Biomass Retention and Recycling to Enhance Sulfate Reduction Kinetics

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ABSTRACT

Biological sulfate reduction represents a more sustainable option for the removal of sulfate from acid rock drainage, particularly if the resulting sulfide is used for metal precipitation or is partially oxidized to sulfur. Sulfate reducing bacteria are relatively slow growing and attach poorly to most solid substrates, so washout is a concern at low hydraulic residence times. This paper describes two strategies to achieve high cell concentrations in sulfate reducing reactors and reports on the resulting improvements in sulfate reduction efficiency.

A linear flow channel reactor was fitted with carbon microfibers, which provided a large surface area for attachment, without significantly reducing effective reactor volume. The surface properties of the carbon fibers enhance biomass attachment and colonization of the fibers occurred within two weeks. The hydrodynamic regime within the reactor ensured even substrate distribution without physical agitation. The second reactor system involved coupling a cross-flow microfiltration unit, fitted with a ceramic membrane, to a conventional continuous stirred tank reactor. The retentate, containing the biomass, was recycled back to the CSTR.

The reactors were fed a synthetic solution containing 1 g/L sulfate, with lactate as the carbon source and electron donor, at a COD to sulfate ratio of 0.7. The hydraulic residence time was reduced from 4 days to 0.5 days, with steady state data collected at each residence time. Maximum volumetric sulfate reduction rates of 47.5 and 65 mg/L.h were achieved in the channel reactor and membrane coupled reactor system respectively. These represent improvements of 20% and 50% over a conventional CSTR, where washout of part of the community occurred at HRTs below 1 day.

Keywords: Sulfate reduction, biomass retention, membrane separation, biomass attachment

INTRODUCTION

The contamination of surface and groundwater by acid mine drainage (AMD) and acid rock drainage (ARD) and the consequences for the environment, agriculture and human health are serious concerns in the regions of South Africa impacted by mining activities. Acid drainage is generated via the oxidation of sulfide minerals, typically pyrite, when exposed to oxygen and water. The process is usually catalyzed by iron and sulfur oxidizing microorganisms. In South Africa, mine water can be divided into two broad categories. The first, AMD, originates from the rebound of groundwater through abandoned mine workings, once dewatering has ceased and is characterized by large volumes of heavily impacted water. The volume and composition of the AMD precludes the application of biological treatment options in most cases. The second type, referred to as acid rock drainage (ARD) in this paper, originates from diffuse sources, such as waste rock dumps, tailings impoundments, coal discard heaps and unworked pits. These sites are more numerous, are likely to affect a greater area and can persist for decades. Acid rock drainage, from diffuse sources as well as end-of-pipe sources, is more amenable to biological treatment.

A variety of technologies have been developed for the treatment of AMD and ARD. The established methods are based on oxidation, neutralization, precipitation and sedimentation. The oxidation converts iron and aluminum to their less soluble oxidized form, which makes subsequent precipitation more efficient. The most appropriate treatment is dependent upon the volume of the effluent, concentration type of contaminants and the pH of the water (Gazea et al., 1996). Acid drainage treatment technologies can be divided into two broad categories, active and passive treatment systems.

Active treatment typically involves the installation of agitated reactors or similar units, which require constant energy input. Furthermore, the addition of alkaline chemicals and reagents to treat the acidic effluent can become costly, given that the drainage may persist for several decades, or longer, at decommissioned mine sites (Gazea *et al*., 1996). Many of the active treatment technologies depend on the addition of lime or limestone, which are non-renewable resources. Lime addition to sulfate rich effluents typically results in substantial gypsum precipitation, which needs to be managed. The long-term sustainability of many active treatment technologies is therefore questionable, both from an economic and environmental perspective. There is a diverse range of active treatment technologies, such as chemical precipitation, ion-exchange, membrane technology and biological sulfate reduction.

Passive systems depend on processes that are kinetically slower than those involved in active systems and thus require longer hydraulic retention times (HRTs) and larger areas to achieve similar results (Hedin *et al*., 1994). Passive treatment options include anoxic limestone drains, permeable reactive barriers, natural and constructed wetlands and engineered biological treatment systems.

