Selection of organic materials potentially used to enhance bioremediation of acid mine drainage

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Abstract

Acid mine drainage (AMD), produced when sulfide minerals are subjected to oxygen and water, is one of the major issues in mining industries. Without proper management, AMD's release to the environment would cause seriously prolonged environmental and health issues, such as increases soil acidity and reduces water quality due to extremely low pH, high sulphate concentration, and heavy metal solubility. AMD treatments are divided into two categories, i.e., active treatment, conducted by applying a chemical to the AMD to neutralize pH and precipitate heavy metals; and passive treatment, which relies on biological and biochemical processes. The active treatment may provide an immediate effect, but costly and yet sustainable; meanwhile, passive treatment takes time to establish and to generate an effect, but it is more economical, sustainable, and environmentally friendly. The wetland system is an example of passive treatment. Therefore, this review focuses on passive treatments, especially the selection of organic materials used in constructed AMD wetland treatment. Organic materials play a central role in the wetland system, i.e., to chelate metal ions, remove sulphate from the solution, increase pH, and growth media for microbes, especially sulphate reducing bacteria (SRB) and plants are grown in the system. Overall, organic materials determine the effectiveness of the wetland system to neutralize AMD passively and sustainably.

Keywords:
acid mine drainage
bioremediation
organic material
sulphate-reducing bacteria

Introduction

Acid mine drainage (AMD) produced in mining operations not only damage water and groundwater quality but also threatens human health and plant physiology (Macingova and Luptakova, 2012; Kefeni et al., 2017). This is due to the high acidity and heavy metal concentration (Taberima et al., 2020). Formation of AMD begins with the exposure of rocks containing sulfide minerals, oxidized, accelerated by microbes, and in contact water (Akcil and Koldas, 2006). Several types of sulfide minerals that stimulate the formation of AMD are arsenopyrite (FeAsS), chalcocite (Cu₂S), chalcopyrite (CuFeS₂), galena (PbS), marcasite (FeS₂), millerite (NiS), molybdenite (MoS₂), pyrite (FeS₂), pyrrhotite (Fe₁₋₀₃S), and sphalerite (ZnS) (Skousen et al., 1998; Rambabu et al., 2020). Pyrite is a sulfide mineral commonly found in coal mining areas (Simate and Ndlovu, 2014). Acid mine water is very acidic, has high sulfuric content, also high concentrations of metal elements (Cd, Co, Cr, Cu, Fe, Hg, Ni, Pb, Zn),
metalloids (As, Sb), and other elements such as Al, Ca, Na, Mg, Mn, and Si are high (Lottermoser, 2010).

The reaction equation for the formation of AMD, in general, has been described by Stumm and Morgan (1981). The process of forming acid mine drainage begins with the oxidation of sulfide minerals such as pyrite (FeS₂) by oxygen and water, which is catalyzed by sulfur-oxidizing bacteria such as *Leptospirillum ferrooxidans*, *Thiobacillus ferrooxidans*, and *Thiobacillus thiooxidans* (Schipper, 2004; Johnson and Hallberg, 2005; Cohen, 2006) forms ferrous iron, sulphate, and some protons which produce acidity. Then, the ferrous iron will be oxidized to become ferric iron which consumes one mole of acid, where the ferric iron will oxidize the pyrite abiotically. Ferric iron dissolves more easily, and the rate of pyrite oxidation by ferric iron is two to three times faster than pyrite by oxygen (Williamson and Rimstidt, 1994; Watzlaf et al., 2004). When the pH is between 2.5 to 3, an iron hydrolysis process will occur, which then precipitates iron ions to form Fe(OH)₃ (ferric hydroxide) (Akcil and Koldas, 2006) and causes the pH to become more acidic.

The process of forming AMD will take place as long as the constituent is available in nature. The case in Pennsylvania shows that up to 200 years after the mining operation closed, the state still faces severe AMD problems. In 1989, AMD affected more than 19,300 km rivers and streams and 720 km² lakes and reservoirs in the United States (Kleinmann, 1990); therefore, it is important to mitigate AMD. The mitigation process of AMD can be divided into two, i.e., active and passive neutralization. However, lately, the techniques have been reclassified into abiotic and biological treatment with sub-classes of active and passive systems (Rambabu et al., 2020). Active treatment of AMD is the addition of lime or other alkaline materials, which will increase pH of the water and precipitate dissolved metals in the form of hydroxides and carbonates. The material commonly used for the active treatment is limestone (CaCO₃) or hydrated lime (Ca(OH)₂), where both will react with H₂SO₄ or FeSO₄ to form Ca²⁺ and bicarbonate ions (HCO₃⁻) that can increase the alkalinity of water, heavy metals will be precipitated in the form of metal hydroxide deposits such as Fe(OH)₃, and sulphate will form gypsum (CaSO₄) (Skousen et al., 2017) This treatment requires continuous control and maintenance, high cost, and not suitable long-term application (Simate and Ndlovu, 2014).

Passive treatments, on the other hands, emphasize natural processes to neutralize pH, stabilize and remove pollutants in the AMD (Macingova and Luptakova, 2012). The passive treatment has the advantage of being low maintenance because it does not require continuous input and sludge disposal (Johnson and Hallberg, 2005; Kefeni et al., 2017). The passive treatment also has a lower financial and environmental impact since the technology is durable, low cost, and materials available in nature (Kalin, 2004; Simate and Ndlovu, 2014).

### Passive Treatment

Passive treatment systems no need or need little intervention by workers after initiating the operation (Ochieng et al., 2010). There are passive abiotic and biological systems. The passive abiotic system for AMD commonly relied on the geochemical reaction by using limestone, organic matters, or a combination of the materials. The systems are, among others, open limestone channels (OLC), anoxic limestone drains (ALD), and sulphate reducing bioreactor, limestone leach beds (LLB) (Faulkner and Skousen, 1994; Ford, 2003; Johnson and Hallberg, 2005). Passive biological systems, on the other hand, involving biochemical and biological processes using organic matters, sulphate-reducing bacteria, and plants depend on the method applied. Including in this system are aerobic wetland, anaerobic wetland, SAPS, and sulphate reducing bioreactor (Skousen et al., 2017). Application of the passive treatment type depends on the initial water pH and chemical properties, predicted maximum flow rate, site characteristics, and land availability.

