Performance of the Hybrid Linear Flow Channel Reactor: Effect of Reactors in Series for Enhanced Biological Sulfate Reduction and Sulfur Recovery

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Abstract

Acid rock drainage (ARD) is a global crisis that will have long-lasting environmental consequences. The application of semi-passive biological sulfate reduction (BSR) is a potential solution for the remediation of persistent low volume ARD effluents. However, major challenges of BSR, including slow reaction kinetics and management of the generated sulfide, still need to be addressed. The development of a hybrid Linear Flow Channel Reactor (LFCR) has shown promise for remediation of sulfate-rich effluents. In this study, the operation of two hybrid LFCRs connected as a dual reactor system was assessed for the improved removal of residual sulfide and COD.

Keywords: Semi-Passive Bioprocess, Biological Sulphate Reduction, Partial Sulphide Oxidation, Sulphur Recovery

Introduction

The generation and discharge of acid rock drainage (ARD) as a result of mining activities in regions rich in sulfidic minerals has significant implications on the receiving ecosystems (McCarthy, 2011). ARD is generally characterised as acidic water containing high concentrations of sulfate, metals and semi-metals. The long term environmental and socio-economic effects has necessitated the need for the development of ARD treatment technologies.

The semi-passive hybrid LFCR process incorporating both biological sulfate reduction and partial sulfide oxidation has shown potential for application as part of a wastewater treatment to address persistent low volume ARD characterised by high sulfate concentrations (Marais *et al*., 2020a). The process facilitates the formation of an anaerobic zone, within the bulk volume, and an aerobic zone at the air-liquid interface. Sulfide generated within the anaerobic zone by SRB is subsequently oxidised to elemental sulfur by sulfur oxidising bacteria (SOB) near the liquid interface, resulting in the formation of a floating sulfur-rich biofilm (FSB). The FSB is intermittently recovered and serves as a value end product that can be applied in agriculture as a fertiliser.

Biological treatment of sulfate-rich waste streams is dependent on the initial feed sulfate concentration as well as its loading rate. In these processes the sulfate loading rate can be mediated by dilution rate (HRT) or feed sulfate concentration. During wastewater treatment, initial sulfate concentrations can vary based on environmental parameters and source of the waste stream. Sulfate-rich contaminated wastewater can range between 1 - 10 g/L and can impact the performance of an applied treatment (Brahmacharimayum *et al*., 2019). Several studies have evaluated the effects of feed sulfate concentrations on BSR under different reactor configurations and operating parameters (Erasmus, 2000; Moosa *et al*., 2002; Al-zuhair *et al*., 2008; Oyekola *et al*., 2010). These studies highlighted that the sulfate reduction rate increases as feed sulfate concentration increases. However, a decline in overall sulfate conversion efficiency has been attributed to the limitation of SRB to adequately reduce sulfate at high loading rates that exceed its metabolic potential. In addition, several studies reported a decline in BSR performance due to sulfide

inhibition. Previous studies have evaluated the effects of hydraulic residence time on the performance of the hybrid LFCR at laboratory scale treating synthetic sulfate-rich wastewater (Marais *et al*., 2020b). From these investigations, some limitations of the process were identified which included the sulfur recovery inefficiency associated with the management of the FSB as well as the untreated residual sulfide and COD released in the final effluent.

The effects of feed sulfate concentration have yet to be evaluated within the hybrid LFCR system. Therefore, an investigation into the effects of feed sulfate concentration on the performance of the hybrid LFCR is critical to further characterise the process. In this study, the operation of two hybrid LFCR units connected in series was assessed for the improved removal of residual sulfide and COD. The aim of the study was to minimise secondary pollution (residual COD, sulfate and sulfide) and increase overall sulfur recovery.

