known that the pH, TSS, and Mn of AMD do not meet the South Sumatera Indonesian coal mining wastewater quality standards. As seen in Table 2, AMD at the location is very acidic at 3.32, with TSS content of 444 mg/L, and Mn 4.47mg/L.

#### **Results of organic matter screening**

This study tested five plant biomasses around the study site that are thought to increase the pH of AMD. Dried areca nut husks, ELW, and EFB have the fastest ability to increase the pH of AMD (pH 7), but in the screening of organic materials to treat AMD, organic materials that are abundant and easy to obtain are needed, so EFB and ELW were selected as organic materials in this experiment. The results of organic matter screening are illustrated in Figure 3.

#### Results of sediment and organic matter characterisation

#### Sediment

The results of the initial characterisation of sediments before use in the CW were observed

Table 2. Characteristics of AMD at the coal mining site

Parameter	Value	Quality standard of South Sumatera Indonesian coal mining liquid waste
рН	3.32	6–9
TSS (mg/L)	444	300
Mn (mg/L)	4.47	4



Figure 3. Results of organic matter screening

Parameter	Methods	Value	Status	Reference
C-Organic (%)	Walkley & Black	4.46	Optimum	
N-Total (%)	Kjeldahl	0.12	Very low	-
Ratio C/N	_	37.16	Very high	[Abuve et al., 2021;
P (ppm)	Spectrophotometer	371	Very high	Fitrihidaiati et al 2021
K (ppm)	Flame photometer	4.279	Very high	
Cation exchange capacity (CEC) (cmol <sup>(+)</sup> /kg)	N NHOAc pH 7.0	19.93	Optimum	
Mn (ppm)	AAS	266	Toxic	OSWER Directive 9285.7-71 [Bitondo et al., 2013]
Microorganisms total CFU/g (106)	MPN	10.68	_	_
Porosity (%)	Gravimetry (ring sample)	60	Good	[Riskawati et al., 2021]

**Table 3.** Results of the initial characterisation of sediments

Notes: determination of the status of each soil trait refers to the above references

(Table 3). Based on Table 3, it is known that the sediment has a high content of C-organic, P, and K with a high-very high category. The high content of C-organic, P, and K in sediments can have a positive influence on plants and microorganisms for growth and development. The microorganisms contained in this sediment are certainly the SRB group because they can live in an acidic environment and contain sulfates and metals. Based on Table 3, the SRB population is known. The SRB population in the sediment is 10.68 CFU/g (×10<sup>6</sup>), which means that each gram of sediment contains  $10.68 (\times 10^6)$  cells forming SRB colonies. Because the sediment already contains SRB, there is no need to inoculate sulfate-reducing bacteria into the reactor. The addition of organic media to the sediment can maintain and increase the SRB population. In Table 3 it is known that the sediment also contains high Mn, so that metals not only come from AMD but also from sediments, as a result, organic matter and plants not only absorb and adsorb metals from water but also from sediments.

**Table 4.** Results of the initial characterisation of organic materials

Parameter	Methods	ELW	EFB
C-organic (%)	LOI	52.65	55.60
N-total (%)	Kjeldahl	1.53	0.41
C/N	_	34	135
Water content (%)	Gravimetry	25.57	8.51

Notes: ELW (eucalyptus leave waste); EFB (oil palm empty fruit bunches).

#### Organic matter

The results of the initial characterisation of organic matter before use in CW were observed (Table 4). Based on Table 4, it is known that both organic materials have a C-organic content above 50%. The C-organic and N-total content of ELW is higher than that of EFB and the C/N of EFB is much higher than that of ELW. The high C/N ratio indicates that the material takes a long time to decompose. A high C/N ratio can be caused by low nitrogen content [Pranata et al. 2022]. So it can be seen that the organic matter content in EFB is more difficult to be decomposed and utilised by sulfate-reducing microorganisms compared to ELW and it can be assumed that ELW is better than EFB in terms of its ability to provide a carbon source for SRB.

