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Assessing the Performance of Constructed Wetland for Acid Mine Drainage Treatment Using Sugarcane Molasses as a Carbon Source

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Abstract

Acid mine drainage (AMD) is a widespread environmental issue at mining sites globally. AMD is caused by pyrite oxidation which produces an acidic discharge > 2500 mg/L of sulfate, dissolved heavy metals at high amounts and low pH (2 - 4.5). AMD has serious health impact on living organisms. The present study aimed to assess the performance of locally available, high-organic matter containing by-products, specifically sugarcane molasses (SCM), as a carbon source for AMD treatment. In batch experiments containing SCM and control, 99% sulfate and nitrate reductions were reached. Heavy metal removal efficiencies > 94% were achieved for Cu, Fe, Mn, and Zn, in the SCM inoculated columns, while efficiencies > 42% were recorded in the control columns. The column experiments removed 99% of the initial Al concentrations but were not very effective in COD reduction to acceptable limits. The findings reported by this study verify that high carbon containing substrates can be used to remediate metal and sulfate contaminated water in mining areas.

Keywords

Acid Mine Drainage, Constructed Wetland, Heavy Metals, Sugar Cane Molasses, Sulfate Reducing Bacteria, Sulfate Reduction

1. Introduction

Acid mine drainage (AMD) release is a widespread environmental problem at mining sites globally. AMD is caused by pyrite oxidation on uncovered mineral surfaces, which produces an acidic discharge with more than 2500 mg/L of sulfate, high amount of dissolved heavy metals such as manganese, iron, and aluminum, and low pH (2 - 4.5) (Neculita et al., 2007; Pérez et al., 2018; Rajak &

Singh, 2019).

It has been demonstrated that AMD is toxic to aquatic organisms, because it reduces the pH below acceptable level for life sustenance and increases the solubility of toxic metals in water (Peng et al., 2017). Heavy metals have also negative impact on plants and animal life and the high degrees of contact may result in serious health concern such as miscarriages, brain damage, blood, kidney and reproductive disorders (in animal) (Rajak & Singh, 2019).

AMD can be treated by active or passive technology before being discharged in order to avoid negative impact on the receiving environment (Ighalo et al., 2022). Active treatment involves the application of alkaline chemical so as to raise pH of mine water, precipitate metals and neutralize acidity. However active treatment is expensive because of the cost of corrosion resistant equipment's needed, manpower and chemicals (Skousen et al., 2017).

Passive treatment is an interesting option, particularly when water is highly contaminated, due to its low capital and running costs, low sludge production, as well as ease of installation and use (Neculita et al., 2007). Chemical, biological, and physical removal techniques are used in passive treatment systems that have ability to reduce sulfate concentration, generate alkalinity, reduce dissolved metal concentration, and neutralize pH levels (Rambabu et al., 2020).

Constructed wetlands are biological systems with the purpose of enhancing variety of chemical, microbial, natural physical and plant-mediated processes. Aerobic and anaerobic/compost wetlands are the two types of wetlands so far employed to treat AMD (Marwa et al., 2013).

The selection of a balanced reactive combination of organic matter constituted of an organic carbon and nitrogen (C, N) source for sulfate reducing bacteria (SRB) is critical for the effectiveness and long-term sustainability of AMD treatments in passive technology (Vasquez et al., 2016). Normally AMD contains dissolved organic carbon in concentrations that are relatively low (<10 mg/L). Organic matter is very important because it is a carbon source that serves as media for material exchange, the source of energy that supports some of the most essential biological reactions in constructed wetlands and nitrogen is essential for cell structure (Chen et al., 2021; Singh & Chakraborty 2022).

Typical materials used as source of organic carbon include peat, straw and hay, spent mushroom composite, cow manure, horse manure, poultry manure and leaves from eucalyptus and pine needles. The mentioned organic substrates serve as a source of energy for development and growth of sulfate reducing bacterial (Oberholzer et al., 2022). Despite the success of these materials in AMD treatment, agricultural needs, animal food, and accessibility remain a concern.

The present study is aimed at using sugarcane molasses (SCM) which is a by-product of sugarcane processing as a source of carbon. The choice of this materials is based on the fact that because of its high carbon content comparatively low quantities shall be needed. It is also a readily available material from sugar processing factories in Tanzania.