In general, aerobic wetlands are effective for removing metal elements from net-alkaline water. Anoxic limestone drain is suitable for AMD with low concentrations of Al, Fe³⁺, and dissolved oxygen (DO). Meanwhile, anaerobic wetlands, OLC, SAPS, sulphate reducing bioreactor, and LLB could treat net-acidic water with a higher concentration of Al, Fe³⁺, and dissolved oxygen (DO) (Ziemkiewicz et al., 2003; Skousen et al., 2017). In the long term, sustainability needs maintenance, and the high annual rainfall in the humid tropic wetland system is more appropriate for AMD treatment.

### Constructed Wetland Systems

Constructed wetland is engineered to improve the quality of polluted water by involving aquatic plant components, sediments (soil, rock, gravel, organic materials, or others), and microorganisms, with the sun as an energy source in a system with minimum maintenance (Vymazal, 2008; Seadira et al., 2014; Pat-Espadas et al., 2018). Wetlands should have organic-rich substrates containing humic and fulvic acids. Both of these acids can exchange dissolved metals (Wildeman et al., 1991). The sedimentary anoxic zone supports chemical and microbial reduction processes, converting previously soluble iron and sulphate to insoluble hydrogen and sulfide (Fennessy and Mitsch, 1989). Wetlands can improve the quality of water contaminated with heavy metals, nutrients, organic materials, and micro-pollutants using biophysical, biochemical, microbial, plant-assisted processes, including processes of oxidation, reduction, precipitation, sedimentation, biofiltration,
adsorption, complexation, chelation, plant active metal uptake, and microbial immobilization mechanisms (Seadira et al., 2014; Pat-Espadas et al., 2018).

Plants are components of constructed wetland with functions, such as (i) consolidation of the substrate by plant roots and increasing the residence time of water in wetland; (ii) stimulation of microorganism processes through the provision of sites by plants for microbial attachment, removing oxygen from their roots, providing a source of organic material for heterotrophic microbes; (iii) plants provide food and protection for wildlife so that they can form a habitat for wildlife; (iv) wetlands with crops are more aesthetics; (v) metal accumulation (Munawar, 2007). Plants also indirectly contribute to forming a layer of organic litter and particulates that will absorb metals and provide a symbiosis between bacteria that promote metal reduction, adsorption, and deposition mechanisms (Pat-Espadas et al., 2018).

Sulphate-reducing bacteria in a constructed wetland play an essential role in reducing sulphate to sulfide, where sulfide subsequently reacts with metal elements to form metal sulfides then precipitates. This process occurs under anaerobic conditions (Cohen, 2006). The bacteria use sulphate, sulfide, or thiosulphate ions as electron acceptors to get energy in their metabolic processes, while the organic material is an electron donor (Hards and Higgins, 2004). Metabolism of the bacteria requires low molecular weight of carbon sources, such as acetate, ethanol, lactate, pyruvate, etc. An important mechanism for SRB activity is the sulphate reduction process which takes place under anaerobic conditions and optimal environmental factors for sulfide formation.

An organic substrate is needed in a constructed wetland to serve as a growth media and source of nutrients for plants, as well as a carbon source for SRB (Acharya and Kharel, 2020). There are organic compounds effective for sulphate removal, i.e., soluble sugars, starch, amino acids, proteins, cellulose, hemicellulose, and lignin (Skousen et al., 2017). Following is the reaction of sulfide formation and sulphate reduction process, which then reacts with metal cations to form metal sulfides:

$$2\text{CH}_2\text{O} + \text{SO}_4^{2-} \rightarrow \text{H}_2\text{S} + 2\text{HCO}_3^- \quad (8)$$

$$\text{Me}^{2+} + \text{H}_2\text{S} \rightarrow \text{MeS} + 2\text{H}^+ \quad (9)$$

In this reaction, the oxidation of organic materials (electron donor) occurs by reducing sulphate to hydrogen sulfide, which is a biocatalyst by sulphate-reducing bacteria (Widdel, 1988). Also, there is a deposition of dissolved metals where \(\text{Me}^{2+}\) represents cationic metals, such as copper (Cu), iron (Fe), nickel (Ni), and zinc (Zn) to form sulfide deposits, insoluble metals (Drury, 1999).

### Organic Materials for Remediation of AMD

The selection of organic materials used is important because of its effect on plants’ growth, SRB, and determine the required retention time of the AMD. The best organic material provides a short retention time. The shorter the retention time means, the smaller the size of the constructed wetland needed to treat AMD. Table 1 shows the types of organic materials examined to neutralize AMD and the retention time provided.

| Method | System | Retention time | Results | Reference |
|--------|--------|----------------|---------|-----------|
| 300 mL AMD was treated with mixtures of wood chips (3%), leaf compost (30%), poultry manure (20%), silica sand (5%), bacterial source (37%), 2% limestone (2%), and urea (3%) | Batch | Several hours less than 1 day | pH from 5.5-6 to >8.5 | Cocos et al., 2002 |
| Combinations of 25 g of oak chips (OC), 28 g of spent oak from shiitake farms (SOS), 35 g of spent mushroom compost (SMC), 50 g of sludge from a wastepaper recycling plant (SWP), and 30 g of organic-rich soil (ORS) in 200 mL reactors. | Column | Less than 10 weeks | pH increased from 6.8 to >8 | Chang et al., 2000 |
| 300 mL AMD was treated with mixtures of wood chips (3%), leaf compost (30%), poultry manure (20%), silica sand (5%), bacterial source (37%), 2% limestone (2%), and urea (3%) | Batch | Several hours less than 1 day | pH from 5.5-6 to >8.5 | Cocos et al., 2002 |
| 300 mL AMD was treated with mixtures of wood chips (3%), leaf compost (30%), poultry manure (20%), silica sand (5%), bacterial source (37%), 2% limestone (2%), and urea (3%) | Batch | Several hours less than 1 day | pH from 5.5-6 to >8.5 | Cocos et al., 2002 |