#### **Results of AMD treatment**

рΗ

In Figure 4 it can be seen that there is an increasing trend in pH in each treatment. In all

control treatments on the first day of the processing process, the pH decreased, but on the 3rd day, the pH had increased from the previous day. The control treatment with a 12-day retention time (K0T1) showed a faster increase in pH value compared to the control treatment with a 6-day retention time (K0T2). The slow increase in pH and the decrease in pH in the control treatment can be caused by the sludge used, which may still be PAF (potential acid forming) material, which is material that still has the potential to produce acid. Based on research conducted by Situru et al. [2019], PAF material is a material that has the potential to form AMD.

In CW using eucalyptus leaf waste organic matter and oil palm empty fruit bunches, the water coming out of the outlet met the environmental quality standards on the first day (pH 7–9). In CW using organic media ELW and EFB with a retention time of 12 days and a retention time of 6 days, respectively, showed a rapid trend of increasing pH. CW M1T1; M2T1; M1T2; and M2T2 were respectively able to increase the pH from the initial pH of 3.32 to 7.17; 7.35; 7.01; and 7.19. Based



Figure 4. Result of pH analysis at the outlet (D = day; RT = retention time; K0T1 = control 12-day RT; K0T2 = control 6-day RT; M1T1 = ELW 12-day RT; M2T1 = EFB 12-day RT; M1T2 = ELW 6-day RT; and M2T2 = EFB 6-day RT)

on statistical analysis, a significant value was obtained for the effect of treatment (media variation) on pH value (Sig.) < 0.05, thus indicating that there is a significant effect of media variation on changes in pH of AMD and a significance value was also obtained for the effect of retention time variation (Sig.) < 0.05, which also indicates that there is a significant effect of retention time variation on changes in pH of AMD (Fig. 4).

The results of this study indicate that there is an effect of organic matter addition, type of organic matter, and retention time on increasing the pH of AMD. EFB and ELW have the potential to be used as organic materials in AMD treatment. The increase in pH in each treatment is due to the mutual interaction between sulfate-reducing bacteria in sediments, organic matter, and plants. SRB uses organic compounds or H<sub>2</sub> molecules as electron donors (electronic donors) to drive the reduction process of sulfate (external electron acceptor) to sulfide. Sulfate (SO<sub>4</sub><sup>2-</sup>), thiosulfate (S<sub>2</sub>O<sub>3</sub><sup>2-</sup>), and sulfite (SO<sub>3</sub><sup>2-</sup>) in AMD, which are the cause of acidity, can be used by SRB as terminal electron acceptors in its metabolic respiration. This process is known as dissimilatory sulfate reduction. SRB reduces sulphate to sulphide, while the organic matter is oxidised to CO<sub>2</sub>, and acidity is utilised on an ongoing basis, increasing the alkalinity of AMD [Ayala-Parra et al., 2016; Punjungsari et al., 2017]. Sulfate reduction is a reduction process carried out by microbes under anoxic (low  $O_2$ ) conditions that occur when sulphate and biodegradable organic matter are present. Sulfate-reducing bacteria utilise O entering the anoxic environment as a sulfate component (SO<sub>2</sub>) for metabolic processing of biodegradable organics, converting bound S into hydrogen sulfide (HS) gas or into precipitable solid-phase sulfides [Zipper et al., 2018].

Organic matter is also able to neutralise AMD because it contains basic elements, namely Na, K, Ca, and Mg so that H<sup>+</sup> ions as a source of AMD acidity can be bound by several basic elements



Figure 5. Result of TSS analysis at the outlet (D = day; RT = retention time; K0T1 = control 12-day RT; K0T2 = control 6-day RT; M1T1 = ELW 12-day RT; M2T1 = EFB 12-day RT; M1T2 = ELW 6-day RT; and M2T2 = EFB 6-day RT)

from organic matter [Nugraha et al., 2020]. Plants can naturally bind sulfide in various forms (carbonates, amino acids, esters, polysaccharides) which can then produce  $H_2S$  and will precipitate in the tissue if Fe is present [Robin et al., 2021]. In addition to the role of SRB, organic matter, and plants, FABA is also a supporting component to increase the pH of AMD. Fly ash-bottom ash (FABA) contains several chemical elements, one of which is silica (SiO<sub>2</sub>) [Fadhilah et al., 2022]. Silicate minerals are the main source of acid neutralisers in the environment [Nugraha and Rolliyah 2021].