2. Materials and Methods

2.1. Principle of Sulfate and Metal Reduction

SRB use electron donors such as sulfate (SO_4^{2-}), nitrate (NO_3^-), and carbon dioxide gas (CO_2), to oxidize simple organic compounds found in the substrate. Due to the anaerobic environment the sulfate reduction process leads to oxidation of organic compounds and precipitation of metal sulfides (MeS). The precipitation of metal is mainly through the reaction with sulfides produced by the SRB, to produce metal sulfides (Kaksonen et al., 2004). Metal sulfides are known to be stable and unaffected by change in pH of the water. The following equations apply for sulfate and metal (using Zn as an example), the simple complete chemical reactions can be written as:

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \tag{1}$$

$$Zn^{2+} + H_2S \rightarrow ZnS + 2H^+ \tag{2}$$

The reduction process occurs due to the presence of SRB in the wetland substrate coupled with sufficient organic material to stimulate their activity (Marwa et al., 2013).

2.2. Carbon to Nitrogen (C/N) Ratio

To support the development of anaerobic bacteria and the preservation of a stable environment in the constructed wetland, a balance of C and N nutrients ratio (i.e. 29.89) is required during anaerobic waste breakdown. Carbon feeds anaerobic bacteria, and nitrogen is essential for cell construction (Iqbal et al., 2014; Hance et al., 2020).

2.3. Substrate Materials

Substrate materials for constructed wetland can be from natural sources (including soil, granite rocks, manganese sand, quarts, gravel and sand, anthracites and zeolite) and synthetic sources (including activated carbon, hallow bricks, ceramic sponge iron and steel slugs) (Wang et al., 2018). In this study the support media (substrate) consisted of inert granite gravels with a particle size of 12 - 20 mm. The inert gravel provides physical support for plant roots and microbial communities but does not contribute any reactive chemical properties based on this study (Kim et al., 2016).

2.4. Sources of Microorganism (SRB)

The SRB are a group of anaerobic prokaryotes that can exist in a wide range of lakes, marshes, underground pipelines, paddy fields, industrial and domestic wastewater, petroleum deposits and other anoxic habitats (Liamleam & Annachhatre, 2007; Xu & Chen, 2020). In this study, the inoculum used was domestic wastewater from Nelson Mandela—African Institution of Science and Technology (NM-AIST) premises was used as a source of SRB. The inoculum was not axenic (not a pure culture of SRB). The study used domestic wastewater, which contains a diverse microbial population, including SRB, along with many other microbial species. The test for isolating SRB or confirming their dominance in the microbial community is important. Without performing microbial community analysis (e.g., DNA sequencing, or selective culturing techniques), it is impossible to determine whether SRB were the dominant group or if other microbial populations played significant roles in the present study. However, the wastewater has been added into the columns and left for three months to ensure acclimation of the microbes including SRB population before the AMD treatment.

2.5. Carbon Source Materials

Domestic wastewater supplemented with SCM was used as a nutrient and carbon source for SRB, respectively. SCM was collected from Tanganyika Planting Company Limited (TPC) in Moshi, Tanzania. SCM are amongst the most common by-products of the sugar refining process that contain, high levels of sugar, C/N ratio, and phosphate which are necessary for the microorganism culture required for the processes that remove heavy metal from wastewater (Park et al., 2010; Iqbal et al., 2014; Özbaş & Balkaya, 2014; Loreto et al., 2021). This study has focused on using constructed wetlands whereby SCM will be used as a carbon source because it has a high organic matter content and also nutrients such as P (483.19 mg/L) and N (554 g/kg) (Mordenti et al., 2021; Thanapornsin et al., 2022). **Table 1** shows the composition of the SCM from the specified company that has been used in this study as a carbon source for SRB.

Table 1. The composition of SCM.

Composition	Concentration	Units
Protein	6.77	g/100mL
Phosphorus (P)	483.19	mg/L
Potassium (K)	28133.60	mg/L
Sodium (Na)	1405.29	mg/L
Magnesium (Mg)	4498.04	mg/L
Iron (Fe)	150.74	mg/L
Manganese (Mn)	79.90	mg/L
Copper (Cu)	0.25	mg/L
Zinc (Zn)	29.93	mg/L
Calcium (Ca)	8110.65	mg/L
Molybdenum (Mo)	0.10	mg/L
Nickel (Ni)	10.81	mg/L
Boron (B)	44.78	mg/L
Cobalt (Co)	2.57	mg/L
Sulfur (S)	5214.58	mg/L
Sucrose	445.60	g/L
Glucose	128.20	g/L
Fructose	99.46	g/L

Source: Thanapornsin et al. (2022).

2.6. Source of AMD

A synthetic solution was prepared by using distilled water and sulfate salts of Al, Ca, Cu, Fe, Mg, Mn, and Zn based on highly polluted mine water. Synthetic AMD allows for controlled conditions (variables) that help to isolate the effects of sugarcane molasses as a carbon source without external interferences. A solution with sulfate concentration of about 4780 mg/L was prepared by dissolving the solid samples (indicated in Table 2) in 6 L of distilled water. Table 2 shows the prepared composition of synthetic AMD solution in a column.