Table 1. Organic materials used for treating of acid mine drainage (AMD).
| Method                                                                                                    | System                      | Retention time          | Results                                                                                                    | Reference       |
|----------------------------------------------------------------------------------------------------------|-----------------------------|-------------------------|------------------------------------------------------------------------------------------------------------|-----------------|
| A mixture of 15% creek sediment, 30% calcite, 40% quartz, 15% organic materials (municipal compost, sheep and poultry manures), and oak leaf, added with synthetic AMD of 1:10 | Batch, on the 27th day, 1 g/dm³ of sodium acetate and 1 g/dm³ potassium oxalate was added to the reactor | Less than 30 days       | pH from 2.4 to >6                                                                                           | Gibert et al., 2004 |
|                                                                                                          |                              |                         | Redox potential from +500 mV to <100 mV                                                                   |                 |
|                                                                                                          |                              |                         | Decreases sulphate from 1057.66 mg/L to <768 mg/L                                                          |                 |
|                                                                                                          |                              |                         | Increases sulfide from 0 to >96.21 mg/L in sheep manure treatment                                         |                 |
|                                                                                                          |                              |                         | Municipal compost has the lowest remediation ability                                                       |                 |
| A mixture of 15% creek sediment, 55% limestone, 30% organic material (2 types, i.e., sheep manure and municipal compost), added with synthetic AMD (1:10) | Column, on the 2nd week, 1 g/dm³ of sodium acetate and 1 g/dm³ potassium oxalate in the compost reactor | Less than 30 days       | Increased pH from 2.4 to >7 (sheep manure)                                                                | Gibert et al., 2004 |
|                                                                                                          |                              |                         | Decreases sulphate from 528.33 to <480.3 mg/L                                                             |                 |
|                                                                                                          |                              |                         | Increases sulfide from 0 to >64.14 mg/L in sheep manure treatment                                         |                 |
|                                                                                                          |                              |                         | Municipal compost has the lowest remediation ability                                                       |                 |
| 1000 mL of AMD were treated with 1 g/L pulp mill waste green liquor dregs (GLD)                           | Batch, 6 hours at 250 rpm    |                         | pH from 5.6 to 8-8.84                                                                                      | Sebogodi et al., 2019 |
|                                                                                                          |                              |                         | Increased SO₄²⁻ from 2093 to 2360-2538 mg/L                                                                |                 |
|                                                                                                          |                              |                         | Decreased the acidity of AMD from 183.36 to 21.7-48.72 mg/L                                              |                 |
| 800 mL AMD was treated with 200 g mixtures of organic matters (leaf compost 30%, poultry manure 18%, and maple wood chips 2%), urea 3%, sand 30%, creek sediment 15%, CaCO₃ 2%, | Batch, 5 days                |                         | pH from 3.9-4.2 to 8                                                                                      | Zagury et al., 2006 |
|                                                                                                          |                              |                         | Redox potential <-350 mV                                                                                   |                 |
|                                                                                                          |                              |                         | Sulphate reduction rates ±71 mg/L/d                                                                       |                 |
|                                                                                                          |                              |                         | Sulfur decreased from 0 to 0.2 mg/L                                                                        |                 |
|                                                                                                          |                              |                         | Removed heavy metals Fe (100%), Zn (94%), Mn, Cd, and Ni (99%)                                           |                 |
| 100 g goat manure were packed in 500 mL AMD                                                             | Column, 75 minutes           |                         | pH from 2.51 ± 0.002 to 6.26 ± 0.002                                                                       | Othman et al., 2015 |
|                                                                                                          |                              |                         | Sulfur from 871.6 ± 12.5 to 834.2 ± 8.2 mg/L                                                              |                 |
|                                                                                                          |                              |                         | Sulphate from 1366.7 ± 57.8 to 1300 mg/L                                                                  |                 |
| 11.46 g/L of shrimp shell in 100 mL of AMD                                                              | Column, 24 hours at 136 rpm  |                         | Increased pH from 3.49 to 6.79                                                                            | Gomez et al., 2018 |
|                                                                                                          |                              |                         | Removed Fe (90%) and Mn (75%)                                                                           |                 |
| 0.5 g of eggshell powder (particle size of 53-160 µm) in 25 mL of AMD                                   | Batch, 24 hours at 180 rpm   |                         | Increased pH from 2.43 to 6.58                                                                           | Muliwa et al., 2018 |
|                                                                                                          |                              |                         | Removed Fe (99.9%), Mn (51%) and sulphate (62.5%)                                                         |                 |