Methods and Materials

Microbial culture and system operation

The SRB culture has been maintained at the University of Cape Town (UCT) on modified Postgate B medium (Marais *et al*., 2020a). The SOB culture was developed from the SRB culture and has been maintained as floating sulfur biofilms in LFCRs (van Hille *et al*., 2015). An 8 L LFCR was operated continuously at a 4 day hydraulic residence time (HRT) with a feed sulfate concentration of 1000 mg/L and supplemented with of 60% (v/v) sodium lactate solution. This provided a chemical oxygen demand (COD) to sulfate ratio of 0.7.

Dual hybrid linear flow channel reactor system

A detailed description of the reactor design and laboratory demonstration of the 8 L hybrid LFCR have been described in a previous investigation (Marais *et al*., 2020b). Key features of the hybrid LFCR (Fig. 1) includes carbon micro fibres as support matrices for enhanced biomass retention, a heat exchanger for temperature control, sampling ports positioned along the length of the reactor and a mesh-screen positioned just below the air-liquid interface for harvesting the FSB. A second LFCR unit was connected downstream of an 8 L LFCR that had been operational for over 488 days (Marais *et al*., 2020b). An image of the laboratory set-up

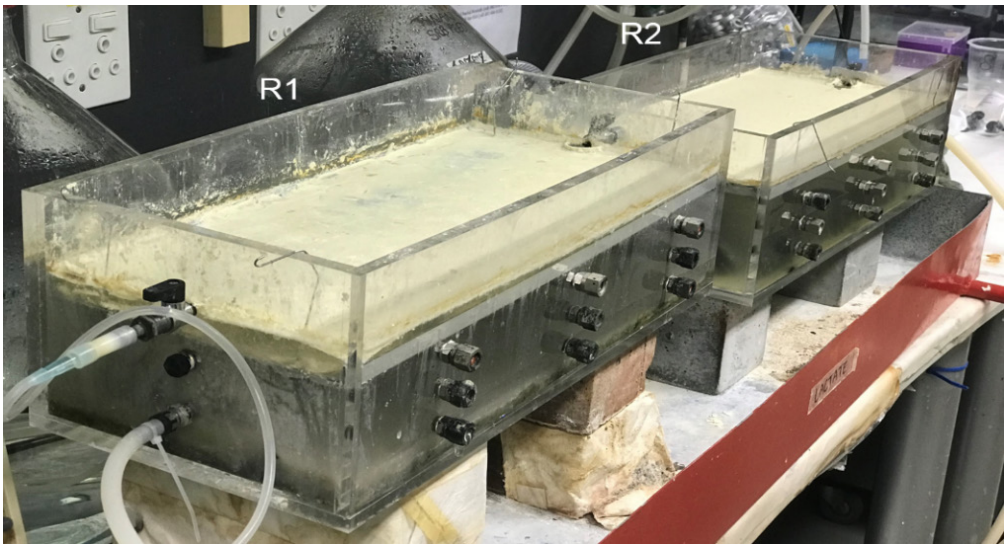

Fig.1 *Dual reactor laboratory set-up showing the two identical 8 L LFCR units connected in series, the primary reactor (R1) was elevated to ensure passive gravitational flow into the secondary reactor (R2). The photograph was taken 21 days after biofilm disruption showing a matured FSB at the surface of both reactors.*

of the dual reactor system is shown in Fig. 1. The secondary reactor was inoculated by overflow collected from the primary reactor. Disruption and harvesting of the FSB were performed intermittently in both reactors. An additional biofilm disruption in the secondary reactor was only performed independently of the primary reactor during operation at 5 and 10 g/L feed sulfate concentrations. The objective was to demonstrate the effect of regulating the frequency of biofilm disruption on sulfide conversion and sulfur recovery.

Analytical techniques

Dissolved sulfide was quantified using the colorimetric N,N-dimethyl-p-phenylenediamine method (APHA, 2012). Residual sulfate concentrations were measured by the barium sulfate method (APHA, 2012). Volatile fatty acid (VFA) analysis was determined using HPLC on a Waters Breeze 2 HPLC system (van Hille *et al.*, 2015). Redox potential and pH were measured on a Metrohm pH lab 827 redox meter relative to a Ag/AgCl reference electrode and a Cyberscan 2500 micro pH meter, respectively. Elemental composition analysis was performed through a CHNOS Elementar Vario EL Cube Elemental Analyser.