Table 2. Composition of synthetic AMD.

Element	Chemicals applied	Desired element concentrations (mg/L)	Concentration of chemical (mg/L)	Amount of Sulfate (mg/L)
Al	$Al_2(SO_4)_3{\cdot}16H_2O$	130	1517	693.31
Ca	$CaSO_4$	680	2312	1641.52
Cu	$CuSO_4{\cdot}5H_2O$	207	809.4	310.81
Fe	$FeSO_4{\cdot}7H_2O$	209	1036	357.76
Mg	$MgSO_4$	286	1430	1144
Mn	$MnSO_4{\cdot}H_2O$	68	209	119.13
Zn	$ZnSO_4{\cdot}7H_2O$	354	1540	513.5
NO_3^-	KNO_3		1530	
COD			548	
Total concentration of sulfate (mg/L)				4780.03

2.7. Sampling and Analysis

Synthetic AMD samples were collected from constructed wetlands after 3, 5, and 10 days for the analysis of physical and chemical parameters over a maximum period of 10 days. All parameters were analysed at each interval. Physical parameters such as pH, electrical conductivity (EC), temperature, and dissolved oxygen (DO) were determined in-situ by using a Multiparameter type HANNA (HI 9829). The treated AMD wastewater samples were collected using water sampling technique. The samples were collected by using clean and clear plastic bottles, and then stored in the plastic bottles of 50 mL for laboratory chemical analysis. The chemical parameters such as nitrate (NO_3^-), sulfate (SO_4^{2-}), COD, Fe, Ni, Zn, Al and Mn were tested and determined by different methods.

The concentrations of NO_3^- and SO_4^{2-} in mg/L were analyzed by using a spectrophotometer (Hach DR900) in the presence Nitrover Reagent Powder Pillow (Nitrate Method 8039) and Sulfover Reagent Powder Pillow (Method 8051), respectively. The concentrations of the selected heavy metals with exception of aluminum were analyzed by using the atomic absorption spectrometer (AAS) APHA Standard Method 3111B. The concentration of Al was analyzed by using a spectrophotometer (Hach DR900 via Method 8012) in the presence of AluVer 3 Al Reagent Powder Pillow (Xu & Chen, 2020; Njau et al., 2023). Wastewater samples for metal analysis were preserved with 1 mL concentrated nitric acid (20%) and samples for COD analysis were preserved with 1 mL of concentrated sulfuric acid.

2.8. Contact Time

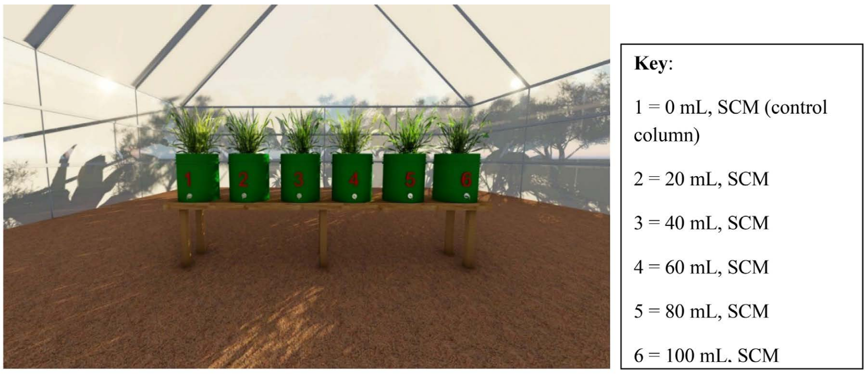
In this study (a batch experiment), the contact time is equivalent to the time the solution is left undisturbed in the system. Since the synthetic AMD and SCM were added to the columns at the begin and samples were taken at intervals (3, 5, and 10 days), the contact time for each measurement corresponds to these time intervals. The inflow or outflow was not determined during the experiment, the solutions remained in the columns without disturbance. This ensures that the mentioned time periods (3, 5, or 10 days) accurately represent the contact time. The contact time directly impacts the efficiency of sulfate reduction, heavy metal removal, and other processes. Longer contact time suggest to allow more interaction between solution, SCM, and SRB, improving treatment performance. However, in the present study the contact time is simply the elapsed time until sampling.