Biological treatment has the potential to be more cost effective and sustainable than the physical and chemical processes mentioned above. The biological treatment of ARD is centered on the activity of sulfate-reducing bacteria (SRB), which are able to reduce sulfate to sulfide, coupled to the oxidation of an electron donor, typically an organic carbon molecule. Sulfate reduction may be assimilatory, where the sulfide is incorporated in sulfur-containing amino acids, or dissimilatory, where the sulfide is released to the external medium. The latter process forms the basis of ARD remediation processes. A generalized reaction for dissimilatory sulfate reduction is shown below (Zagury et al., 2007; Oyekola et al., 2009).

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \quad (1)$$

The sulfate is reduced to sulfide, coupled to the simultaneous generation of alkalinity, predominantly as bicarbonate (HCO_3^-). From an ARD treatment perspective the alkalinity acts to neutralize the acidity while the sulfide is available for the precipitation of metals as metal sulfides (Johnson and Hallberg, 2005).

A number of commercial processes, based on biological sulfate reduction, have been developed, but their widespread application has been constrained by three factors. These are the cost of the electron donor, the relatively slow growth of sulfate reducers and the associated kinetic constraints and the management of the sulfide product. The research presented in this paper addresses the second constraint by investigating novel reactor configurations aimed at biomass retention and recycling. The first and third constraints have been addresses by previous and ongoing research within CeBER (van Hille and Mooruth, 2013; Harrison et al., 2014) and by others (Janssen et al., 1995; Molwantwa and Rose, 2013).

METHODOLOGY

Sulfate reducing bacteria (SRB) culture

The SRB mixed microbial community was obtained from the Department of Microbiology, Biochemistry and Biotechnology at Rhodes University, originally from the anaerobic compartment of a facultative pond at the Grahamstown sewage treatment works, and has been maintained at UCT since 1999. The stock culture has been maintained on modified Postgate B medium consisting of: 0.5 g/L KH_2PO_4, 1 g/L NH_4Cl, 2 g/L $MgSO_4.7H_2O$, 1 g/L Na_2SO_4, 1 g/L yeast extract, 6 mL/L 60% sodium lactate solution (Sigma), 0.3 g/L sodium citrate. The same solution was used to feed all experimental reactors. Unless otherwise stated, all regents were analytical grade, sourced from Merck.

Reactor units

Continuously stirred biological sulfate reduction reactor

Continuous experiments were performed in glass reactors with a working volume of 1 L. The reactor height was 200 mm, with a liquid volume height of 118 mm, and diameter 104 mm.

Agitation was provided by an overhead stirrer powering a four-bladed marine impeller (58 mm diameter) at 300 rpm. The reactor was fitted with four vertical baffles (10 mm width) to prevent vortex formation. Temperature was controlled at 30°C by pumping heated water through the external jacket or placing the reactors in a temperature-controlled water bath. Feed solution was continuously pumped into the reactor using a variable speed peristaltic pump.

Sulfate reduction reactor with microfiltration unit

The reactor configuration for the cross-flow microfiltration consisted of the standard 1 L glass reactor coupled to a microfiltration unit. The contents of the reactor were pumped through the membrane unit (ceramic membrane with a 0.2 μm pore size) at a rate of 1.7 L/min, meaning the entire volume passed through the membrane every 35 seconds. As a result, the reactor could be considered well mixed and additional agitation by the impeller was not required. This was confirmed through mixing studies.

Linear flow channel reactor (LFCR) with carbon microfibers

The channel reactor provided a flow-through reactor with medium stratification in place of homogeneity. It was constructed from Perspex (11 mm thickness). The front wall of the reactor was fitted with three sets of sample ports, located 60 mm, 120 mm and 180 mm from the inlet. At each distance, there were three ports, 25 mm, 55 mm and 90 mm from the base of the reactor. Each port was fitted with a GC septum and samples were withdrawn with a hypodermic needle. The reactor was fitted with three feed ports (25 mm, 60 mm and 95 mm from the base) in the left wall and three effluent ports (15 mm, 50 mm and 85 mm from the base) in the right wall. When the top outlet port was utilized the liquid height in the reactor was 85 mm, giving a working volume of 2.125 L. The reactor was fitted with a lid and an airtight silicon seal. A port fitted 10 mm below the lid in the left and right hand walls allowed the headspace to be flushed. A strip (38 mm wide) of carbon microfibers (AMT Composites, Cape Town) was attached to the bottom of the lid so that the fibers were submerged in the liquid. The strip had a bundle of microfibers (180 mm long) attached at 7 mm intervals on each side. The reactor was operated continuously by pumping feed in from the uppermost feed port and collecting effluent from the uppermost effluent port.

