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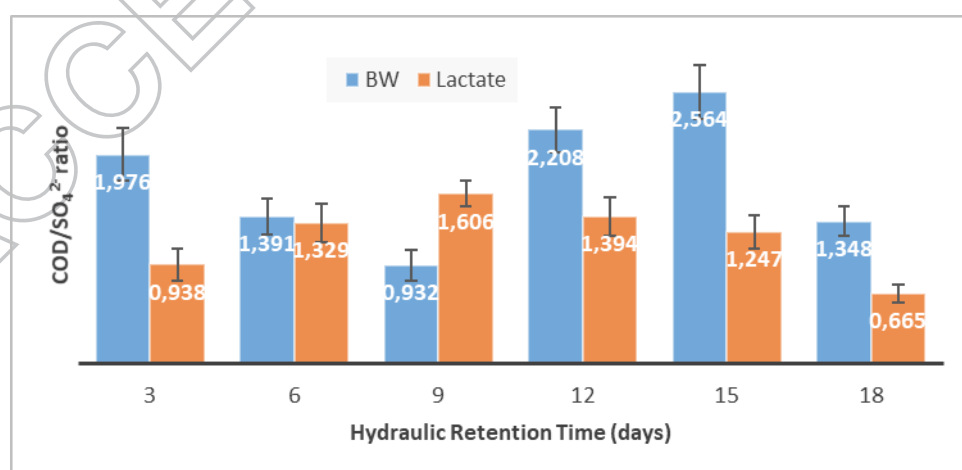
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Abstract

This study investigated the effect of carbon sources ($n = 2$) on the performance of a microbial community in an anaerobic moving bed biofilm reactors (MBBR) treating acid mine drainage (AMD). The 1.5 L anaerobic MBBR was operated across a range of hydraulic retention times – HRT's (3-18 days), using different substrates, i.e. brewing wastewater and lactate as sole carbon sources and electron donors. Maximum sulphate reduction and chemical oxygen demand (COD) consumption rate was 21.94 and 24.28 mg $\text{SO}_4^{2-} \text{L}^{-1} \text{h}^{-1}$, and 0.473 and 0.697 mg COD $\text{L}^{-1} \text{d}^{-1}$ for brewing wastewater and lactate supplemented bioreactors, respectively, at a HRT of 3 days. The maximum COD/ SO_4^{2-} ratio was found to be 2.564 in bioreactor supplemented with brewing wastewater at HRT of 15 days. The metal removal above 70% followed the order; $\text{Be}^{2+} > \text{Fe}^{2+} > \text{Sr}^{2+} > \text{Pb}^{2+} > \text{Mg}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Li}^{1+} > \text{Ca}^{2+}$ in comparison to the system supplemented with lactate, $\text{Be}^{2+} > \text{Fe}^{2+} > \text{Sr}^{2+} > \text{Mg}^{2+} > \text{Cu}^{2+} > \text{Li}^{1+} > \text{Zn}^{2+} > \text{Pb}^{2+}$ after HRT of 18 days. Complete removal of beryllium (II) was observed irrespective of the carbon source. The results clearly showed that brewing wastewater can be deployed as a nutritional supplement in environmental remediation of AMD.



Keywords: Acid mine drainage, anaerobic, brewing wastewater, lactate, microbial community, MBBR

Introduction

Previous and present mining activities, including various industries, discharge large quantities of acidic wastewater into the environment. Acid mine drainage (AMD) is a major challenge associated with active and abandoned mining sites, particularly whereby remediation strategies are non-existent which culminates into intense microbial oxidation of sulphide minerals resulting in leaching of potential toxic elements. This AMD usually contains a high concentration of sulphate (350 – 13,000 mg SO₄²⁻/L) and heavy metals (10⁻³ – 10⁴ mg/L) [1]. Although, sulphate discharges are not a major threat to the environment as they are non-volatile, chemically inert and non-toxic, high sulphate concentration can cause an imbalance in the natural sulphur cycle [2, 3]. Traditionally, lime neutralisation is used to reduce the acidic properties of the wastewater, followed by precipitation of heavy metals as hydroxides [4]. However, large quantities of amorphous metal-sludge generated, are difficult to dewater due to high sulphate concentration associated with the AMD [5, 6]. In addition, reverse osmosis is another method that is prevalent in AMD treatment technologies due to the high quality of the treated water which can then be recycled. However, operating with water recycle rate of between 35% and 85%, could lead to huge volumes of concentrates as well as brines, which is a major threat to the ecosystem [7]. Therefore, there is a need for an environmentally friendly treatment technique for AMD.