| 4500 mL AMD was treated                                                                                 | Column, Less than 30 days   |                         | Increased pH from 2.4                                                                                    | McCullough       |
| Method | System | Retention time | Results | Reference |
|--------|--------|----------------|---------|-----------|
| with 200 g of organic garden waste and municipal sewage sludge combined (1:1) with 32:1 (AMD:organic materials) | | days | to >5 • Redox potential <-300 mV • Reduced Fe to 0.1%, Mn to 2%, Zn to <2%, Cr, Cu, Ni, dan Pb decreased to until it approaches the limit | and Lund, 2011 |
| 0.5 g of sediment and 0.25 g organic matters (50% crab shell and 50% spent mushroom compost) to treat 100 mL AMD | Batch | Within 3 days | • Increased pH from 3.13 to 6.32 • Removed Mn from 10.4 to 6 ppm, Fe from 15 to <10 ppm, Al from 8.1 to <1 ppm, Zn from 0.5 to 0.01 ppm • Removed sulphate by 39% (at 63 days) | Newcombe and Brennan, 2010 |
| 30 g sand, 50 g stream sediment, 50 g substrate (100% chitin) into 50 L AMD with three phases of flow rates 0.25, 0.5, and 1 mL/min, respectively | Continuous-flow column | 7 days | • pH from 3.13 to 7.15 • The alkalinity of 6700 mg/L as CaCO₃ • Removed heavy metals: Mn 96% and Fe 100% | Newcombe and Brennan, 2010 |
| 50 mL AMD were treated with 50 mL synthetic Fischer Tropsch wastewater (FTWW) and sulphate-reducing bacteria inoculum | Anaerobic bioreactor | Less than 200 hours | • pH from 2 to >6 • The alkalinity of 1000 mg/L as CaCO₃ • Sulphate concentration reduced from 3000 to <500 mg/L, COD from 5450 to <2000 mg/L • Increased sulfide to >50 mg/L | Magowo et al., 2020 |
| Used of organic matters (corncob 90.6 g, wood 90.6 g, and cow manure 422.9 g), limestone sand 38.1 g, silica sand 373.8 g, and sediment from a pond as bacterial inoculum. Average residence time was 5.0 ± 0.3 days based on a flow rate of 376.3 mL/day | Column | Less than 40 days | • Increased pH from 2.85 to near 6 • Remediated 1 g of Fe, 5.5 g of Cu, 0.5 g of Zn, and 21.4 g of sulphate per litre per year | Ruehl and Hiibel, 2020 |
| 1000 mL of AMD were treated with 10 types of organic materials (*Acacia nelotica* and mango woodchips; *Acacia nelotica* and mango sawdust; cow, buffalo and goat manure; sugarcane; and pearl and proso millet) weighing 500 g, and inoculated with 100 mL whey | Bench-scale bioreactor | Several hours less than 1 day | • Increased pH from 2.91 to 7.03 in goat manure treatment • Redox potential from 366-375 mV to >-100 mV • Decreased sulphate concentration from 4103-4276 to 3548 ± 210 mg/L • Reduced acidity from 1200 to >400 mg/L as CaCO₃ • The metal removal efficiency was Fe (99.3%), Cu (99.9%), | Choudhary and Sheoran, 2011 |
| Method                                                                 | System                                      | Retention time | Results                                                                                   | Reference                      |
|----------------------------------------------------------------------|---------------------------------------------|----------------|-------------------------------------------------------------------------------------------|---------------------------------|
| Constructed wetland with coarse gravel at the bottom followed by 15 cm walnut shell (particle size 2-4 mm) and 100 mL activated sludge was added to 7000 mL AMD. The system was planted with *Iris pseudacorus* L. | Lab-scale constructed wetland               | Less than 20 days | - Increased pH from 4 to ±6.5 <br>- Removed Cu, Cd, Zn <1 mg/L; Cr <2 mg/L; and Fe < 20 mg/L | Chen et al., 2020                |
| A system consisted of sandstone, gravel, pebbles as high as 15 cm, and 50 cm substrate (75% soil, 20% goat manure, and 5% wood shavings), planted with *Desmostachya bipinnata*, and 10 mm of goat manure after planting. Fed with AMD up to 200 mm | Construction of bench-scale wetland         | Within 24 hours | - pH from 2.93 to 7.22, and alkalinity from zero mg/L to 204.30 mg/L as CaCO₃  <br>- Decreased electric conductivity (average 31.9), turbidity (average 48.4 %), sulphate (average 24.8%), and acidity (average 94.4%)  <br>- Average removal rates of Fe (95.4%), Cu (90.2%), Zn (77.5%), Pb (89.5%), Co (70.0%), Ni (46.7%), and Mn (58.2%). | Sheoran, 2017                   |
| 66 l AMD with a flow rate of 9.43 L/d treated with a constructed wetland (CW) consisting of 20% bamboo chips, 60% cow manure, 10% soil, and 10% gravel planted with *Typha latifolia* with a density of 25 plants/m² | Lab-scale horizontal subsurface flow CW     | Less than 10 days | - pH from 2 ± 0.25 to >7  <br>- Sulphate removal was 92.1%  <br>- Removed Cr (99.7%), Ni (97.8%), Co(93.7%), Fe (91.6%) and Al (59.7%)  | Singh and Chakrabort, 2020       |
| 300 tons of empty oil-palm fruit bunches and 120 tons of compost as a substrate for the bottom of the pond and planted with *Typha* sp, *Melaleuca cajuputi*, and *Nauclea orientalis* L. The system was fed with AMD at a rate of 0.1 m³/second | Field-scale constructed swamp forest with a surface area of 3300 m² (divided into 4 compartments) and total capacity of 1500 m³ of AMD | 4 hours | - pH (3.5 to 6.27)  <br>- Removed Fe from 1.16 to 0.08 ppm, and Mn from 4.7 to 2.2 ppm | Yusmur et al., 2019; Rahmatia et al., 2019 |
| The components of the constructed wetland installation consist of 3 cm quartz sand, 10 cm paddy mud, 10 cm organic material (straw compost and sawdust as | Lab-scale constructed wetland               | 7 days | - Increased pH from 3 to >6 (at 2 days), The highest pH (7.7) was in the 100% compost treatment with or without SRB | Sandrawati et al., 2019; Suryatmana et al., 2020 |
| Method | System | Retention time | Results | Reference |
|--------|--------|----------------|---------|-----------|
| single or mixed), with and without SRB inoculation with 12 L of AMD and planted with *Vetiveria zizanioides* L. | | | - Decreased Fe concentration from 7.32 to 1.85 mg/L in the 100% sawdust+SRB treatment | |
| | | | - Decreased Mn concentration from 12.89 to 0.06 mg/L in the control treatment (without the addition of organic material or SRB) | |
| | | | - Reduced Sulphate from 337.35 to 25.3 mg/L in the 100% sawdust treatment with or without SRB | |
| Two constructed wetlands, one filled with 50 kg of hardwood charcoal and the other filled with 75 kg of slag with a particle diameter of 25 mm, then filled 60 L of AMD with a discharge of 30 L/min and planted with *Zantedeschia aethiopica* and *Cyperus papyrus* | Subsurface-flow constructed wetlands | Less than 10 hours | - Increased pH from 1.35 to >6 for charcoal and to 7 for slag | Sheridan et al., 2013 |
| | | | - Sulphate concentration decreased from 6000 to <500 mg/L | |
| | | | - Removed >99.99% Fe | |
| | | | - Slag was more effective than charcoal | |
| Limestone (size 20 mm) as the base, covered with a mixture of peat and gravel, then planted with 45 five-month-old *Typha latifolia* at a density of 2.84/m². The system was filled with AMD at a flow rate of 1.5 mL/min for a 5-days | Pilot-scale vertical subsurface-flow constructed wetland carried out in 0.2 m² | 5 days | - Increased pH from 4.2 to 8 | Dufresne et al., 2015 |
| | | | - Removed Fe 98.6%, Mn 75.5%, Ni 88.5%, Zn 96.7% | |
| Media of a mixture of sandy soil (0-4 mm), cow manure, peat, and dolomite was planted with 45 five-month-old *Typha latifolia* at a density of 2.84/m² and filled with AMD at a flow rate of 1.5 mL/min, for a 5-days | Pilot-scale horizontal surface-flow constructed wetland carried out in 0.2 m² | 5 days | - Increased pH from 4.2 to 7.6 | Dufresne et al., 2015 |
| | | | - Removed Fe 86.9% and Zn 91.2%, but increases Mn 35.2% and Ni 6% | |