Effect of feed sulfate concentration on process performance

Process performance was evaluated across feed sulfate concentrations of 1, 2.5, 5, and 10 g/L. The feed COD/SO_4 ratio was maintained at 0.7 over the range of feed sulfate concentrations to ensure sufficient electron donor for complete sulfate reduction. The FSB was disrupted intermittently where the biofilm is physically fragmented and allowed to settle onto the mesh screen just below the liquid surface. During harvesting of the FSB the mesh screen is completely removed from the reactor and the sulfur-rich biofilm material is collected. At the end of each experimental run, between adjusting feed sulfate concentrations, a biofilm harvest was performed. The harvested FSB was dried at 80 °C and stored for elemental analysis.

Results and discussion

The residual sulfate concentration profile across the dual reactor system is shown in Fig.2a. During operation at 1 g/L feed sulfate concentration, the residual sulfate concentration was relatively stable across both reactors, suggesting little sulfate reduction occurred within the secondary reactor. Once operated at 2.5, 5 and 10 g/L feed sulfate concentration, there was a slight reduction in sulfate within the secondary reactor. However, this was not comparable to the performance achieved in the primary reactor despite there being sufficient residual VFA (not shown) and sulfate available to favour BSR activity. Volumetric sulfate reduction rate (VSRR) increased (0.046 to 0.200 mmol/L.h) as feed sulfate concentration was increased from 1 to 5 g/L in the primary reactor before decreasing to 0.110 mmol/L.h at 10 g/L feed sulfate concentration. The sulfate conversion efficiency decreased from 42 to 10% as the feed sulfate concentrations increased from 1 and 10 g/L, respectively.

Effective removal of residual sulfide was achieved in the secondary reactor. Sulfide concentration rapidly decreased after every biofilm disruption (Fig. 2b). As the biofilm regenerated, sulfide concentration increased nearing the expected theoretical sulfide based on sulfate conversion. This was consistent with previous findings, where the FSB forms a barrier that impedes oxygen mass transfer across the air-liquid interface. As the biofilm matures oxygen transfer declines, eventually inhibiting sulfide oxidation. Through controlled intermittent disruption of the FSB, oxygen ingress into the sulfide-rich reactor volume is significantly increased allowing sulfide oxidation to proceed. In the hybrid LFCR the steep oxygen and sulfide concentration gradients that form across the air-liquid interface provide the ideal redox and pH conditions to favour partial sulfide oxidation to elemental sulfur. In this study, the residual sulfate data confirmed that complete sulfide oxidation to sulfate was negligible in both reactors, a strong indication that elemental sulfur production was favoured throughout the study. This was further supported by the consistent elevated pH seen within the secondary reactor which was expected due to the production of hydroxyl ions as a by-product of partial sulfide oxidation.