#### Total suspended solids (TSS)

Total suspended solids (TSS) can include silt, clay, metal oxides, sulphides, algae, bacteria, fungi, and inorganic particles. TSS concentrations can be removed by sedimentation and by the activity of microorganisms, as well as plants [Gargallo et al., 2016]. Figure 5 shows that in CW control K0T1 and K0T2, the first day has shown a decrease in TSS levels. In CW K0T1 was able to reduce the initial TSS level of 444 mg/L to 22 mg/L and in CW K0T2 was able to reduce TSS levels to 40 mg/L.

In CW using organic media (ELW and EFB), each also shows a significant trend of TSS reduction and starting from the first day has been able to reduce TSS levels under the threshold of environmental quality standards. The TSS removal ability decreases as time goes by. On the last day of observation, it can be seen that CW M1T1; M2T1; M1T2; and M2T2 were each able to remove TSS levels from 444 mg/L to; 11 mg/L; 44 mg/L; 20 mg/L; 70 mg/L. The reactor with ELW organic matter and 12-day retention time has the greatest TSS removal ability compared to other treatments. Based on statistical analysis, a significance value was obtained for the effect of treatment (media variation) on TSS value (Sig.) < 0.05, thus indicating that there is a significant effect of media variation on changes in TSS of AMD and a significance value was also obtained for the effect of retention time variation (Sig.) < 0.05, which also indicates that there is a significant effect of retention time variation on changes in TSS of AMD.

The decrease in TSS levels in constructed wetlands can be influenced by the presence of plants. Plant root hairs have a positive charge that can



**Figure 6.** Results of manganese (Mn) analysis at the outlet (QS = quality standard; D = day; RT = retention time; K0T1 = control 12-day RT; K0T2 = control 6-day RT; M1T1 = ELW 12-day RT; M2T1 = EFB 12-day RT; M1T2 = ELW 6-day RT; and M2T2 = EFB 6-day RT)

function in attracting colloidal particles in AMD that have opposite charges such as suspended solids in water. Nugraha et al. [2020] stated that a decrease in TSS content can occur through physical processes such as sedimentation and filtration. The filtration process occurs because wastewater passes through plant root tissue and media.

From the results of the study, it is known that there was an increase in TSS in treatments that used organic materials. The decrease in TSS removal efficiency as retention time progresses can be caused by a lot of biomass that is formed so there is a high possibility of biomass escaping into the effluent [Rokhmadhoni and Marsono, 2019].

#### Manganese (Mn)

Figure 6 shows that both control treatments also experienced an increase in Mn metal levels on the first day due to a decrease in pH. On the following day, there was a decrease in Mn levels in AMD that had passed through the CW control. On the last day of the treatment process, it can be seen that K0T1; and K0T2 were each able to reduce the initial Mn level of 4.47 mg/L to 2.30 mg/L; 2.95 mg/L.

The treatment using ELW and EFB was able to reduce Mn levels in AMD greater than the control treatment and from the first day had met the environmental standards set in Indonesia. The Mn removal ability decreased as time passed. CW M1T1; M2T1; M1T2; and M2T2 were able to remove Mn from a concentration of 4.47 mg/L to 0.16 mg/L; 0.49 mg/L; 0.36 mg/L; 1.42 mg/L, respectively. Reactor with ELW organic matter and 12-day retention time has the greatest Mn removal ability compared to other treatments. Based on statistical analysis, a significance value was obtained for the effect of treatment (media variation) on Mn value (Sig.) < 0.05, thus indicating that there is a significant effect of media variation on changes in Mn of AMD and a significance value was also obtained for the effect of retention time variation (Sig.) < 0.05, which also indicates that there is a significant effect of retention time variation on changes in Mn of AMD.