Biological treatment of wastewater including AMD is an effective alternative for high strength sulphate containing wastewater such as AMD. This is performed by a consortium of bacteria comprising of sulphate reducing bacteria (SRB), and strictly anaerobic bacteria, as well as facultative anaerobic bacteria [8]. AMD is known to be amenable to anaerobic digestion with subsequent removal of heavy metals as metallic sulphides [9]. The controlled biological treatment of AMD offers some advantages which includes permanent removal of sulphur and metals, thus the subsequent production of clean water. Biological reduction of sulphates has been successfully applied for the treatment of AMD on a large scale for many years because it is considered an efficient and low cost process with varying organic carbon sources being used as electron donors [10]. Commonly used electron donors are lactate, ethanol, methanol, formate and hydrogen. Regarding the energy and biomass yield requirements, lactate is reported as the best carbon source [11]. Although the biological treatment of AMD is environmentally benign, the application of this treatment method on a large scale could be hampered by nutrient requirements for the sustainable maintenance of the microbial culture and its proliferation for elongated periods of time.

Agro-industrial waste offers an alternative as an organic carbon source for microbial proliferation. Globally, 1.6 X 10⁹ tonnes of agro-industrial waste are generated annually, and such waste contains remnants of proteins, reducible sugars, trace elements and minerals that could sustain a low cost bioprocess, such as those designed for AMD treatment [12, 13]. Several organic waste materials have

been investigated for such process including animal manure, molasses, leaf mulch, saw dust, wood chips, and sewage sludge [14-16]. The selection of a substrate to use depends on the biodegradability of the substrate. Approximately 2.0×10^8 L of reducible sugar rich wastewater, is generated annually in South Africa from sorghum beer industries, with some nutrient components including proteins, fats and some trace elements [17]. The availability of this quantity of brewing wastewater in South Africa could provide a suitable feedstock for microbial proliferation in the treatment of AMD. Therefore, a comparative analysis of the performance of microbial community supplemented with lactate and brewing wastewater as sole carbon sources in anaerobic MBBR systems treating AMD, was studied to promote the application of agro-industrial waste in environmental remediation.

2.0 Materials and Methods

2.1 Acid mine drainage (AMD) collection sampling

AMD samples were collected from a coal mining location in Mpumalanga Province (South Africa) using a standard sampling approach [18]. The AMD was screened for particulate matter removal prior to storage at 4°C. The physicochemical characteristics of the AMD ($n = 3$) showed that the wastewater was comprised of 8080 ± 10.35 mg $\text{SO}_4^{2-}/\text{L}$, metallic ions (mg/L): 485 ± 3.25 Al^{3+} , 0.07 ± 0.01 Be^{2+} , 422.66 ± 3.17 Ca^{2+} , 0.5 ± 0.11 Cd^{2+} , 2.33 ± 0.21 Co^{2+} , 0.46 ± 0.05 Cu^{2+} , 0.13 ± 0.01 Cr^{3+} , 2308 ± 5.51 Fe^{2+} , 297.6 ± 2.67 Mg^{2+} , 0.53 ± 0.05 Li^{1+} , 60.8 ± 1.89 Mn^{2+} , 5.47 ± 0.43 Pb^{2+} , 1.0 ± 0.11 Sr^{2+} , and 7.93 ± 0.34 Zn^{2+} . The sample temperature was $20 \pm 2^\circ\text{C}$, pH 2.98 ± 0.2 , and conductivity of 7.48 ± 0.5 mS/cm, including, a redox potential of 229.5 ± 3.58 mV and a turbidity of 145 ± 2.24 NTU. An inductively coupled plasma optical emission spectrometer (ICP-OES) (ICP Expert II, Agilent Technologies 720 ICP-OES) was used to measure metal ion concentrations while a COD and Multiparameter Bench Photometer HI 83099 (Hanna Instruments Inc., USA) was used to measure both the COD and sulphate (SO_4^{2-}) concentration in the AMD samples. Comparatively, the sulphate ion concentration and conductivity observed in this location are within the range reported ($1265 - 14070$ mg $\text{SO}_4^{2-}/\text{L}$) and ($2.41 - 11.85$ mS/cm), respectively, at different points along different paths of the AMD in the surrounding communities of Mpumalanga province [19], meanwhile [20] a much higher sulphate concentration (20980 mg $\text{SO}_4^{2-}/\text{L}$) and 12.41 mS/cm conductivity from waste coal dump in Witbank South Africa. For the metallic ions, Fe (II) concentration was the highest in all cases as seen in this study with pH < 3 [19, 20].