Analytical methods

All pH testing was done on a Cyberscan 2500 micro pH meter. The meter was calibrated daily using standard (pH of 4.0 and 7.0) buffer solutions. Aqueous sulfide was quantified using the colorimetric DMPD method (APHA, 2005). Aqueous sulfate concentrations were measured by HPLC using a Waters Breeze 2.0 system equipped with a Waters IC-Pak A HR (Anion High Resolution) column and a conductivity detector. The system was run isocratically using a sodium borate-gluconate mobile phase at a flow rate of 1 mL/min. Sample injection volumes of 100 μL were used. To quantify the ion concentrations standard solutions (20, 40, 60, 80 and 100 mg/L) were prepared using sodium sulfate (Na_2SO_4) salt. A full volatile fatty acids (VFAs) analysis was conducted to quantify the concentration of lactic, acetic, propionic, iso-butyric, butyric, iso-valeric and valeric acids. The concentration of each VFA was determined using HPLC on a Waters Breeze

2 HPLC system equipped with a Bio-Rad Organics Acids ROA column and a UV (210 nm wavelength) detector. The system was run isocratically using a mobile phase of 0.01 mol/L H_2SO_4 at a flow rate of 0.6 mL/min. Sample injection volumes of 100 μL were used. To quantify the VFA concentrations, standard solutions (100, 200, 300, 400 and 500 mg/L for each acid) were prepared.

Experimental programme

Baseline

Baseline data were generated in a standard CSTR operated at 30°C, using the modified Postgate B medium as the feed source. The reactor was started at a HRT of four days and sampled at least once per HRT. Once steady state had been established and monitored for at least three HRTs, the feed rate was increased, reducing the HRT to three days. The process was repeated a number of times, collecting data at each HRT (**Table 1**).

The reactors were sampled by removing 10 mL of solution and immediately using 20 μL to perform a sulfide assay. The pH and redox potential were measured using the remaining sample. A subsample (2 mL) was transferred to a 2 mL Eppendorf tube, to which 40 μL of zinc chloride solution was added. The tubes were mixed on a vortex mixer, then centrifuged at 14000 × *g* for seven minutes. The supernatant was used to prepare samples for analysis of anions and VFAs by HPLC.

Table 1 Summary of operating conditions for baseline CSTR, membrane unit and channel reactor. HRTs refers to the total number of hydraulic residence times under each set of conditions

	Baseline CSTR and membrane reactor			Channel reactor		
Desired HRT (days)	Mean flow rate (mL/min)	Mean HRT (days)	HRTs	Mean flow rate (mL/min)	Mean HRT (days)	HRTs
4	0.170	4.10	11.96	0.361	4.09	2.47
3	0.219	3.17	7.61	0.465	3.18	5.64
2.5				0.594	2.49	4.37
2	0.339	2.05	19.24	0.713	2.07	5.29
1.5	0.466	1.49	9.49	0.950	1.55	6.79
1	0.661	1.05	17.69	1.412	1.05	7.68
0.75	0.900	0.77	10.29	1.869	0.79	13.33
0.5	1.347	0.52	7.90	2.535	0.58	18.07
1.3				1.135	1.30	4.03

Membrane unit

The purpose of coupling the microfiltration unit to the continuous reactor was to separate biomass from suspension and recycle it back to the reactor, while discharging a cell-free permeate. This was chosen to build up a very high cell density. It was hypothesized that the high cell density would

support efficient sulfate reduction at HRTs below one day, owing to the de-coupling of the mean cell retention time (MCRT) and HRT.

The reactor coupled to the membrane filtration unit was set up and operated similarly to the reactor used to collect the baseline data. The feed rates, overall hydraulic retention times and number of hydraulic retention times under each set of conditions was identical to the control reactor (**Table 1**). Once steady state had been achieved at a four day HRT the pump was turned on and reactor contents were pumped through the membrane. Permeate was pumped from the membrane unit at the same rate as feed was pumped into the reactor to maintain a fixed operating volume across the system. Under normal operating conditions no effluent was collected from the overflow port of the reactor. However, if the membrane became fouled, reducing the transmembrane flux, or the permeate pipe became blocked, the volume in the reactor would accumulate and discharge through the overflow port. When this occurred, the membrane needed to be de-fouled.