Four possibilities can cause a decrease in dissolved metal concentrations in constructed wetlands, namely: interaction between sulfide ( $S^{2-}$ ) produced in the sulfate reduction process with 2-variable metals (such as  $Mn^{2+}$ ,  $Cu^{2+}$ , and  $Zn^{2+}$ ); metal absorption process by plant tissue; metal adsorption process by organic matter; biosorption process by microorganisms contained in the wetland environment.

The change of sulfuric acid, which is the cause of acidity in AMD, into hydrogen sulfide by the activity of SRB will then react with metal cations (Me<sup>2+</sup>) ("Me" stands for metal) in AMD to form solid-phase metal sulfide MeS<sub>(s)</sub> or solid phase metal hydroxide Me(OH)<sub>2(s)</sub> [Punjungsari, 2017]. Mineral sulfide (MeS) or metal sulfide is very insoluble and will precipitate. It can only dissolve under acidic and/or strongly oxidising conditions because its solubility constant (Ksp) is very low. Me<sup>2+</sup> and sulfide are produced and H<sup>+</sup> will be utilised simultaneously in the chemical process causing an increase in pH in AMD [Ayala-Parra et al., 2016]. Microbial cell surfaces are mostly negatively charged with functional groups, such as hydroxyl, carboxyl, and phosphoryl, which can directly adsorb metal cations, which can potentially be used for bioremediation [Violante, 2013; Ortiz-Castillo et al., 2021]. SRB not only have significant ecological functions, but also plays an important role in the bioremediation of contaminated sites [Li et al., 2018].

In addition to SRB activity, the decrease in Mn levels in AMD can also be caused by the adsorption ability of organic materials composed of cellulose. Plants contain cellulose, which is an organic compound that is the main component of all plants [Jaffar et al., 2020]. Cellulose contained in plants can be utilised in water treatment techniques to remove several contaminants including toxic metals [Albukhari et al., 2019]. Cellulose extracted from bacteria has been reported to be highly efficient in water treatment [Oyewo et al., 2020; Manzoor et al., 2018].

EFB can reduce metal content because it consists of lignin components that contain functional groups and can bind metal ions. The functional groups are hydroxyl (-OH) and carbonyl (-CO = O) groups. In addition, EFB also contains cellulose and hemicellulose which also play a role in reducing metal levels because they contain groups (-OH) that interact with metals. Flavonoids and tannins also have hydroxyl functional groups (-OH) so they can also bind to metals. Phenol compounds (flavonoids and tannins) can function as metal-chelating agents. Apart from EFB, ELW also contains lignin, cellulose, and phenol compounds so it also can adsorb metals [Mardhiati, et al., 2021]. Kaur et al. [2018] reported that the phytochemical compound content of eucalyptus leaves consists of phenol compounds, tannins, and flavonoids.

In addition to the presence of SRB activity and the adsorption ability of organic matter, the decrease in Mn levels in AMD is also caused by the activity of mangrove plants. Mangrove plants *Rhizophora* are metal tolerant. Mangrove plants *Rhizopora stylosa* can bioaccumulate metals Pb, Mn, Cu, Cr, Ni, Fe, Na, and Zn [Robin et al., 2021; Bourgeois et al., 2020]. Mangrove plant *Rhizophora mucronate* can accumulate metals Ni > Mo > Zn > Cu > Cr > Co > Mn > Al > V > Fe [Aboulsoud and Elkhouly, 2023]. Mangrove plants *Rhizophora mangle* is actively able to translocate (from bottom to top) Cd-Cu-Hg-Zn-Mn metals from two different sediment fractions into its leaves [Martínez-Colón et al., 2023]. After the 22-day experiment was completed, it was found that all contaminant parameters in AMD were below the threshold of environmental quality standards applied in Indonesia using constructed wetlands system with a combination of organic waste, FABA bricks,



Figure 7. Metal mass balance (in percentage) after 22 days of trial period of vertical subsurface flow (VSSF) system

and plants. So that this is expected to be a solution to treating AMD globally.