2.2 Isolation and growth media preparation

Biotechnological approach using sulphate reducing bacteria (SRB) is considered an effective method for simultaneous removal of sulphate and heavy metals in wastewater. The sulphate acts as the electron acceptor while a carbon substrate serves as the electron donor in an anaerobic process that leads to the formation of bicarbonate ions and sulphides [21]. The metal ions are precipitated by the sulphides while

the bicarbonate raised the pH of the wastewater [22, 23]. The use of refined carbon substrate such as lactate has significant effect both on the SRB growth and economic viability of the process in an industrial scale [23, 24]. Hence, the use of an agro-industrial waste that is usually thrown away, to mitigate the cost.

The isolation of sulphate reducing bacteria (SRB) was done using a modified Postgate (1984) isolation media as described in a previous study, in two different reactors with lactate (L) in one and brewing wastewater (BW) in the other reactor, as carbon sources and electron donors [1]. The darkening of the medium indicated a successful incubation and proliferation of the microbial consortium of SRB. The experiments were conducted as triplicate.

2.3 Composition of the brewing wastewater as a carbon source

Brewing wastewater was collected from a sorghum beer manufacturer in Potchefstroom, South Africa. The analysis of the wastewater using High Performance Liquid Chromatograph (HPLC, Agilent 1290 Infinity) equipped with a 300 m x 7.8 mm Aminex HPX-97H column [25] revealed the presence of mainly fat (0.26 ± 0.03 g/100mL), protein (0.33 ± 0.01 g/100mL), lactose (0.5 ± 0.02 g/100mL), glucose (0.5 ± 0.02 g/100mL), fructose (0.5 ± 0.03 g/100mL), sucrose (0.5 ± 0.02 g/100mL), maltose (0.5 ± 0.03 g/100mL) and dry matter (0.42 ± 0.02 g/100mL).

2.4 Process overview: anaerobic moving bed biofilm reactor

The experiments were performed in different 1.5 L anaerobic MBBRs fitted with overhead stirrers at a speed of 200 rpm. High density polyethylene (HDPE) Mutag Biochip 25TM bio-carriers – 15% working volume were used [26]. The bio-carriers have an active surface area of $4,850 \text{ m}^2/\text{m}^3$, diameter of 25 mm, and a 1.1 mm thickness. The bioreactors were operated at 35°C in a circulating waterbath and pH 7 ± 0.5 was maintained for the growth media until the AMD was introduced into the bioreactors.