2.9. Experimental Set-Up and Procedures

The batch experiments using plastic pots (bed column) planted with *Cyperus altenifolius* were conducted in a ventilated greenhouse of an area about 120 m^2 located at the NM-AIST campus. *Cyperus altenifolius* were planted into the gravels three months before this experiment to ensure plant maturity and optimal bioremediation capacity. *Cyperus altenifolius* it is a well-known bioremediator for metals. *Cyperus altenifolius* was selected in this study due to its ability to tolerate metal toxicity in harsh environments, as typically found in AMD, uptake and stabilize heavy metals (such as Fe, Mn, Zn, and Cu) in polluted environments, including wetlands and wastewater treatment systems, enhance microbial activity, particularly that of SRB by providing a habitat in its root system, as well as local availability makes it a preferred choice for wetland systems. The experimental design used was a completely randomized design for the column experiments with six pots (columns) as illustrated in **Figure 1**. Each pot had a diameter of 30 cm and height of 45 cm. The pots were fitted with a drain for collecting samples and drain the pot when necessary. Gravel of size 12 - 20 mm was filled into pots' height of 40 cm. Five pots were inoculated with SCM at increasing volumes: 20 mL, 40 mL, 60 mL, 80 mL, and 100 mL (corresponding to volume ratios of 0.0033, 0.0066, 0.0100, 0.0133, and 0.0167 v/v). One pot was kept as a control, with no SCM added. The initial AMD's pH of both inoculated and control columns was 7. The pH adjustment was done using 2 M hydrochloric acid and 2M hydroxide. The independent variable was the SCM volume applied to the columns to assess how varying SCM volumes influenced response variable parameters like sulfate reduction, heavy metal removal (Cu, Fe, Mn, Zn, Al), nitrate reduction, and COD levels over time (3, 5, and 10 days). Each column represented a unique treatment level without replication, indicating a single-trial experiment. This allows for assessing the dose-response relationship between SCM volume and AMD remediation efficiency and comparing treatment performance against a control column (no SCM). The ranges of the evaluated operating conditions (such as SCM volume, heavy metal concentrations, contact time, sulfate concentration, nitrate concentration, and COD concentration) are presented in Table 3.

Table 3. Ranges of the experimental parameters.

Parameters	Range
SCM volume (v/v)	0 - 0.0167
Contact time (days)	0 - 10
Sulfate concentration (mg/L)	4780
Heavy metal concentration (mg/L)	68 - 680
Nitrate concentration (mg/L)	1530
COD concentration (mg/L)	548 - 5226



Key:

1 = 0 mL, SCM (control column)

2 = 20 mL, SCM

3 = 40 mL, SCM

4 = 60 mL, SCM

5 = 80 mL, SCM

6 = 100 mL. SCM

Figure 1. Batch experimental setup.

Data analysis, interpretation, and efficiency of heavy metal removal

The graphics were analyzed by using excel software. The efficiency of pollutant removal was analyzed according to Equation (3).

$$R\% = \frac{(C_0 - C_t)}{C_0} * 100 \tag{3}$$

where C_0 and C_t (mg/L) were initial and final concentrations of heavy metals solution at time t (h), R (%) represents the removal efficiency respectively.

3. Results and Discussion

3.1. Sulfate Removal

The theoretical conversional reaction of sulfate to hydrogen sulfide under SRB has led into the production of free hydrogen ions that increased an acidic property of the solution. During experiment, the negative change of the solution initial pH (from 7 - 3.2) has been observed to increase with experimental treatment time from 0 to10 day. Figure 2 shows the effect of SCM in the sulfate reduction. Sulfate reduction was observed in all tested columns, including those inoculated with SCM and the control column. Sulfate reduction from the columns was considered as a direct indicator of the SRB activity. The concentrations of residual sulfate (4780 mg/L, initial sulfate concentration at day 0) analyzed between day 3 and 10 were found to decrease in solution with the increase in SCM composition in the tested columns. The sulfate reduction process was attained due to the presence of sufficient organic material in SCM that stimulate the microbial activity, including that of SRB. The minimum residual sulfate concentration of 5 mg/L (99% removal efficiency) in treated solutions was observed from column containing SCM (0.0167 v/v) at day 10 of the experiment implying that the microbes had enough time to degrade the pollutant. This level is below the accepted discharge limit set by world health organization (WHO) which is 400 mg SO_4^-/L. Despite the slight differences between the inoculated columns and the control column in sulfate reduction, the SCM at a very low concentration applied influenced the sulfate-reducing activity of the microbes including SRB. "The findings of this study were supported by (Jong & Parry, 2003) who used the SRB in an anaerobic packed-bed reactor in treatment of AMD.