**Organic materials as a stimulator of SRB**

The selection of organic materials used in a constructed wetland is an essential factor in AMD bioremediation's success. The composition of organic material must be considered carefully since it determines the effectiveness of the AMD remediation process (Gibert et al., 2002). The use of organic materials can be mixed with various elements needed, such as research conducted by Nurcholis et al. (2018) adding biostimulants (macro-and micro-nutrients, beneficial microbes, plant growth hormones) to organic materials could increase pH, remove dissolved heavy metals, and reduce TSS.

Table 1 clearly shows the effect of different organic materials, individually or in a mixture, on pH, sulphate concentration, and metal elements in the treated AMD. Surprisingly, a combination of oil palm empty fruit bunch and compost provide retention time of only 4 hours (Yusmur et al., 2019; Rahmatia et al., 2019). The organic substrate should create a reducing environment and function as an available carbon source (low C/N) (Prasad et al., 1999; Gibert et al., 2004; Zagury et al., 2006). As in the research of Zagury et al. (2006), a combination of leaf compost, maple wood, and poultry manure has created a condition of reduction as evidenced by the Redox potential number of -350 mV, also in the
research of McCullough and Lund (2011), namely by adding organic garden waste able to reduce the Redox potential up to -300 mV. When the reduction conditions have been created, the iron ion, which was initially in the form of ferric (Fe$^{3+}$), will be reduced to ferrous ions (Fe$^{2+}$) and release one OH$^{-}$ molecule where OH$^{-}$ ions play a role in increasing the pH of the water.

Apart from the occurrence of the iron reduction process, the low Redox potential also supports the presence of sulphate-reducing bacteria (Prasad et al., 1999). Materials that contain easily-available substances are consumed relatively easily and rapidly by SRB, such as goat manure (Gibert et al., 2004; Choudhary and Sheoran, 2011; Othman et al., 2015; Sheoran, 2017), poultry manure (Cocos et al., 2002; Zagury et al., 2006; Dufresne et al., 2015), cow manure (Ruehl and Hiibel, 2020; Singh and Chakraborty, 2020), and mushroom compost (Chang et al., 2000; Newcombe and Brennan, 2010). Zagury et al. (2006) has demonstrated that poultry manure has easily available substances (63% soluble sugar, hemicellulose, amino acids, and proteins), indicating the highest available organic carbon. According to Postgate (1984), the main sources of carbon are volatile fatty acids, such as acetate, propionate and butyrate, and short-chain fatty acids, such as lactate, pyruvate, and malate. Occasionally, long-chain fatty acids and certain aromatic could be used as substrates of SRB. Fermentation products such as ethanol, methanol, and acetate could also be used as a source of carbon. Choudhary and Sheoran (2011), for example, added whey in their research to accelerate AMD's remediation. Whey is a fermented product and contains lactose and lactic acid, both of which are sources of energy to supply the growing needs of SRB (Widdel, 1988).

The selection of organic materials can also be based on the C/N ratio. A C/N ratio of around 10 indicates that the organic material is biodegradable (Prasad et al., 1999). Another study stated that the maximum C/N ratio for SRB growth is between 45-120 (Gibert et al., 2004). Lignin content has been known to negatively correlated with the nutrient and energy supply to bacteria. The higher lignin content of organic material, the slower it is decomposed and less supply of nutrient and energy to bacteria, that also means lesser bacterial activity, including SRB.

From all treatments tested, it was found that the addition of organic material was able to reduce levels of dissolved metals in water (Table 1), such as Fe, Mn, Ni, Zn, Cr, Co, and Pb. Some metal elements, e.g., Fe will be immediately precipitated as (oxy) hydroxides (Gibert et al., 2004), such as goethite, hematite, magnetite, and lepidocrocite at neutral pH. Carbonate minerals such as otavite (Cd), siderite (Fe), and smithsonite (Zn) are also deposited (Amos and Younger, 2003). This deposition will occur at the start of the reactor, even prior to acclimation of SRB (Amos and Younger, 2003). Indeed, when the SRB is present, more metals will be precipitated in the form of metal sulfides. However, the study of Dufresne et al. (2015) showed negative results for Mn, where the dissolved Mn content in the effluent increased compared to the influent. This is because Mn is one of the most challenging metals to remove due to the complexity of the interactions that govern its solubility (Karathanasis et al., 2009; Song et al., 2012). Furthermore, Edwards et al. (2009) and Emmanuel and Rao (2008) observed that Mn is difficult to remove. Under anaerobic conditions, Mn remains in the Mn$^{2+}$ form, which dissolves more easily and is, therefore, very difficult to remove. Besides, the formation of MnS deposits is very difficult and can be easily released (Singh and Chakraborty, 2020). The removal of metal from AMD followed the order: Al$>$Fe$>$Cu$>$Zn$>$Cd$>$Mn, which corresponds to the electronegativity of the respective ions (Younger et al., 2002).

**Organic materials as a metal chelating agent**

Organic material with a low C/N ratio indicates that the material has wholly decomposed and formed humus. Humus substance has a role in ion-exchange, surface adsorption, and complexes or chelates several metal ions (Allison, 1973). The lower the lignin content of organic materials, the higher the humic acid content. Functional groups such as carboxylic acids, alcohol, phenols, and carbonyl are the main functional groups found in humic acid. Humic acid is a polyelectrolyte macromolecular organic substance with groups such as $-COOH$ or $-OH$, so that it can form complexes with metal ions. At pH 3.5-9, humic acid forms a flexible linear polyelectrolyte colloid system which causes weakened hydrogen bonds, deprotonization of humic acid functional groups and increases the number of negative charges of humic acid functional groups, which can function as ligands in humic acid, so that it can chelate metals that are positively charged such as Fe, Mn, Al, Cd, Zn, Cu, Ph, and others (Klucakova and Pavlikova, 2017). This explains the role of organic material as an adsorbent in the process of reducing metals in AMD. It is clear that organic materials with a low C/N ratio, such as poultry manure, cow manure, sheep manure, leaf compost, oak leaf, and spent mushroom compost, are not only as a carbon source for sulphate-reducing bacteria but also as metal chelating agents. At low pH, organic materials containing high lignin and cellulose are not able to adsorb heavy metals. This is proven by research that in the pH range of 3.0-5.5, ethylenediamine modified rice hull sorbed more Cu(II) than natural rice hull with the sorption in the pH range of 4-5.5. This was because the natural rice hull consists of cellulose, lignin, carbohydrate, and silica (Tang et al., 2003).