Each reactor contained 900 mL Postgate media with the reactor being seeded with a 10% (v/v) inoculum. Fresh medium was fed into the reactor weekly for 4 weeks. All glass lids and fittings were sealed with vacuum grease, and the reactors were purged with nitrogen gas in order to maintain anaerobic conditions. After establishing viable microbial growth, raw AMD was pumped into the reactors at a feed rate of 1.6 L/h and the systems were operated at different hydraulic retention times (HRTs). The microbial growth assessment was based on optical density (OD) at a wavelength of 600 nm using a GENESYSTM 10S UV-Vis spectrophotometer (Thermo Fisher ScientificTM, Waltham, MA, USA). For each HRT, microbial growth was measured (OD) at interval over a period of time until stationary phase is reached. Microbial growth rate was calculated as the slope of the plot of OD against time for the exponential phase of microbial growth. The sulphate concentration and chemical oxygen demand (COD) were measured using a COD and Multiparameter Bench Photometer HI 83099 (Hanna Instruments Inc., USA). The metal ions concentration in the samples were measured after HRT of 18

days using the inductively coupled plasma optical emission spectrometer (ICP-OES) (ICP Expert II, Agilent Technologies 720 ICP-OES, California, USA). The control experiments were not inoculated with microbial consortium. All measurements were in triplicate.

3. Results and discussion

3.1 Microbial growth rate

Among the many factors affecting the microbial proliferation in AMD systems is reactor configurations. Different reactor types such as continuously stirred tank reactor, gas-lift bioreactor, submerged membrane bioreactor, fluidised bed bioreactor, and up-flow anaerobic granular sludge bed bioreactor, amongst others have been reported for AMD treatment [27]. In this study, an anaerobic moving bed biofilm reactor (MBBR) was used for the treatment of AMD at different hydraulic retention times (HRTs). A steady microbial growth was observed for both carbon sources (L and BW), see – Fig. 1. At lower HRTs (3 to 9 days HRT), the systems supplemented with BW showed a lower microbial growth due to the time taken to degrade the agro-industrial waste; however, a higher growth rate was observed as the HRT increased (12-18 days HRT). This can be attributed to the residency time increases at higher HRTs for which the excess and residual carbon sources were readily available to the microbial community. In the system utilising the refined carbon source (L), there was an initial rapid increase in the microbial growth at the initial stage (i.e. lower HRT) – 3 to 9 days, due to the availability of the processed carbon source; however, as the HRT increased, the microbial growth decreased because of the depletion of the carbon source. The maximum microbial growth rate (μ_{max}) were 0.3134 and 0.3317 per day for BW and L at HRT of 3 days. These values are within the range of available kinetic parameters for SRB communities [28-31].