The reactor was sampled as described for the control reactor, while permeate was sampled by collecting the outflow for 5-10 minutes. Reactor and permeate samples were analyzed for pH, redox potential, anions and VFAs.

Linear flow channel reactor

The channel reactor was initially operated at a 5.5 day HRT (0.27 mL/min) to allow for colonization of the microfibers. This was achieved successfully over a period of 20 days. From day 20, the feed rate was increased to achieve a HRT of 4 days. Samples (2 mL) were removed daily from the middle (FM) and lower (FB) sample ports in the first and third (BM and BB) rows. The pH and sulfide concentration were measured immediately, after which the remainder of the sample was treated with 40 µL of zinc chloride (100 g/L) and centrifuged at 14000 × g for seven minutes to remove sulfide as zinc sulfide. The supernatant was filtered through a 0.22 µm nylon membrane filter and retained for HPLC analysis (VFAs and anions). Effluent from the reactor was collected in a sealed bottle over varying time intervals and the volume quantified to confirm the HRT. A portion of the collected effluent was treated for HPLC analysis, while the rest was used to measure pH and redox potential. The operating conditions of the channel reactor are summarized in **Table 1**.

RESULTS AND DISCUSSION

The data presented in this section represent a summary of steady state data at the different retention times across the three reactors. Steady state was assumed when the change in key parameters, particularly residual sulfate concentration, was less than 10% for three successive HRTs following a change in system conditions.

Baseline date

The steady state profiles for sulfate consumption and measured sulfide are shown in Figure 1. The theoretical sulfide concentration, based on the molar concentration of sulfate reduced, is also

shown. The sulfate reduction is relatively consistent across the range of HRTs from four days down to one day, with between 870 and 920 mg/L of the 1000 mg/L feed being consumed. Based on molar stoichiometry, the theoretical sulfide concentration was just below 300 mg/L. However, the actual measured sulfide concentration was significantly lower, suggesting either a loss of sulfide to the surroundings or further reaction of the sulfide.

The reactor unit was sealed and any offgas passed through a sodium hydroxide sulfide scrubber, where gaseous hydrogen sulfide (H_2S) would be converted to aqueous bisulfide (HS^-). Analysis of the bisulfide concentration indicated no significant loss of H_2S from the reactor. This is consistent with the steady state pH (7.4 ± 0.15). Under these conditions the majority of the aqueous sulfide would exist as the HS^-. The result suggests partial oxidation or precipitation of a portion of the sulfide, although no metal sulfide precipitate could be observed. An experiment to quantify the abiotic sulfide oxidation under similar solution chemistry conditions (data not shown) indicated that only a small portion of the missing sulfide could be accounted for by abiotic oxidation. Visual observation of the reactor showed an elemental sulfur deposit at the air/water interface. This deposition was clearly visible when the reactors were taken down, suggesting biologically mediated partial oxidation to sulfur.

There was a significant decrease in the amount of sulfate reduced at a HRT of 12 hours (dilution rate of 0.083/h), with the conversion efficiency falling to around 50%. Despite the reduction in the sulfate conversion, the conversion of lactate remained at 99%. Based on the amount of sulfate reduced, the expected residual lactate concentration at this HRT should have been over 1000 mg/L. However, a residual concentration of below 30 mg/L was detected. This indicates that the sulfate conversion was not limited by lactate concentration.