**Organic materials as plant growth substrates**

The media (organic substrate) plays an essential role in removing metals, providing a surface for bacterial activity, and helping plant growth (Spiers and Fietje, 2000; Benito et al., 2005; Parde et al., 2020). Several
materials have been used in AMD treatment experiments at laboratory scales of to the field applications. Table 1 listed goat manure and wood-shaving (Sheoran, 2017), empty oil palm fruit bunches and compost (Yusmur et al., 2019; Rahmatia et al., 2019), bamboo chips and cow manure (Singh and Chakraborty, 2020), straw compost and sawdust (Sandrawati et al., 2020), soil, cattle manure and peat (Dufresne et al., 2015), charcoal and slag (Sheridan et al., 2012), and walnut shell (Chen et al., 2020) is used as a basic substrate which is then planted with hyperaccumulator types of plants which can increase the pH to neutral and remove metals by up to 90%.

Organic materials serve as a substrate for growing plants in the wetland system, such as demonstrated by researchers, as shown in Table 1. The role of plants in the wetland system is to absorb metals (Munawar, 2007), the existence of a plant root system that releases root exudates containing organic compounds (such as acetate), which are the primary metabolites of microbe. Another study conducted by Muddarinsia and Siahaan (2014) stated that the addition of organic matter as a planting medium was able to increase the absorption of Hg metal in Lindernia crustacea, Digitaria radicosa, and Cyperus kyllingia. More importantly, plants also have the potential to provide a long-term source of organic material to replenish materials consumed, in an organic manner, gradually by the metabolism of SRB in the substrate (Younger et al., 2002).

In selecting a suitable substrate material, it must have the following properties: (i) it has sufficient fibre to maintain permeability; (ii) contains SRB (mostly in the compost of mammal manure); (iii) is alkaline or does not easily release strong organic acids into solution; (iv) must not contain viruses that are potentially harmful (Younger et al., 2002). Ruehl and Hiiibel (2020) state that with a large fraction of simple organic carbon, such as manure in a system, initially bioremediation activity will be high, but then gradually reducing until those simple carbons are exhausted. Therefore, mixing readily biodegradable substrates with long-term degradable substrates would enhance long-term efficiency (Willquist et al., 2015).

Conclusion and Recommendation

Conclusion

Passive treatment, especially wetland systems, is a promising long-term and sustainable treatment for AMD. Organic materials play important roles in the wetland system; they immediately react with AMD to increase pH, reduce sulphate and metal ions in the water, as a substrate of plant, and SRB that speeds up neutralization processes AMD and sustaining the system. Researches show that any organic materials could be used for treating AMD; however, they have different retention time and capacity to increase pH, remove sulphate and metal from the water.

Recommendation

Research conducted by Yusmur et al. (2019) has shown the effectiveness of empty oil-palm fruit bunch for AMD treatment need to be disseminated to mining industries, especially in Indonesia. Indonesia has a palm oil plantation area of 2.82 million hectares with a yield of 48.4 million tons (BPS, 2019), and every 1 ton of palm oil will produce waste in the form of empty fruit bunches as many as 23% or 230 kg. This is an abundant source of organic materials to treat AMD across the country.

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References

Acharya, B.S. and Kharel, G. 2020. Acid mine drainage from coal mining in the United States—an overview. Journal of Hydrology 588:125061, doi: 10.1016/j.jhydrol.2020.125061.
Akcil, A. and Koldas, S. 2006. Acid Mine Drainage (AMD): causes, treatment and case studies. Journal of Cleaner Production 14:1139-1145.
Allison, F.E. 1973. Chapter 15 An ion exchange material, chelating agent, and buffer. Developments in Soil Science 3:301-314.
Amos, P.W. and Younger, P.L. 2003. Substrate characterisation for a subsurface reactive barrier to treat colliery spoil leachate. Water Research 37:108–120.
Benito, M., Masaguer, A., De Antonio, R. and Moliner, A. 2005. Use of pruning waste compost as a component in soilless growing media. Bioresource Technology 96(5):597–603.
Badan Pusat Statistik (BPS). 2019. Indonesian Oil Palm Statistic. Indonesia: Jakarta.
Chang, I.S., Shin, P.K. and Kim, B.H. 2000. Biological treatment of acid mine drainage under sulphate-reducing conditions with solid waste materials as substrate. Water Research 34(4):1269-1277.
Chen, J., Li, X., Jia, W., Shen, S., Deng, S., Ji, B. and Chang, J. 2020. Promotion of bioremediation performance in constructed wetland microcosms for acid mine drainage treatment by using organic substrates and supplementing domestic wastewater and plant litter broth. Journal of Hazardous Materials 404(Pt A): 124125, doi: 10.1016/j.jhazmat.2020.124125.
Choudhary, R.P. and Sheoran, A.S. 2011. Comparative study of cellulose waste versus organic waste as substrate in a sulfate reducing bioreactor. Bioresource Technology 102(6): 4319-4324.
Cocos, I.A., Zagury, G.J., Clement, B. and Samson, R. 2002. Multiple factor design for reactive mixture selection for use in reactive walls in mine drainage treatment. Water Research 36:167-177.
Cohen, R.R.H. 2006. Use microbes for cost reduction of metal removal from metals and mining industry waste streams. Journal of Cleaner Production 14:1146-1157.
Drury, W.J. 1999. Treatment of acid mine drainage with anaerobic solid-substrate reactors. *Water Environment Research* 71:1244-1250.

Dufresne, K., Neculita, C.M., Brisson, J. and Genty, T. 2015. Metal retention mechanisms in pilot-scale constructed wetlands receiving acid mine drainage. *Proceedings of the 10th iCARD (International Conference on Acid Rock Drainage)-IMWA* (International Mine Water Association). April 21-24 2015, Santiago, Chile.

Edwards, J.D., Barton, C.D. and Karathanasis, A.D. 2009. A small scale sulfate reducing bioreactor for manganese removal from synthetic acid mine drainage. *Water, Air, & Soil Pollution* 203:267-275.

Emmanuela, K.A. and Rao, A.V. 2008. Adsorption of Mn (II) from aqueous solutions using pithacelobium dulce carbon. *Rayan Journal of Chemistry* 1(4):840-852.

Faulkner, B.B. and Skousen, J.G. 1994. Treatment of acid mine drainage by passive treatment systems. In: Bureau of Mines SP 06A. *Proceedings of International Land Reclamation and Mine Drainage Conference*. April 24-29 1994, U.S. p.250-257.