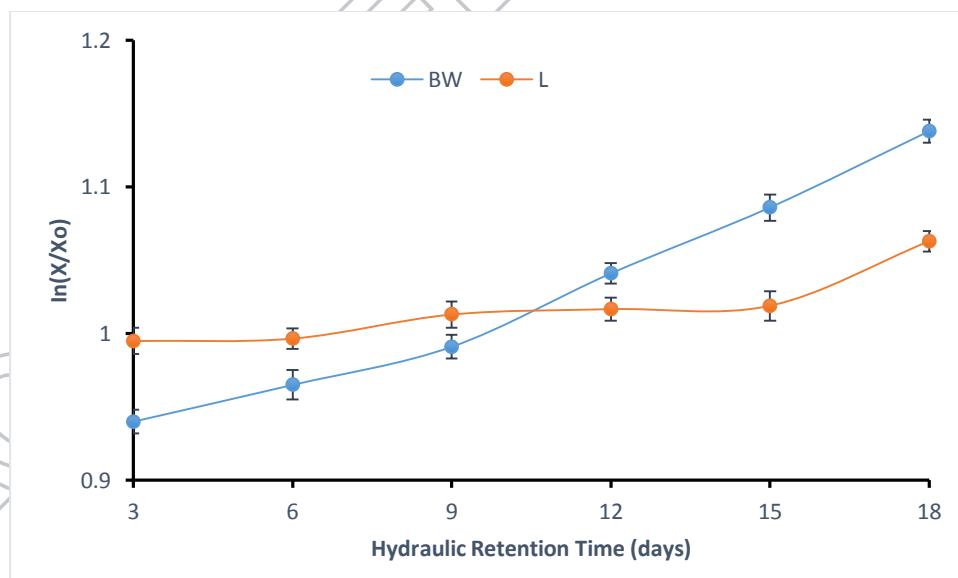


Fig. 1: Maximum microbial growth rate for each hydraulic retention time

3.2 Effect of HRT on pH and redox potential

Previously, bioreactors treating AMD were usually neutralised and the pH had a direct effect on the microbial community that thrives within a bioreactor [27]. However, the presence of acid tolerant microorganisms has offered the possibility of treating AMD directly without prior neutralisation [32-34]. In this study, the pH of the process increased with an increasing HRT, an indication that the microbial community generated alkalinity itself which allows for metal precipitation – Fig. 2. This observation is similar to the report on microbial community treating AMD which were dominated by *Thiobacillus* spp. and thus raised the pH of the reactors [35]. Thermodynamically, Gibbs free energy is the major driving force of microbial growth. Previous reports have shown that higher Gibbs free energy is obtained at low pH during sulphate reduction [36]. This explains the sulphate reduction observed at low pH in this study. Higher pH was observed in BW than L supplemented reactors, although below a neutral pH. Conversely, there was a reduction in the redox potential for both reactors, an indication of microbial growth over time. The movement of various elements in biological systems depend on the redox reaction conditions. The potential difference in the solution leads to the oxidation/reduction reaction in the system [9, 37].

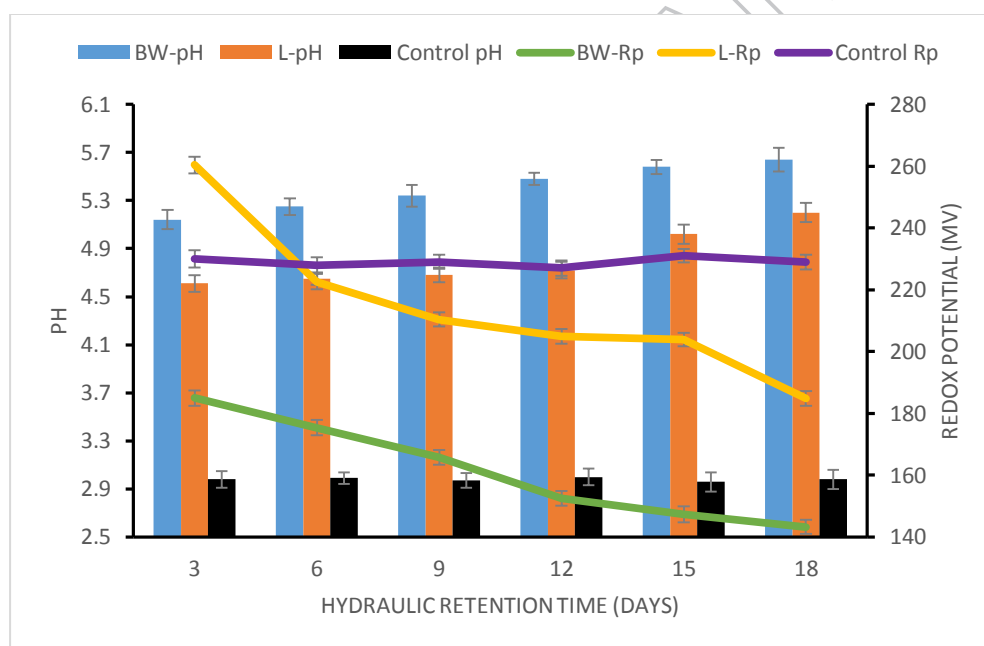


Fig. 2: Effect of HRT on pH and redox potential

3.3 Sulphate reduction and COD/sulphate ratio

There are several factors affecting sulphate reduction efficiency in an anaerobic MBBR. The effects of hydraulic retention time on the performance of bioreactors have been widely studied. It was generally accepted that metal sulphides precipitate between 3 and 5 days, and that any HRT below 3 days will not allow time for microbial activity to neutralise acidity and facilitate subsequent metal precipitation [38]. Hence, the reactors were set-up at an initial HRT of 3 days. From Fig. 3, the rate of sulphate