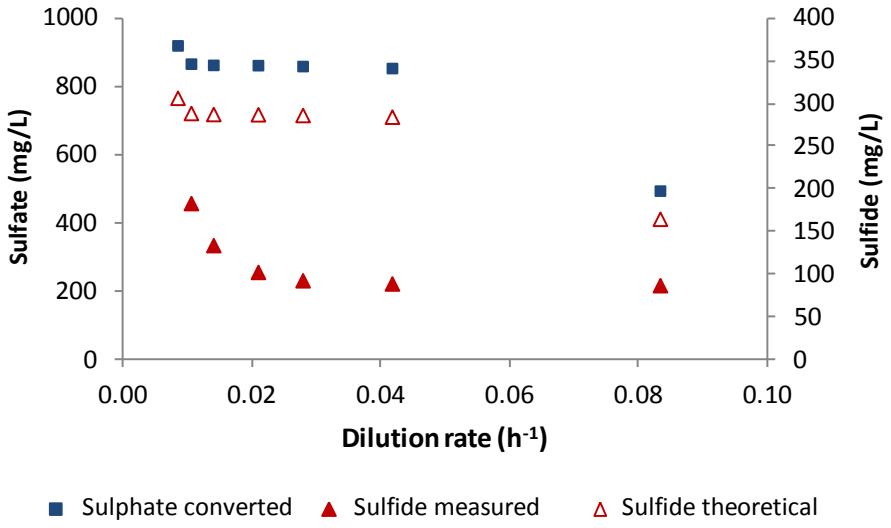

Figure 1 Sulfate conversion and measured and theoretical sulfide concentrations as a function of dilution rate. HRT plotted as dilution rate (1/HRT)

This observation also suggests that while sulfate reducers oxidized lactate at a rate near their μ_{max} another group of microorganisms characterized by higher μ_{max} and K_s values for lactate utilization were able to proliferate due to increased lactate loading at the high volumetric loading rate. Consequently, there was no accumulation of lactate. The decline in sulfate conversion was most likely a consequence of wash out of a portion of the sulfate reducing community when the reactor was operated at a dilution rate greater than their μ_{max}. These data are similar to those obtained by Moosa et al. (2002, 2005), Oyekola et al. (2009; 2010; 2012) and Baksaran and Nemati (2006). The study showed that the decrease in sulfate reduction efficiency coincided with a decrease in acetate formation and increase in propionate formation (Oyekola et al., 2009). Propionate production is an indication of lactate fermentation (Heimann et al., 2005). In addition Oyekola et al. (2012) performed a qualitative assessment of microbial community structure, which confirmed that the diversity of sulfate reducers decreased with increasing dilution rate.

Membrane unit

The redox potential in the reactor decreased from an initial value of around -350 mV to a steady state between -390 and -400 mV during the first 30 days of operation, as the sulfate reduction efficiency increased prior to attaining steady state at a four day HRT (day 30). During the same time the pH increased from around pH 7 to pH 7.4-7.5.

The redox potential of the permeate was typically similar to that of the bulk reactor fluid, except during periods where permeate flow decreased due to fouling or blockage of the tube draining the membrane unit. Under these circumstances oxidation of the permeate was near complete, resulting in a less negative redox potential. The pH of the permeate from the microfiltration membrane was consistently higher than the pH measured in the reactor, typically by 0.5 pH units.

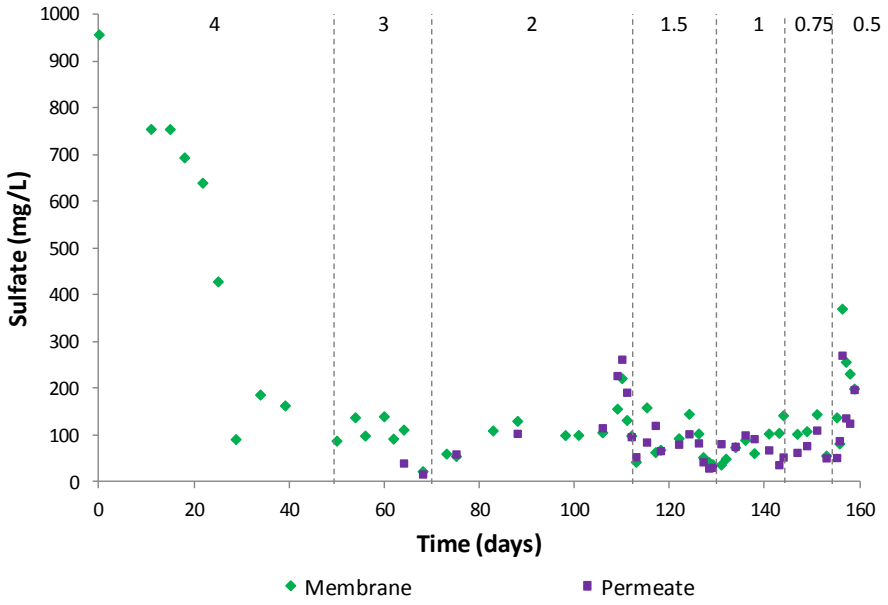

Figure 2 Residual sulfate data from the continuous reactor with microfiltration unit. Changes in HRT are shown by the dashed lines with the nominal mean HRT indicated. 'Membrane' refers to the sample from the bulk reactor fluid

The residual sulfate data (Figure 2) gave an indication of the efficiency of the system across the range of HRTs. The system took around 30 days to reach steady state at the initial four day HRT. The residual sulfate at steady state was approximately 140 mg/L, representing a removal efficiency of over 85%. This was consistent with the baseline data under similar operating conditions. The residual sulfate was relatively unchanged down to a HRT of 0.75 days and increased slightly at 0.5 days. The sulfate concentration measured in the permeate was similar or slightly lower in most cases, indicating that while partial oxidation of sulfide to sulfur occurred in the drainage tube, complete oxidation to sulfate did not.