Fennessy, S. and Mitsch, W.J. 1989. Design and use of wetlands for renovation of drainage from coal mines. In *Ecological Engineering: An Introduction to Ecotechnology*. Mitsch, W.J. and Jorgensen, S.E. (ed.) New York: John Wiley and Sons.

Ford, K.L. 2003. Passive treatment systems for acid mine drainage. U.S. Bureau of Land Management Papers.

Gibert, O., De Pablo, J., Cortina, J.L. and Ayora, C. 2002. Treatment of acid mine drainage by sulphate-reducing bacteria using permeable reactive barriers: a review from laboratory to full-scale experiments. *Reviews in Environmental Science and Biotechnology* 1(4):327-333.

Gibert, O., De Pablo, J., Cortina, J.L. and Ayora, C. 2004. Chemical characterization of natural organic substrates for biological mitigation of acid mine drainage. *Water Research* 38:4166-4196.

Gomez, D.N., Rodrigues, C., Lapoll, F.R. and Recio, M.A.L. 2018. Adsorption of heavy metals from coal acid mine drainage by shrimp shell waste: Isotherm and continuous-flow studies. *Journal of Environmental Chemical Engineering* 7(1):102787, doi: 10.1016/j.jece.2018.11.032.

Hards, B.C. and Higgins, J.P. 2004. Bioremediation of acid rock drainage using SRB. Ontario: Jacques Whit Environment Limited.

Johnson, B.D. and Hallberg, K.B. 2005. Acid mine drainage remediation options: a review. *Science of the Total Environment* 338:3-14.

Kalin, M. 2004. Passive mine water treatment: the correct approach? *Ecological Engineering* 22:299-304.

Karathanasis, A.D., Edwards, J.D. and Barton, C.D. 2010. Manganese and sulfate removal from a synthetic mine drainage through pilot scale bioreactor batch experiments. *Mine Water Environment* 29:144–153.

Kefeni, K.K., Msagati, TAM. and Mamba, B.B. 2017. Acid mine drainage: Prevention, treatment options, and resource recovery: A review. *Journal of Cleaner Production* 151:475-493, doi: 10.1016/j.jclepro.2017.03.082.

Kleinnmann, R.L. 1990. Acid mine drainage in the United States. *Proceedings of the 1st Midwest Regional Reclamation Conference*.

Klucakova, M. and Pavlikova, M. 2017. Lignitic humid acids as environmentally-friendly adsorbent for heavy metals. *Journal of Chemistry* 7169019, doi: 10.1155/2017/7169019.

Lottermoser, B.G. 2010. *Mine Wastes Characterization Treatment And Environmental Impacts*, 3rd edition. London (GB): Springer.

Macingova, E. and Luptakova, A. 2012. Recovery of metals from acid mine drainage. *Chemical Engineering* 28:109-114.

Magowo, W.E., Sheridan, C. and Rumbold, K. 2020. Bioremediation of acid mine drainage using Fischer-Tropsch waste water as a feedstock for dissimilatory sulfate reduction. *Journal of Water Process Engineering* 35:101229.

McCullough, C.D. and Lund, M.A. 2011. Bioremediation of acidic and metalliferous drainage (AMD) through organic carbon amendment by municipal sewage and green waste. *Journal of Environmental Management* 92:2419-2426.

Muddarisna, N. and Siahaan, B.C. 2014. Application of organic matter to enhance phytoremediation of mercury-contaminated soils using local plant species: a case study on small scale gold mining locations in Banyuwangi of East Java. *Journal of Degraded and Mining Lands Management* 2(1): 251-258, doi:10.15243/jdmlm.2014.021.251.

Mulawi, A.M., Leswift, T.Y. and Onyango, M.S. 2018. Performance evaluation of eggshell waste material for remediation of acid mine drainage from coal dump leachate. *Minerals Engineering* 122:241-250.

Munawar, A. 2007. Utilization of local biological resources for passive treatment of acid mine drainage. *Jurnal Ilmu Tanah dan Lingkungan* 7:31-42 (in Indonesian).

Newcombe, C.E. and Brennan, R.A. 2010. Improved passive treatment of acid mine drainage in mushroom compost amended with crab-shell chitin. *Journal of Environmental Engineering* 136:616-626.

Nurcholis, M., Wijaya, M. and Ratminah, W.D. 2018. Application of biostimulant and CaO to remediate acid mine drainage on the coal mining land in Lampung Sumatra Island. *Journal of Degraded and Lands Management* 5(4): 1347-1354, doi: 10.15243/jdlm.2018.054.1347.

Ochieng, G.M., Sseego, E.S. and Nkwonta, O.I. 2010. Impacts of mining on water resources in South Africa: A review. *Scientific Research and Essays* 5(22): 3351–3357.

Othman, A., Sulaiman, A. and Sulaiman, S.K. 2015. The study on the effectiveness of organic material in acid mine drainage treatment. *Journal Teknologi* 77(1):79-84.

Parde, D., Patwa, A., Shukla, A., Vijay, R., Killeidar, D.J. and Kumar, R. 2020. A review of constructed wetland on type, technology and treatment of wastewater. *Environmental Technology and Innovation* 21:101261, doi: 10.1016/j.eti.2020.101261

Pat-Espadas, A.M., Portales, R.L., Amabilis-Sosa, L.E., Gomez, G. and Vidal, G. 2018. Review of constructed wetlands for acid mine drainage treatment. *Water* 10:1-25.

Posigate, J.R. 1984. *The Sulphate Reducing Bacteria*, 2nd edition. Cambridge: Cambridge University Press.

Prasdat, D., Wai, M., Be rube’, P. and Henry, J.G. 1999. Evaluating substrates in the biological treatment of acid mine drainage. *Environmental Technology* 20:449-458.

Rahmatia, C., Hilwan, I., Mansur, I. and Noor, I. 2019. Metal retention mechanisms in pilot-scale constructed wetlands receiving acid mine drainage. *Journal of Degraded and Mining Lands Management* 8(3): 2779-2789 (2021)

Open Access
Rambabu, K., Banat, F., Pham, Q.M., Ho, S.H., Ren, N.Q. and Show, P.L. 2020. Biological remediation of acid mine drainage: Review of past trends and current outlook. *Environmental Science andEcotechnology* 2:1-14.