reduction reduces with an increasing HRT. This was attributed to the depletion of readily available carbon and/or energy source as well as reducing microbial activities in the bioreactor [39]. Improved reactor performance was observed in the bioreactor supplemented with refined carbon source (L) at low HRT. At elevated HRT, higher sulphate reduction rates were observed in system supplemented with agro-industrial waste compared to the refined carbon source, which can be traced to the availability of diverse soluble sugars in the BW. Therefore, we can conclude that low HRT is an appropriate operational strategy for a system supplemented with the refined carbon source while a system operating on agro-industrial waste requires a longer HRT. A similar pattern was reported for sulphate removal in AMD by mixed SRB in an up-flow anaerobic packed bed reactors, with the highest sulphate reduction rate being after 2 days [9]. The highest average sulphate reduction rate in this study was 21.94 and 24.28 mg SO₄²⁻/ (L h) for BW and L, respectively, and compares well with approaches previously used in the treatment of AMD [9, 22, 39, 40].

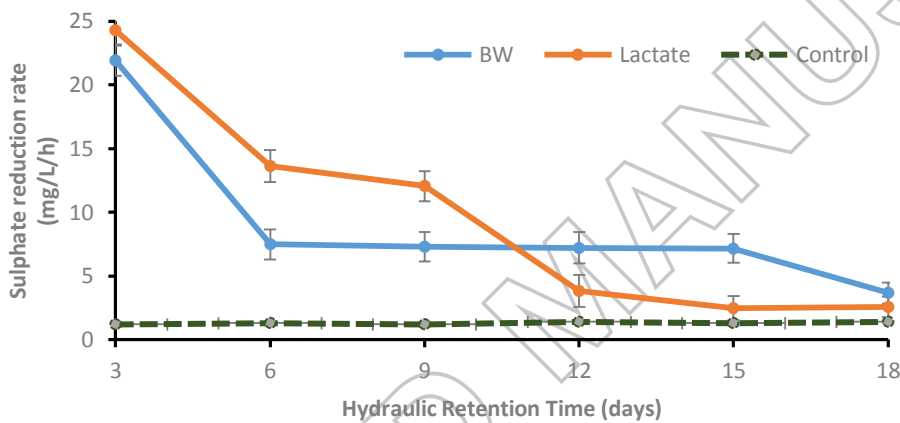
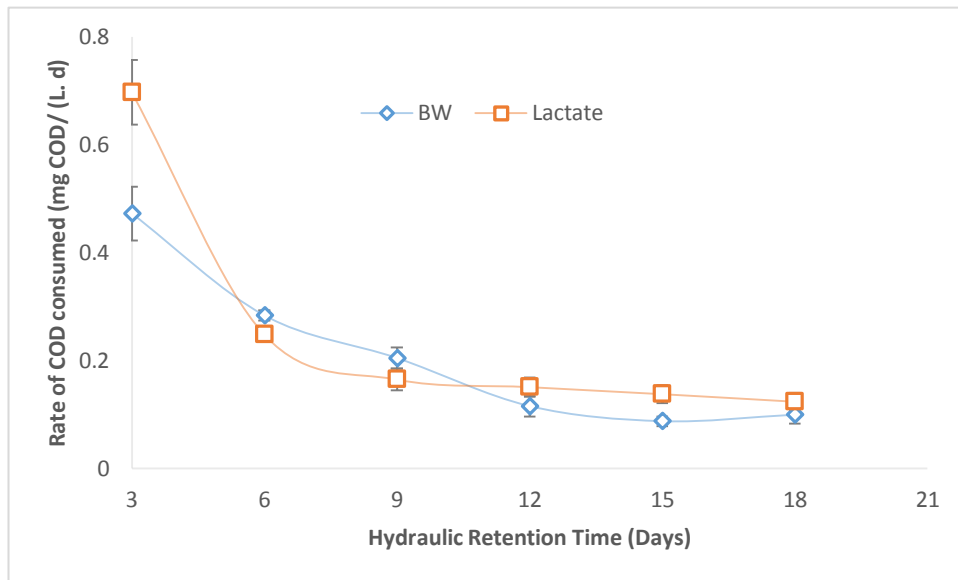