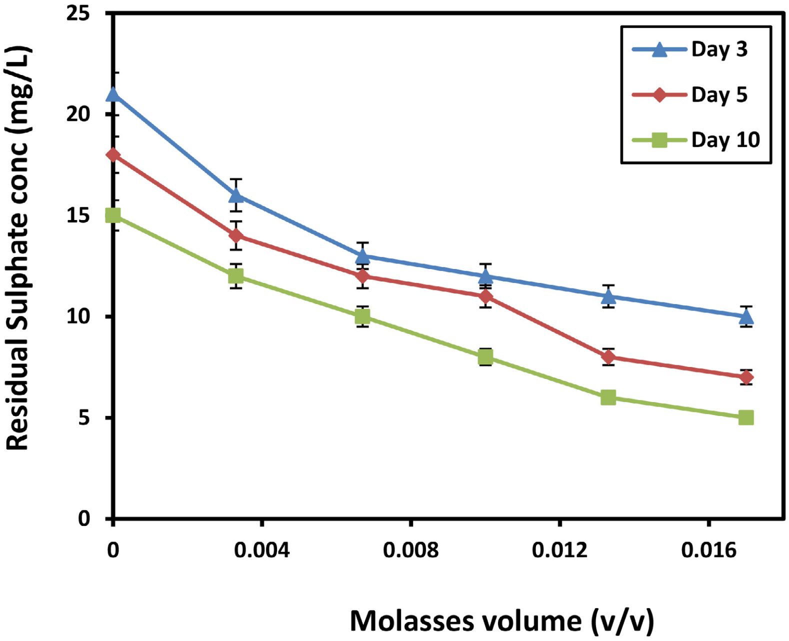


Figure 2. The effect of SCM on sulfate reduction at constant pH (7.0), initial sulfate concentration (4780.03 mg/L) and varied SCM dosage (0-0.0167 v/v).

3.2. Heavy Metal Removal

Heavy metals could be removed from the AMD solution through precipitation process as considered in Equation (2). **Table 4** shows the analysis of heavy metal concentrations for aqueous phase of treated water. The findings relate to a system inoculated with SCM and control containing initial metal concentrations ranges from 68 - 354 mg/L.

Table 4. Heavy metal analysis in both inoculated with SCM (0.0167 v/v) and control columns.

Heavy Metal	Initial concentration (mg/L)	Final concentration in control	Removal %	Final concentration in inoculum	Removal %
Cu	207	73.00	64.73	9.17	95.57
Fe	209	85.71	58.99	10.76	94.85
Mn	68	39.26	42.26	3.50	94.66
Zn	354	83.03	76.55	11.98	96.62
Al	130	0.1725	99.87	0.0495	99.96

Figure 3 shows that the concentrations of Cu, Fe, Mn and Zn were significantly reduced to a range of 1.5 - 12 mg/L after 3 to 10 days of treatment with a 0.0167 v/v SCM concentration. The results indicate removal efficiencies of 95.57%, 94.85%, 94.66% and 96.62% for the initial concentrations of Cu, Fe, Mn, and Zn, respectively, were achieved. The control column exhibited heavy metal removal efficiencies ranging from 42.26 to 76.55% efficiency in treated wastewater, slightly