#### Mn accumulation in CW

Most heavy metals are present in the environment and are naturally occurring. Manganese is the most abundant toxic heavy metal in nature, and is found in various levels of oxidation in nature [Mitra et al., 2022]. Based on Figure 7, it can be seen that the AMD treatment system using constructed wetlands that utilises the potential of organic media and Rhizophora sp. mangrove plants can reduce Mn levels from AMD and sediment. Data showed that 3 Rhizophora sp. mangrove plants in each CW were able to absorb Mn around 29-63%. Likewise, ELW organic media can adsorb Mn around 50-58%, and EFB organic media can adsorb around 45-53% of the total Mn charged in CW, so that the remaining Mn in the sediment is around 0.7-35% and in the water is around 0.3-4.56% of the total Mn charged in CW. In this treatment system, there are also unaccounted (missing) Mn levels. This can occur because Mn metal can be removed due to other chemical reactions or accumulated in the cells of soil microorganisms. The percentage of unaccounted Mn metal ranges from 4–21% of the total Mn in CW.

#### **Contaminant removal efficiency**

CW is a sustainable and cost-effective technology commonly used for wastewater reclamation, an alternative to energy-efficient treatment systems. In addition to being a prospective clean energy producer with very low operational costs, CW

 Table 5. Results of contaminant analysis at the inlet after 22 days

Treatment	pН	TSS (mg/L)	Mn (mg/L)
K0T1	4.63	30	2.26
K0T2	4.25	45	2.63
M1T1	7.15	11	0.18
M2T1	7.34	48	0.51
M1T2	7.03	27	0.34
M2T2	7.20	97	1.54

can provide a range of ecosystem services as well as carbon footprint reduction [Kataki et al., 2021].

Every time AMD flows through the reactor, there will be a removal of contaminants in the water, so the recirculation process will also affect the contaminant levels in the inlet basin. As the treatment time and recirculation process progresses, it will cause a reduction in water contaminant levels in each inlet basin. After 22 days of treatment, the concentration of AMD contaminants in each inlet basin were observed (Table 5). Based on the results of the study, the efficiency of contaminant removal in each treatment were observed (Fig.

Table 6. BCF and TF of mangrove Rhizopora sp.

Treatmen <b>t</b>	BCF	<i>Rhizopora</i> sp.	TF	
K0T1	1.06	Dhaat		
K0T2	1.09	RIDOL	0.6	
M1T1	0.74	Stom		
M2T1	0.80	Stelli		
M1T2	0.72			
M2T2	0.75	Leave		



Figure 8. Removal efficiency of acid mine drainage contaminants

Organic matter	Removal contaminant in AMD	Reference
Sugarcane slag, corn cob, and sunflower straw	Cr <sup>6+</sup> , Cr <sup>3+</sup> , and SO <sub>4</sub> <sup>2-</sup>	Wang et al. [2021]
Bamboo chips and cow manure	Fe, Mn, Al, Co, Ni, and Cr	Singh and Chakraborty [2020]
Potato oil, brewery residue, peat, and straw	Sulfate (SO <sub>4</sub> <sup>2-</sup> )	Nielsen et al. [2019]
Cow manure, corncob, and pinewood	$Fe^{2+}$ , $Cu^{2+}$ , $Zn^{2+}$ , and $SO_4^2$	Ruehl and Hiibel [2020]
Biochar	Chalcopyrite	Yang et al. [2020]

Table 7. Previous research studies on AMD treatment with organic matter

8). The removal efficiency of TSS levels in a row from high to low, namely M1T1 (97.52%) > M1T2 (93.92%) > K0T1 (93.24%) > K0T2 (89.86%) > M2T1 (89.20%) > M2T2 (78.15%). Mn removal efficiency from high to low was M1T1 (95.97%) > M1T2 (92.40%) > M2T1 (88.59%) > M2T2 (65.55%) > K1T1 (49.44%) > K1T2 (41.16%).