The low residual sulfate, even at a HRT of 0.5 days, was indicative of very efficient performance. The recycling of the majority of the biomass, particularly under conditions where slower growing species would be washed out, resulted in a very high biomass concentration that could sustain efficient sulfate reduction at a high volumetric loading rate. The average VSRR measured across the 0.5 day HRT was 64.18 mg/L.h, over 50% higher than that achieved during the baseline study.

The sulfide concentration in the bulk fluid was similar to that in the baseline CSTR, while the permeate was consistently lower, fluctuating around 100 mg/L when the system was operating efficiently. The lower sulfide concentration, coupled with the increased pH suggested the partial oxidation of some of the sulfide to elemental sulfur. The silicone tubing used to drain the permeate is permeable to oxygen. Under oxygen limiting conditions the partial oxidation of sulfide is favored, according to the reaction below (Kuhn *et al*, 1983)

$$2HS^- + O_2 \rightarrow \tfrac{1}{4}S_8 + 2OH^- \quad (2)$$

The generation of hydroxide ions accounts for the increase in pH. As the pH increased, a portion of the remaining sulfide could be converted to polysulfides. The permeate was typically pale yellow color, consistent with the presence of some polysulfide. Sulfur production in the outlet pipes resulted in relatively frequent blockages, resulting in effluent draining from the reactor overflow port, rather than exiting as permeate. The problem was more pronounced at the shorter HRTs. At the 1.5 day HRT 76% of the total effluent was made up of permeate and 24% as overflow from the reactor. The proportion exiting as permeate fell to 67%, 69% and 44% at the 1 day, 0.75 day and 0.5 day HRTs respectively.

The sample from the reactor clearly contained suspended biomass, which became more apparent as the experiment progressed. The appearance of attached biofilm on the reactor walls and the presence of elemental sulfur in the bulk fluid prevented accurate quantification by either dry mass or optical density. The permeate sample was consistently cell free, indicating that the cells were recycled back to the reactor with the retentate. The absence of cells in the permeate was confirmed by light microscopy.

Linear flow channel reactor

The system experienced some perturbations during the initial operation and steady state, with respect to sulfate reduction, was achieved day 40 and on day 45 the HRT was reduced to 4 days. The sulfide data (Figure 3) show that a relatively high sulfide concentration was maintained in the reactor, even at a 0.5 day HRT.

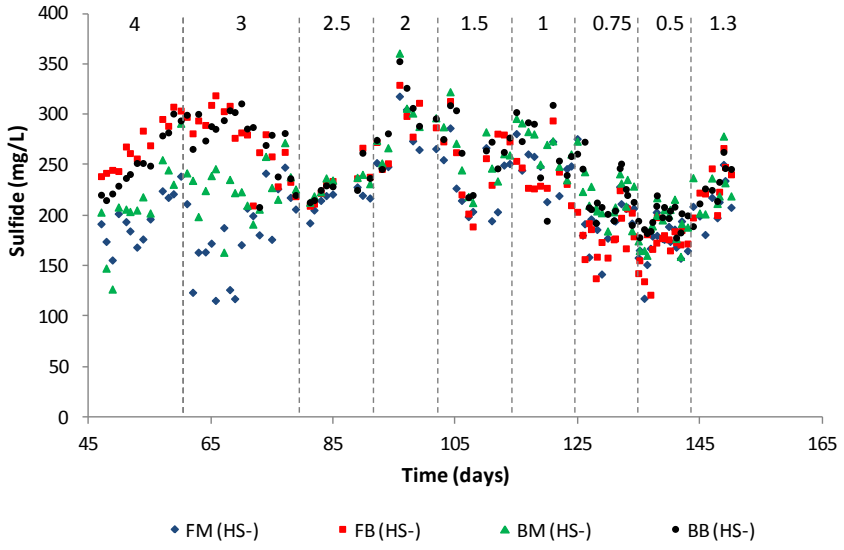

Figure 3 Aqueous sulfide concentration as a factor of HRT, measured at four points in the reactor. Changes in HRT are represented by dashed lines and mean HRT is indicated