Ruehl, M.D. and Hibel, S.R. 2020. Evaluation of organic carbon and microbial inoculum for bioremediation of acid mine drainage. *Minerals Engineering* 157:1-7.

Sandrawati, A., Suryatmana, P., Putra, I.N. and Kamaluddin, N.N. 2019. Effect of types of organic matter and sulfate-reducing bacteria on Fe and Mn concentrations in the remediation of acid mine drainage. *Soilres* 17(1): 38-44 (in Indonesian).

Schiper, A. 2004. Biogeochemistry of metal sulfide oxidation in mining environments sediment and soils. In: Amend, J.P., Edwards, K.J. and Lyons, T.W. (eds). *Sulfur Biogeochemistry – Past and Present.* Amerika (USA): Geological Society of America 379:49-62.

Seadira, T., Baloyi, J., Raphulu, M., Moutloali, R. and Ochieng, A. 2014. Acid mine drainage treatment using constructed wetland. *International Conference on Chemical, Integrated Waste Management and Environmental Engineering.*

Sebogodi, K.R., Johakimu, J.K. and Sithole, B.B. 2019. Beneficiation of pulp mill waste green liquor dregs: Applications in treatment of acid mine drainage as new disposal solution in South Africa. *Journal of Cleaner Production* 246:118979.

Sheoran, A.S. 2017. Management of acidic mine waste water by constructed wetland treatment system: A bench scale study. *European Journal of Sustainable Development* 6:245–255.

Sheridan, G., Harding, K., Koller, E. and Pretto, A.D. 2013. A comparison of charcoal- and slag-based constructed wetlands for acid mine drainage remediation. *Water SA* 39(3):369–374.

Simate, G.S. and Ndlovu, S. 2014. Acid mine drainage: Challenges and opportunities. *Journal of Environmental Chemical Engineering* 2(3):1785-1803.

Singh, S. and Chakraborty, S. 2020. Performance of organic substrate amended constructed wetland treating acid mine drainage (AMD) of North-Eastern India. *Journal of Hazardous Materials* 397:122719.

Skousen, J., Rose, A., Geidel, G., Foreman, J., Evans, R. and Hellier, W. 1998. Handbook of Technologies for Avoidance and Remediation of AMD. The National Mine Land Reclamation Centre, West Virginia, Morgantown, WV.

Skousen, J., Zipper, C.E., Rose, A., Ziemkiewicz, P.F., Nairn, R., McDonald, I.M. and Kleinmann, R.L. 2017. Review of passive systems for acid mine drainage treatment. *Mine Water and the Environment* 36:133-153.

Song, H., Yim, G.J., Ji, S.W., Neculita, C.M. and Hwang, T.W. 2012. Pilot-scale passive bioreactors for treatment of natural acid mine drainage: efficiency of mushroom compost vs. mixed substrates for metal removal. *Journal of Environmental Management* 111:150-158.

Spiers, T.M. and Fietje, G. 2000. Green waste compost as a component in soilless growing media. *Compost Science & Utilization* 8(1):19–23.

Stumm, W. and Morgan, J.J. 1981. *Aquatic Chemistry: An Introduction Emphasizing Chemical Equilibria in Natural Waters.* New York: John Wiley & Sons, Inc.

Suryatmana, P., Sandrawati, A., Putra, I.N. and Kamaluddin, N.N. 2020. The potential of sulfate-reducing bacteria and types of organic matter in acid mine drainage treatment using a system constructed wetland for *Vetiveria zizanioides* L. *Soilres* 18(2):36-43 (in Indonesian).

Taberina, S., Junaudi, E., Sarwom, R., Lindongi, L.E. and Mulyanto, B. 2020. The acid mine drainage (AMD) impact of tailings and non-tailings on the ecosystem changes in the ModADA sedimentation area, Timika. *Journal of Degraded and Mining Lands Management* 7(2): 2085-2094, doi: 10.15243/jdm1m.2020.072.2085.

Tang, P.L., Lee, C.K., Low, K.S. and Zainal, Z. 2003. Sorption of Cr (VI) and Cu (II) in aqueous solution by ethylenediamine modified rice hull. *Environmental Technology* 24(10):1243-1251.

Vymazal, J. 2008. Constructed wetland for waste water treatment: a review. In Sagupta, M. and Dalwani, R. (eds). *Proceeding of Taal 2007: The 12th World Lake Conference.* p 965-980.

Watzlaf, G.R., Schroeder, K.T., Kleinmann, R.L., Kairies, C.L. and Nairn, R.W. 2004. *The Passive Treatment of Coal Mine Drainage.* Pittsburg: US Department of Energy.

Widdel, F. 1988. Microbiology and ecology of sulfate and sulfur-reducing bacteria. In: Zehnder AJB (ed.) *Biology of Anaerobic Microorganisms.* New York: John Wiley and Sons.

Wildeman, T., Gusek, J., Dietz, J. and Morea, S. 1991. *Handbook for Constructed Wetlands Receiving Acid Mine Drainage.* US EPA, Cincinnati, OH.

Williamson, M.A. and Rimstidt, J.D. 1994. The kinetics and electrochemical rate-determining step of aqueous pyrite oxidation. *Geochimica et Cosmochimica Acta* 58(24):5443-5454.

Willquist, K., Bjorkmalm, J., Sjostrand, K., Lagerkuist, A., Ericson, R., Hagemalm, M. and Liu, J. 2015. Biological treatment toolbox for Sweedish mine drainage. SR Report.

Younger, P.L., Banwart, S.A. and Hedin, R.S. 2002. Passive Treatment of Polluted Mine Waters. In: *Mine Water, Environmental Pollution.* Springer: Dordrecht, The Netherlands.

Yusmur, A., Ariansyah, M. and Mansur, I. 2019. Mitigation and management direction of acid mine drainage through artificial swamp forest in post-mining land. *Journal of Natural Resources and Environmental Management* 9(3):566-576 (in Indonesian).

Zagury, G.J., Kulnieks, V.I. and Neculita, C.M. 2006. Characterization and reactivity assessment of organic substrates for sulphate-reducing bacteria in acid mine drainage treatment. *Chemosphere* 64:944-954.

Ziemkiewicz, P.F., Skousen, J.G. and Simmons. J. 2003. Long-term performance of passive acid mine drainage treatment systems. *Mine Water and the Environment* 22:118-129.