### Bioconcentration factor (BCF) and translocation factor (TF) of mangrove

After 22 days of the treatment process, mangrove plants that have known manganese metal concentrations in their tissues, can be known for their bioconcentration and translocation abilities. BCF and TF values in mangroves were observed (Table 6). The BCF value of Mn in these plants during the 22-day AMD treatment process was < 1 to > 1 (0.72–1.09). The BCF value of Mn > 1indicates that these plants are accumulators of Mn metal, namely plants that can hoard high concentrations of the metal in their plant tissues and can even exceed the concentration in the soil.

Mangrove plants are analysed for translocation ability as a control mangrove plant after the acclimatisation process as a sample that will represent the translocation ability of mangrove *Rhizophora* sp. in general. Based on the table above, it can also be seen that the TF value of Mn is equal to < 1. If the TF value < 1 then the mechanism that occurs in plants is a phytostabilisation mechanism which is a process carried out by plants to transform pollutants in the soil into non-toxic compounds without absorbing the pollutants into the plant body. The results of the transformation of these pollutants in the soil, thus making heavy metals harmless.

These results are in line with previous studies. Aboulsoud and Elkhouly [2023] reported that *Rhi-zophora mucronate* had BCF values exceeding one and TF values less than one for Mn metal. *Rhizophora mucronate* can be considered a good phytostabiliser of Mn heavy metals capable of reducing their mobility through accumulation by the roots. Previous research studies that used organic matter in removing contaminants in AMD was observed (Table 7).

#### CONCLUSIONS

The development of a method for neutralising AMD using the potential of organic waste in CW mangrove plants has been successfully carried out because it can neutralise AMD with quite good efficiency. After 22 days of AMD treatment process, the CW system with 12 and 6 days of hydraulic retention time effectively increased the pH and removed TSS and Mn of AMD. pH increased in the order of: M2T1 (7.34) > M2T2 (7.20) > M1T1 (7.15) > M1T2 (7.03) > K0T1 (4.63) > K0T2 (4.25). TSS removal efficiency in the order: M1T1 97.52% > M1T2 93.92% > K0T1 93.24% > K0T2 89.86% > M2T1 89.20% > M2T2 78.15%. Mn removal efficiency in the order: M1T1 95.97% > M1T2 92.40% > M2T1 88.59% > M2T2 65.55% > K1T1 49.44% > K1T2 41.16%. So it is known that the 12 - day retention time oil palm empty fruit bunches (EFB) reactor (M2T1) can increase the maximum pH (from 3.32 to 7.34) and the 12-day retention time eucalyptus leaf waste (ELW) reactor (M1T1) can remove the maximum TSS and Mn (from 444 to 11 mg/L; from 4.47 to 0.18 mg/L), respectively. Rhizophora sp. showed bioaccumulation ability > 1 (accumulator) and translocation < 1 (phytostabiliser). The accumulation of Mn in CW was, respectively, in mangrove Rhizophora sp. (29-63%), ELW (50-58%), and EFB (45-53%), remaining in the sediment (0.7-35%), in the water (0.3-4.56%), and undetectable (4-21%) of the total Mn in CW. The ineffective and inefficient performance of artificial wetlands so far may be due to inappropriate media selection and composition, as well as design planning and operation of the wastewater conveyance system. The media type and composition, as well as the assembly and operation of the system in this study successfully neutralised AMD

with good efficiency and a relatively short time. The addition of alkaline materials, namely FABA bricks and mangrove plants, also had a good effect in this study. To the best of the author's knowledge, an AMD treatment system utilising a combination of potential wetland sediments from the mining site (which naturally contain sulfate-reducing bacteria), organic waste, FABA bricks, and mangrove plants has not yet been conducted. However, there is still a need for further research on the neutralising capacity of organic matter and FABA bricks, as well as the survival of mangrove plants in acidic freshwater.

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