At the longer HRT there was some inhomgeneity in the reactor, a function of the hydrodynamics within the reactor, with higher concentrations in the lower half of the reactor. While the sulfide levels did decrease to an extent as the HRT decreased, a relatively steady state was observed at each HRT and the decreased performance with decreasing HRT, below the critical maximum specific growth rate of the cells, observed in the CSTR, did not occur owing to a de-coupling of the hydraulic and biomass dilution rates. Biofilm growth on the carbon fibers was clearly visible, demonstrating biomass retention.

Comparison of performance across reactor configurations

The performance in each of the different reactor configurations can be compared by considering the volumetric sulfate reduction rate relative to the volumetric sulfate loading rate. At a feed sulfate concentration of 1 g/L, the performance was similar at HRTs from four days down to one day, with a sulfate reduction efficiency of between 85-95%. Significant divergence in performance was observed at lower HRTs, where washout of a portion of the sulfate reducing community occurred in the stirred tank reactors as the dilution rate exceeded the maximum specific growth rate. The two systems that were characterized by either recycling of the biomass (BSR reactor coupled to membrane filtration unit) or efficient retention of the biomass (carbon microfiber channel reactor) maintained higher VSRRs, owing to the requisite decoupling of the MCRT and HRT. At the HRT at which washout is first observed in the CSTR (0.5 day), for the channel reactor the VSRR was approximately 20% higher than the baseline study, while for the membrane system it was over 50% higher, despite the challenges associated with elemental sulfur formation. Maree and co-workers (2004) achieved very efficient sulfate reduction by recycling sludge from a clarifier back to a well mixed primary reactor, although the clarifier volume was equal to that of the reactor, which has capital cost and process footprint implications.

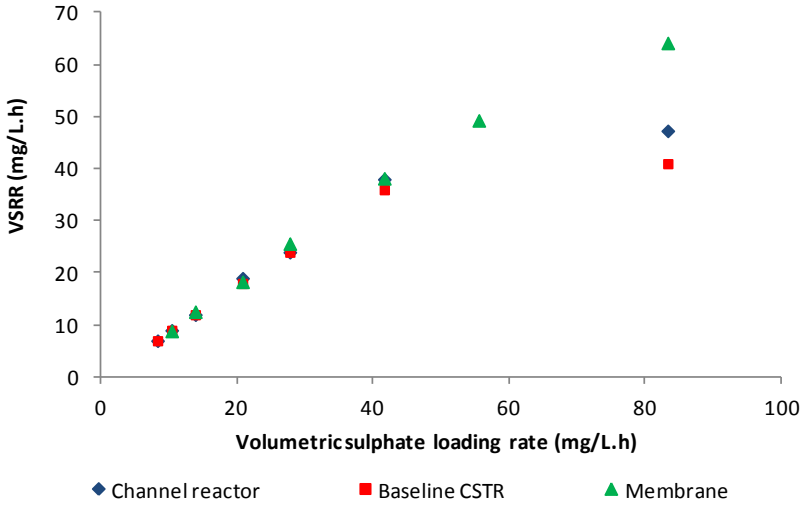

Figure 4 Comparison of the VSRRs across the three reactor configurations at a feed sulfate concentration of 1 g/L

CONCLUSION

Decoupling of mean cell and hydraulic retention times was achieved successfully in both the LFCR, where biofilm formation on the carbon microfibers was very efficient and ensured biomass retention and the reactor fitted with the microfiltration membrane, where the membrane permeate was consistently cell free, demonstrating biomass recycle. The maintenance of the biomass within the reactor resulted in significantly improved performance at low HRTs, with the volumetric sulfate reduction rate in the channel and membrane coupled reactors being 20% and 50% higher respectively than the baseline CSTR data at a HRT of 0.5 days.

In both systems, the complete elimination of oxygen was not possible, resulting in the partial oxidation of a portion of the sulfide formed to elemental sulfur. In the channel reactor this occurred primarily in a floating biofilm, similar to that observed in the dedicated sulfide oxidation reactor, with some sulfide oxidation also occurring in the effluent pipe. This suggests that sulfide oxidation and sulfur recovery could be coupled with sulfate reduction in this configuration.