Fig. 3: Variation in sulphate reduction rate

In addition, the bioreactor supplemented with L, removed the COD more efficiently than the bioreactor supplemented with BW under the various HRT investigated, with the highest average rate of 0.473 and 0.697 mg COD/ (L d) for BW and L, respectively, at low HRT of 3 days, see – Fig. 4a. When the HRT increased, the rate of COD consumption decreased for both bioreactors. This trend was similar to previous reports in literature reviewed [22, 40]. Subsequently, COD/SO₄²⁻ ratio was affected as the HRT changed – Fig. 4b. The lowest COD/SO₄²⁻ ratio was 0.665 in systems supplemented with L at a HRT of 18 days while the highest was 2.564 in systems supplemented with BW at a HRT of 15 days. The COD/SO₄²⁻ ratio increased from 0.938 to 1.606 between HRT of 3 and 9 days before declining to 0.665 at HRT of 18 days in systems supplemented with the refined carbon source (L). The decline was further evidence that the substrate was depleted at higher HRT, which subsequently reduced the rate of sulphate removal. In contrast, the COD/SO₄²⁻ ratio, decreased from 1.976 to 0.932 between HRT of 3 and 9 days before ascending to 2.564 at HRT of 15 days in bioreactors supplemented with agro-industrial waste (BW). The availability of multiple soluble sugars in the BW, maybe responsible for the higher

COD/SO₄²⁻ ratio at elevated HRTs, resulting in better sulphate reduction rates. Generally, bio-carriers are known to improve the rate of COD removal in wastewater treatment. When HRT was increased, the rate of COD consumed increased due to improved biofilm formation in the MBBR, with subsequent pollutant removal [26, 41].

A



B

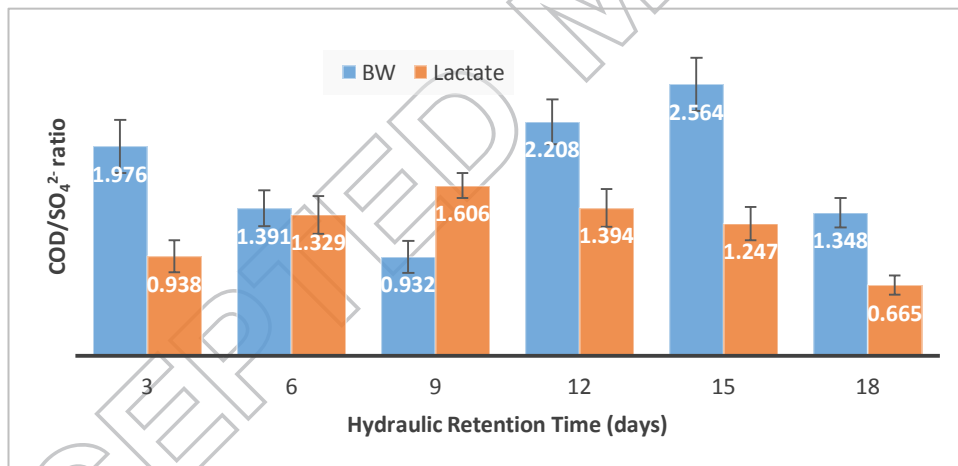


Fig. 4: Effect of hydraulic retention time on rate of COD consumed (A) and COD/SO₄²⁻ (B)

3.4 Metal ions removal

At the end of hydraulic retention time of 18 days, bioreactors effluent was analysed for heavy metal reduction. There was complete removal of an alkaline earth metal, beryllium (Be²⁺), in both bioreactors. This may be attributed to the insolubility of beryllium sulphide in water. The removal of other metals was incomplete and only 95.7% and 95.9% of initial Fe²⁺ in BW and L, respectively were removed – Fig. 5. This is comparable to previous reports of 95% Fe²⁺ removal and Jong & Parry (2003) who obtained 82.3% removal of Fe²⁺ using an anaerobic reactor for treatment of metal-laden wastewater [42].