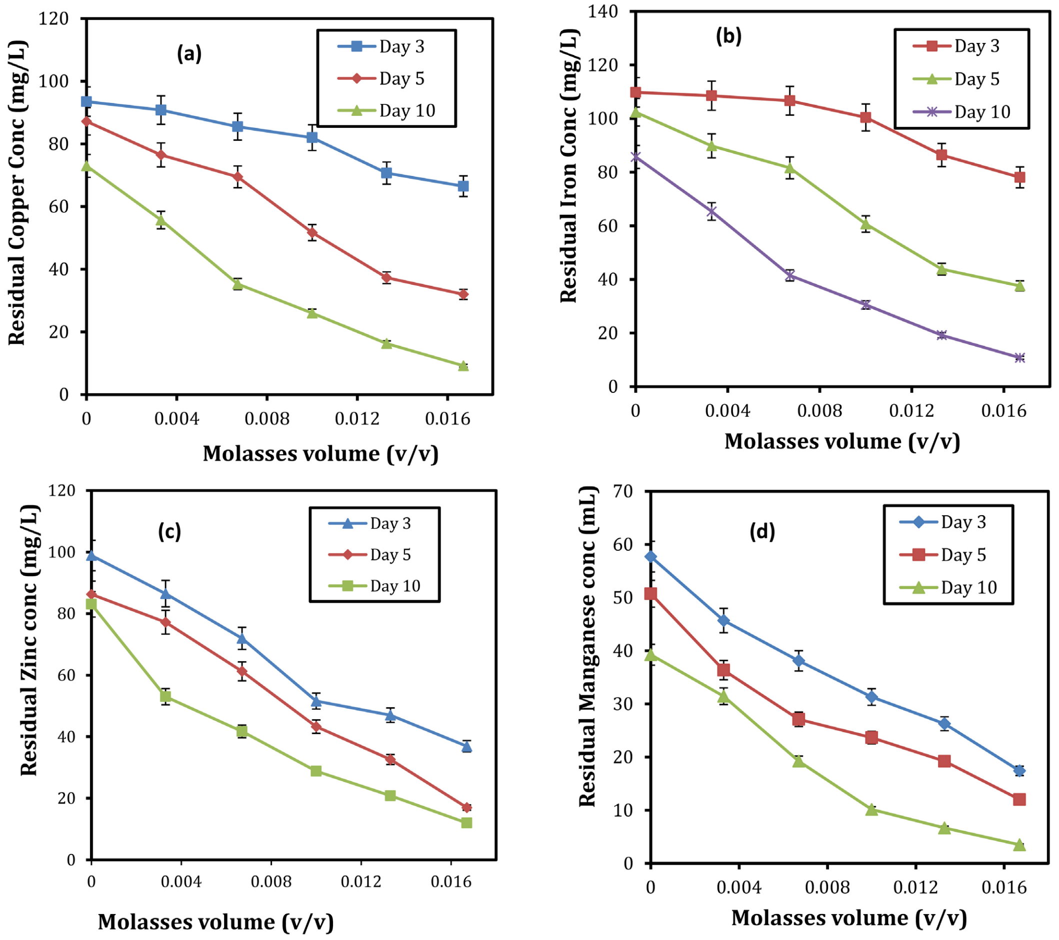


Figure 3. The effect of SCM on reduction of heavy metals: copper (a), iron (b), zinc (c), and manganese (d), at constant pH (7), initial metal's concentrations (207, 209, 354, and 68 mg/L, respectively) and varied SCM dosage (0 - 0.0167 v/v).

lower than that observed in the SCM inoculated columns. The control column showed lower removal efficiency, indicating the critical role of SCM in enhancing SRB activity.

This indicates that SCM improved the biological activity of SRB by providing essential C and N nutrient sources over time. C and N are important since they support the development of anaerobic bacteria and the preservation of a stable environment in the constructed wetland, also C feeds anaerobic bacteria and N is essential for cell construction. When sulfate reduction process takes place, sulfides are produced by SRB which precipitate metals to form metal sulfides (stable compounds) hence metal reduction. The findings of this study were supported by (Jong & Parry, 2003) who reported > 97.5% removal of Fe, Ni, Zn, and Cu, when anaerobic columns were used in treatment of heavy-metals and sulfate contaminated water.

Moreover, in **Figure 4**, the greatest reduction of aluminum concentrations from 130 to 0.0495 mg/L in solution was observed with an increase in contact time from day 3 to 10 across all inoculated and control columns. A slight increase of the residual aluminum concentrations was observed with an increase in SCM dosage. The reason was suggested to the formation of unstable aluminum sulfides that require longer stabilization time to precipitate. Increasing SCM dosage enhances sulfide production, promoting aluminum sulfide formation, but insufficient contact time caused these compounds partially dissolved. Moreover, the higher organic load from SCM can influence the aluminium solubility by forming complexes with organic ligands, and maintaining aluminium in the dissolved phase longer.