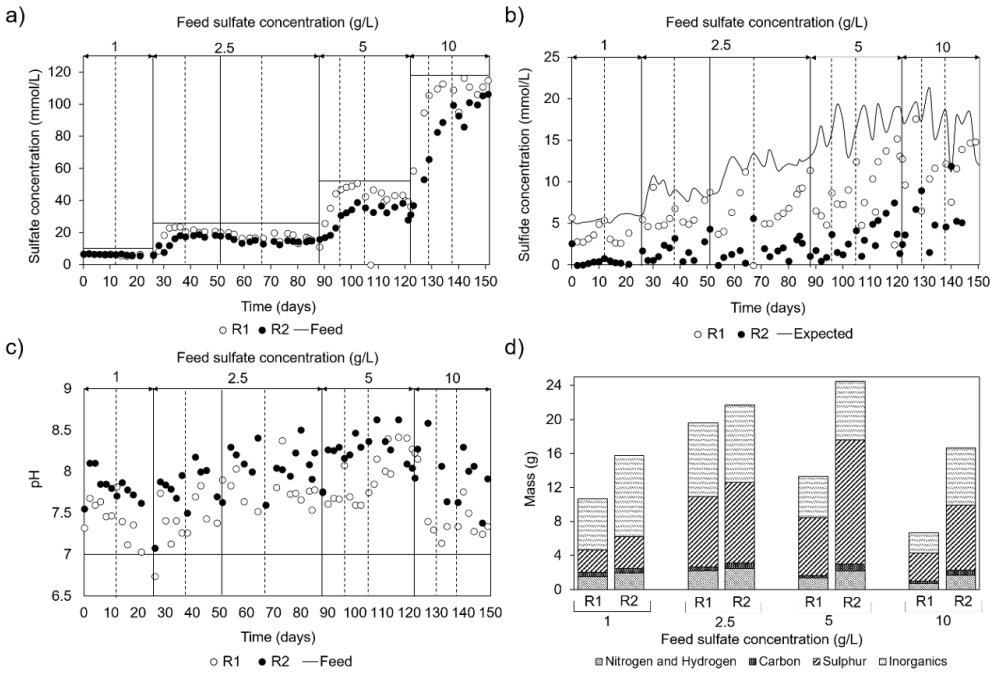

Fig. 2 *Effect of feed sulfate concentration on performance of the dual hybrid LFCR system. The a) residual and feed sulfate concentration, b) measured and expected sulfide concentration and c) pH profile over time as well as d) the FSB harvested in the primary and secondary reactors shown. Vertical dotted and solid lines represent biofilm disruption and harvesting events.*

A marked decrease in pH occurred within the primary reactor during operation at 10 g/L feed sulfate concentration, which ranged between 7.1 and 7.3 (Fig. 2c). These results coincided with the notable decrease in sulfate conversion. In sulphidogenic reactors the pH plays a critical role in the inhibition of sulfide to SRB activity (Moosa *et al*., 2006; van den Brand *et al*., 2016). Undissociated $H_2S_{(g)}$ has the strongest inhibitory effect due to its ability to permeate the cell membrane and resulting in denaturation of enzymes. The quantity of $H_2S_{(g)}$ is largely determined by the pH where hydrogen sulfide exists as a mixture of $H_2S_{(g)}$ and HS^- between pH 6 to 8 (Moosa & Harrison, 2006). Below pH 6, undissociated $H_2S_{(g)}$ dominates while at a pH >7.5, the $H_2S_{(g)}$ fraction of the total sulfide in solution is minimal (van den Brand *et al*., 2016). Therefore, the observed decline in SRB acidity is most likely a consequence of sulfide inhibition due to high sulfide concentrations (> 14.9 mmol/L) concomitant with the decrease in pH below 7.5 (Fig.2b and c).

VFA concentration analysis (not shown) confirmed lactate metabolism in the dual reactor system occurred predominantly via the incomplete oxidation pathway coupled to sulfate reduction. As feed sulfate concentration increased there was a decrease in lactate conversion from 100 to 18% in the primary reactor, at 1 and 10 g/L feed concentration, respectively (Table 1). Lactate conversion increased at every feed concentration with the addition of the secondary reactor. The presence of residual propionate concentrations throughout the study indicated that lactate fermentation occurred. The incomplete oxidation of lactate led to an accumulation of predominantly acetate in the overflow released into the secondary reactor. Since acetate was the most abundant carbon source, the activity

Table 1 Effect of feed sulfate concentration on overall process performance of the hybrid LFCR comparing single and dual reactor operation.