The sulfur formation presented more of a problem in the membrane system as particulate sulfur blocked the permeate drainage line and peristaltic pump tubing, restricting permeate flow, as well as forming a layer on the outer surface of the membrane, reducing transmembrane flux. As a consequence, a significant amount of the accumulated biomass was lost as overflow from the reactor. Despite this, the membrane coupled system resulted in the most efficient sulfate reduction. Subsequent experiments have shown that active pumping of permeate is not required and this has eliminated the problem.

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REFERENCES

APHA (American Public Health Association) (2005) *Standard Methods for the Examination of Water and Wastewater,* 21st Edition. American Public Health Association, Washington, D.C.

Gazea, B., Adam, K. & Kontopoulos, A. (1996) 'A review of passive systems for the treatment of acid mine drainage', *Minerals Engineering*, vol 9, pp 23—42.

Harrison, S.T.L., van Hille, R.P., Mokone, T., Motleleng, L., Smart, M., Legrand, C. & Marais, T. (2014) Addressing the challenges facing biological sulphate reduction as a strategy for AMD treatment: Analysis of the reactor stage: raw materials products and process kinetics, Water Research Commission, Pretoria, South Africa.

Hedin, R.S., Nairn, R.W. & Kleinmann, P.L.P. (1994) *Passive Treatment of Coal Mine Drainage*. US Bureau of Mines Information Circular 9389, US Department of the Interior, Bureau of Mines, Pittsburgh, PA., pp 1—35.

Heimann, A.C., Friis, A.K. & Jakobsen, R. (2005) 'Effects of sulfate on anaerobic chloroethene degradation by an enriched culture under transient and steady-state hydrogen supply' *Water Research*, vol 39, pp 3579—3586.

Janssen, A.J.H., Sleyster, R., van der Kaa, C., Jochemsen, J., Bontsema, J. & Lettinga, G. (1995) 'Biological sulphide oxidation in a fed-batch reactor', *Biotechnology and Bioengineering*, vol 47, pp 327—333.

Johnson, D.B. & Hallberg, K.B. (2005) 'Acid mine drainage remediation options: a review', *The Science of the Total Environment*, vol 338, pp 3—14.

Maree, J., Greben, H. & de Beer, M. (2004) 'Treatment of acid and sulphate-rich effluents in an integrated biological/chemical process', *Water SA,* vol 30, pp 183—190.

Molwantwa, J.B. & Rose, P.D. (2013) 'Development of a Linear Flow Channel Reactor for sulphur removal in acid mine wastewater treatment operations', *Water SA*, vol 39, pp 649—654.

Moosa, S., Nemati, M. & Harrison, S.T.L. (2002) 'A kinetic study on anaerobic reduction of sulphate, Part 1: Effect of sulphate concentration', *Chemical Engineering Science*, vol 57, pp 2773—2780.

Moosa, S., Nemati, M. & Harrison, S.T.L. (2005) 'A kinetic study on anaerobic reduction of sulphate. Part II: Incorporation of temperature effects in the kinetic model', *Chemical Engineering Science*, vol 60, pp 3517—3524.

Oyekola, O.O., Harrison, S.T.L. & van Hille, R.P. (2012) 'Effect of culture conditions on the competitive interaction between lactate oxidizers and fermenters in a biological sulphate reduction system' *Bioresource Technology*, vol 104, pp 616—621.

Oyekola, O.O., van Hille, R.P. & Harrison, S.T.L. (2009) 'Study of anaerobic lactate metabolism under biosulfidogenic conditions' *Water Research*, vol 43, pp 3345—3354.

Oyekola, O.O., van Hille, R.P. & Harrison, S.T.L. (2010) 'Kinetic analysis of biological sulphate reduction using lactate as carbon source and electron donor: Effect of sulphate concentration', *Chemical Engineering Science*, vol 65, pp 4771—4781.

van Hille, R.P. & Mooruth, N. (2013) Investigation of carbon flux and sulphide oxidation kinetics during passive biotreatment of mine water. Research report no. 2139/1/13, Water Research Commission, Pretoria, South Africa.

Zagury, G.J., Neculita, C.M. & Bussière, B. (2007) 'Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: critical review and research needs', *Journal of Environmental Quality*, vol 36, pp 1—16.