The metal removal was as a result of metal sulphides precipitation due to the activities of SRB. Previous reports have shown that Al^{3+} , Cu^{2+} , Fe^{2+} and Pb^{2+} are precipitated at pH below 7 [43, 44]. The metal removal above 70% followed the order; $\text{Be}^{2+} > \text{Fe}^{2+} > \text{Sr}^{2+} > \text{Pb}^{2+} > \text{Mg}^{2+} > \text{Cu}^{2+} > \text{Zn}^{2+} > \text{Li}^{1+} > \text{Ca}^{2+}$ for system supplemented with BW while that of the system supplemented with L followed the order; $\text{Be}^{2+} > \text{Fe}^{2+} > \text{Sr}^{2+} > \text{Mg}^{2+} > \text{Cu}^{2+} > \text{Li}^{1+} > \text{Zn}^{2+} > \text{Pb}^{2+}$. This can be attributed to the relatively high pH (below 7) in both bioreactors, given that metals become insoluble at higher pH. The Al^{3+} removal was the lowest (29%) in the system supplemented with L, which may be due to the fact that Al^{3+} does not form stable sulphides in the presence of water (Jong & Parry 2003). Furthermore, Co^{2+} removal of 19% was observed in system supplemented with BW. Previous reports have shown that optimum Co^{2+} removal occurs at pH of 6 [45, 46]. The low removal of Co^{2+} was as a result of the acidic pH of the systems.

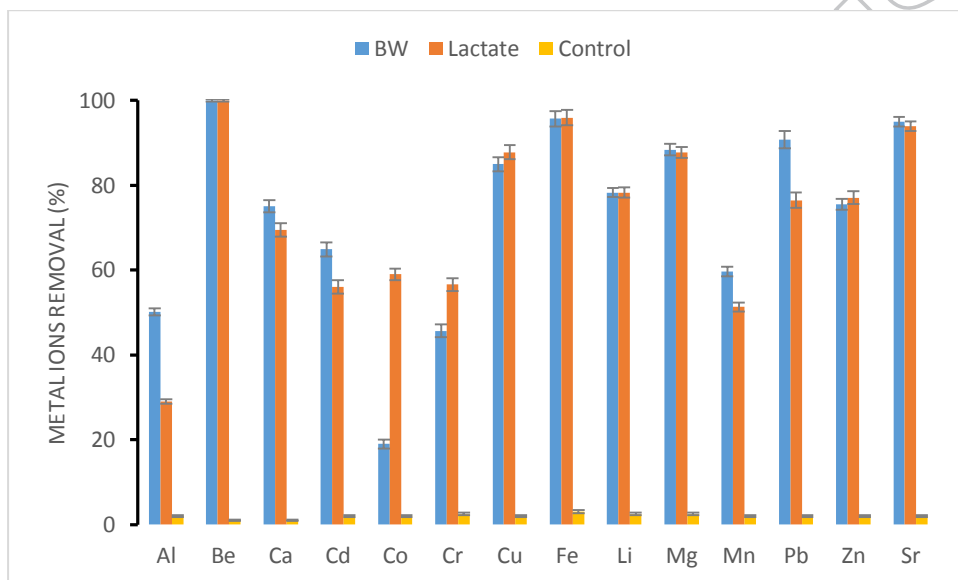


Fig. 5: Metal ions removal after HRT of 18 days

4 Conclusion

The systems showed microbial sulphate reduction and subsequent precipitation of metal ions in systems supplemented with lactate and brewing wastewater. After the adaptation period, the pH of the bioreactors increased, redox potential reduced, and the COD consumption fluctuated with respect to variations in the hydraulic retention time. Higher HRT was required for optimum sulphate reduction in systems supplemented with brewing wastewater while a lower HRT favours optimum sulphate reduction in system supplemented with lactate. Complete removal of beryllium (II) was observed in both bioreactors, followed by iron (II); 95.7% and 95.9% for brewing wastewater and lactate supplemented system, respectively. This study demonstrated the potential of brewing wastewater

relative to lactate, as a suitable carbon source for proliferation of microbial community in an anaerobic MBBR treating acid mine drainage.

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Conflict of interest

The authors declare no conflict of interest.

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