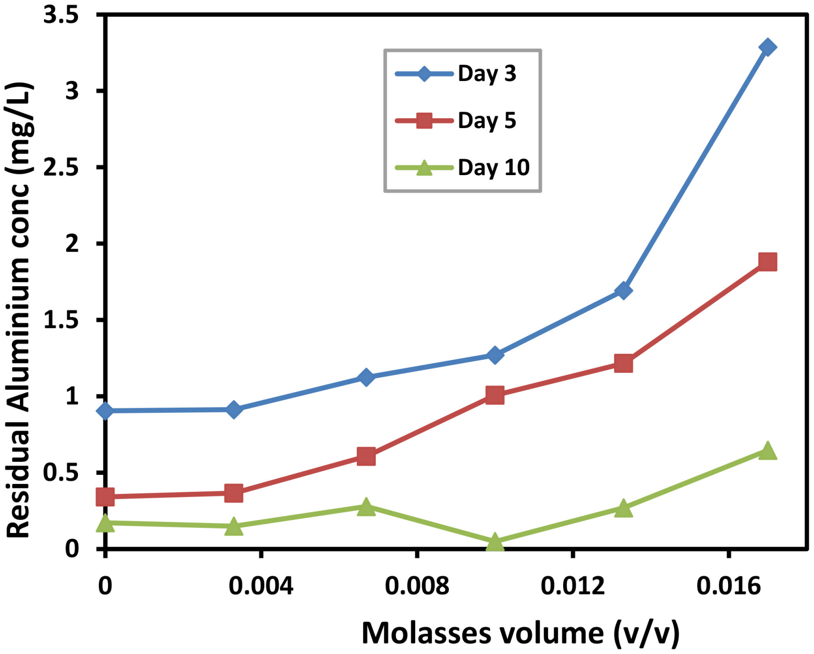


Figure 4. The effect of SCM on residual aluminum at constant pH (7.0), initial aluminium concentration (130 mg/L) and varied SCM dosage (0 - 0.0167 v/v).

3.3. COD Removal

Increase in COD levels resulting from the mixing of AMD and small amounts of SCM were observed from initial contact time (0 day). This can be seen from **Figure 5(a)** that the COD of the AMD increased from 548 to 5226 mg/L when the fraction of SCM was increased from 0 to 0.0167 v/v or 0 to 1.67% SCM. This phenomenon indicates that SCM contain high amount of organic materials. In present study, the residual COD concentration from AMD were found to decrease with the increase in contact time (of up to 10 days) and the volume of SCM at a fixed pH 7. **Figure 5(a)** shows that the columns inoculated with SCM (3 - 10 days) were more efficient in the COD removal relative to control column. This signifies that SCM supply the required carbon sources for SRB activity in the columns to break down the organic materials. The findings were supported by Hance et al. (2020) who reported that the great amount of residual COD was due to accumulation of organic materials within the reactor. **Figure 5(b)** shows that the COD removal efficiency ranging from 30% to 96.8% which was observed with an increase in the fraction of SCM from 0 to 0.0167 v/v. Despite the highest COD removal attained by the columns, the final levels did not reach the limit set by WHO and Tanzania Bureau of Standard (TBS) (60 mg/L) for treated wastewater to be discharged into the environment.

3.4. Nitrate Removal

Figure 6 shows that at initial concentration of nitrate (1530 mg/L), the maximum reduction (99.80%) was found with an increase in treatment time from day 3 to 10 and the SCM volume (0.0167 v/v). The nitrate reduction was observed in all columns inoculated with SCM and the control column. Higher nitrate reduction was observed in columns with SCM than that of the control column although all attained the greatest nitrate removal. The reduction of the nitrate concentrations from the water samples was considered to be due to assimilation of nitrate by microbes including SRB for oxidation of SCM. Also, high reduction of nitrate was due to other reactions in the nitrogen cycle in which nitrate was converted to ammonium (via denitrifying bacteria) and nitrogen gas (via ammonifying bacteria) by the process of ammonification and denitrification, respectively. Moreover, the other possible process for nitrogen species transformation including anammox that lead into anaerobic ammonium oxidation which contributed to nitrogen removal. These processes suggest the presence of other microbial groups (e.g., denitrifiers, ammonifiers, and anammox bacteria) alongside SRB in the inoculum.

4. Conclusion

This study demonstrated the effectiveness of using SCM as a sustainable carbon source for the treatment of AMD in constructed wetlands. Following microbial acclimation, sulfate, nitrate and dissolved concentrations of Cu, Zn, Fe, Mn, and Al) were significantly reduced in the SCM-inoculated columns compared to the control. Results from batch experiments revealed significant reductions in sulfate

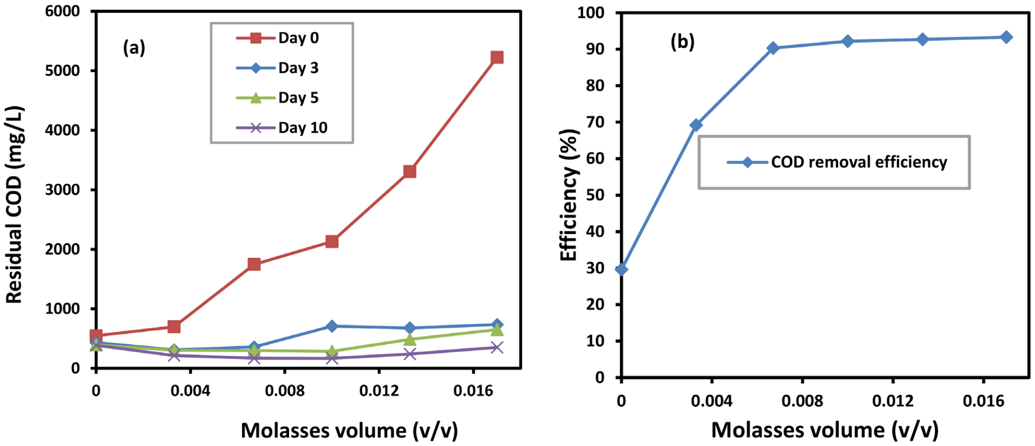


Figure 5. The effect of SCM on residual COD (a) and COD removal efficiency (b) at constant pH (7.0) and varied SCM dosage (0 - 0.0167 v/v).