	Single reactor					Dual reactor system			
Sulfate concentration (g/L)	VSRR (mmol/L.h)	Lactate conversion (%)	Sulfate conversion (%)	Sulfide conversion[a] (%)	Sulfur Recovery[b] (%)	Lactate conversion (%)	Sulfate conversion (%)	Sulfide conversion[a] (%)	Sulfur Recovery[b] (%)
1	0.046	100	42	38	85	100	42	85	93
2.5	0.119	77	44	52	54	100	49	79	68
5	0.200	36	34	39	41	53	36	68	71
10	0.110	18	10	31	51	36	10	61	85

[a] Cumulative sulfide conversion based on the expected theoretical sulfide concentration and final effluent sulfide concentration over the duration of each experimental run

[b] Elemental sulfur recovery from harvested FSB calculated based on sulfide conversion

of acetate utilising bacteria was favoured. However, a decrease in acetate concentration in the secondary reactor was not coupled to biological sulfate reduction which strongly indicated the lack of active acetate utilising SRB within the system. Although there was slight fluctuation in propionate concentration between feed sulfate concentrations, little difference between the primary and secondary reactors was observed over the duration of the study. This suggested that minimal lactate fermentation to propionate occurred within the secondary reactor. Furthermore, residual propionate was not utilised as an alternative carbon source within the secondary reactor.

Given that the secondary reactor relied solely on microbial colonisation via the overflow received from the primary reactor where incomplete lactate oxidation occurred, the inoculum may not have selected for a complete oxidising SRB community that can utilise acetate as a carbon source. Furthermore, the secondary reactor may have required a longer acclimatisation period between inoculation and the start of the study to allow the SRB community to establish and for biomass to accumulate in the reactor. Incomplete substrate utilisation and accumulation of acetate in BSR systems is a major drawback of the process and is well documented. To overcome the low sulfate conversion and acetate utilisation, the secondary reactor could be pre-colonised separately with an active acetate-utilising SRB culture prior to dual operation.

The amount of FSB harvested, and elemental composition analysis is shown in Fig.2d. Recovery in the primary reactor increase from 1 to 2.5 g/L and then decreased during operation at 5 and 10 g/L feed sulfate concentration. Although the amount of biofilm recovered from the secondary reactor was slightly higher compared to the primary reactor, during operation at 1 and 2.5 g/L, the marked increase in FSB recovered during operation at 5 and 10 g/L was a consequence of the additional biofilm disruption events that were performed. This allowed the biofilm to regenerate, outside of the biofilm disruption regime applied to the primary reactor. The results demonstrated that an increase in the frequency of biofilm disruption in the secondary reactor can increase the overall sulfide conversion and sulfur recovery within the hybrid LFCR (Table 1). A comparison of the elemental composition revealed that the FSB recovered from both reactors at each feed sulfate concentration, on average, exhibited a similar composition. However, there was an increasing trend in the proportion of elemental sulfur content (24±0.5 to 56±5.8%; mean±s.d) within the biofilm as the feed sulfate concentration increased from 1 to 5 g/L. This coincided with the observed increase in VSRR as the feed sulfate concentration increased.

Conclusion

The key objective of the study was to evaluate the effect of feed sulfate concentration, minimise secondary pollution (residual COD, sulfide) and increase overall sulfur recovery. The highest VSRR was achieved at a 5 g/L feed sulfate concentration. Although there was decrease in sulfate conversion as sulfate loading increased, the system was able to maintain sulfate reduction at the highest feed sulfate concentration of 10 g/L. Conversion of untreated lactate occurred within the secondary reactor however, there was an accumulation of acetate (residual COD) within the system that was not effectively removed. The addition of the secondary reactor enhanced overall sulfide oxidation efficiency (85%) and sulfur recovery (93%), significantly increasing the overall performance of the hybrid LFCR when compared to single unit operation. In addition, the study demonstrated that the frequency of disrupting the biofilm in the secondary reactor is an important parameter to achieve maximum sulfur recovery through the FSB. Efforts to increase sulfate reduction and COD removal across the dual reactor system could be achieved by separately establishing the secondary reactor on an acetate based feed to select for an active SRB community capable of acetate metabolism.

Acknowledgements

The authors acknowledge the Water Research Commission (K5/2393), the Department of Science and Technology (DST), and the National Research Foundation (NRF) of South Africa for funding. STLH holds the South African Research Chair in Bioprocess Engineering (UID64778). TM acknowledges Post-doctoral fellowship support from the NRF (UID 116722).

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