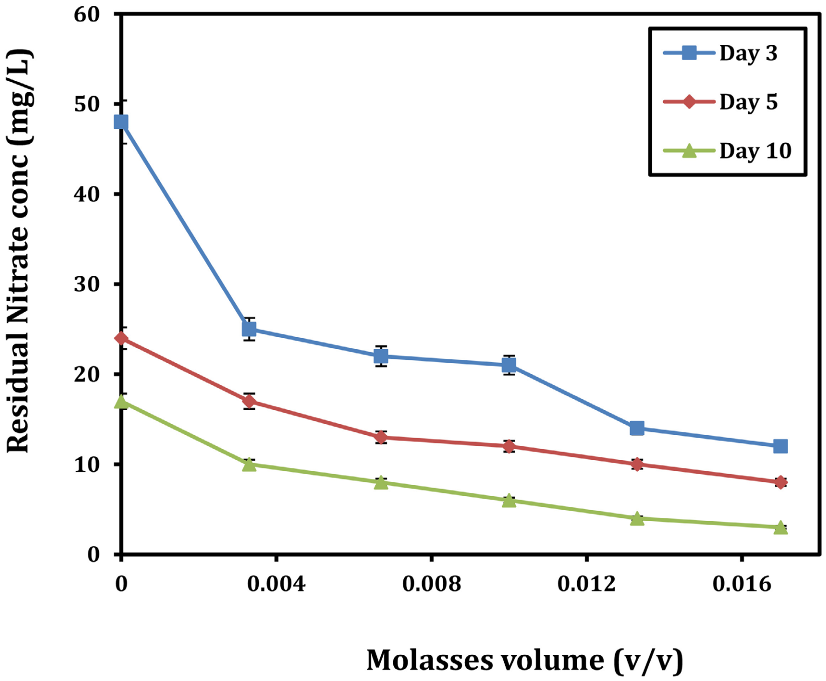


Figure 6. Shows the effect of SCM in nitrate reduction at constant pH (7.0) and varied dosage of SCM (0 - 0.0167 v/v).

and nitrate concentrations, achieving >96% removal efficiency through the microbial activity particularly that of SRB. Heavy metal removal efficiency > 94% for Cu, Fe, Mn, Zn, and Al, confirming the potential of SCM to enhance metal precipitation processes. The residual metal concentrations, except for aluminium, remained above the acceptable discharge limits set by the TBS and WHO. Despite achieving high COD removal efficiency (92.15%), the residual COD concentration (167 mg/L) was higher than the acceptable limits (60 mg/L), suggesting that either longer contact time or supplementary treatments (such as additional aeration step or using biofiltration techniques) are necessary for complete compliance with environmental discharge standards. Therefore, the findings validate the potential application of locally available organic substrates such as SCM for passive AMD remediation, offering a cost-effective and environmentally friendly alternative to conventional treatment methods. Moreover, the absence of detailed microbial profiling limits the understanding of the dominant biological processes.

5. Recommendations

Laboratory trials using raw mine water from the mining sites are recommended for future studies to assess the field application of SCM dosed constructed wetlands. Future studies are needed to investigate COD and the selected heavy metals (Cu, Fe, Mn, and Zn) removal to determine the retention time required to meet the acceptable limits set by TBS and WHO standards. Microbial community analysis to identify and quantify the microbial groups present, the use of isotopic labeling techniques to distinguish between nitrogen transformation processes, and experiments with axenic SRB cultures to isolate their specific contributions are highly recommended. Further conduction of the control experiment where SCM is added to distilled water (without AMD) and measure the concentrations of metals leached into the solution, is recommended. This will allow quantification of the contribution of SCM to the total metal concentrations in the system. The study also recommended the need for further investigation into microbial community dynamics, flow regimes, and scalability of the proposed treatment system. The potential presence of residual organic compounds and other water quality indicators that could impact discharge compliance should be addressed in future studies. Future studies should incorporate microbial profiling techniques, such as 16S rRNA sequencing or selective culturing, to better understand the microbial diversity and the specific role of SRB in the system.

6. Acknowledgement

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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