# Effect of operational parameters on the performance of an integrated semi- passive bioprocess.

TS Marais[1], RJ Huddy[1], RP van Hille[1,2], STL Harrison[1]

*[1]Centre for Bioprocess Engineering Research, Department of Chemical Engineering, University of Cape Town, Private Bag X1, Rondebosch, 7701, South Africa*

*[2]The Moss Group, 13 Bell Crescent, Westlake Business Park, Westlake Drive, Tokai, 7945, South Africa*

**Abstract** Contamination of South Africa water resources in mining areas by metals and salinity, including acid rock drainage (ARD), is a major risk. Remediating this is important to minimise the impact on the environment and surrounding communities. This paper investigates system performance of an integrated semi-passive bioprocess for simultaneous sulphate reduction and partial sulphide oxidation within a single linear flow channel reactor (LFCR) unit, as a function of the operating conditions of hydraulic residence time (HRT), electron donor and reactor size. The work aims to contribute to the characterisation of a novel integrated bioprocess, from an engineering and microbial ecology perspective.

**Key words** semi-passive process, biological sulphate reduction, partial sulphide oxidation

## Introduction

In South Africa, historical gold and coal mining and related activities have left a legacy of acid rock drainage (ARD) which threatens the public water supply (McCarthy 2011). Impacted water is characterised by high levels of acidity, sulphates and potentially toxic metals with low concentrations of organic material (McCarthy 2011). The potential long-term nature of ARD generation and predicted increases in unmet water demand in South Africa drive the need for economically sustainable treatment operations to address the ARD problem over extended periods of time.

Despite extensive research demonstrating the technical feasibility and potential of biological sulphate reduction (BSR) for ARD treatment, relatively few commercial processes have been developed. The application of these technologies has been limited to niche applications, mainly due to the relatively slow kinetics of sulphate reducers, high cost of electron donor (e.g. ethanol, methanol and volatile fatty acids) and challenges in managing the resulting sulphide product, which is significantly more toxic than sulphate (Rose 2013). Active BSR systems are well described throughout literature. Passive BSR systems are limited to traditional wetlands or packed bed reactors with the drawback of unpredictability in system performance (Zagury et al. 2007). To address the ARD challenges in the context of South Africa, a low-cost technical solution for application in remote areas without highly trained personnel is required to deliver predictable performance. This has led to the development of an integrated semi-passive process based on a linear flow channel reactor (LFCR) which enables simultaneous sulphate reduction and partial sulphide oxidation, with the recovery of a value adding elemental sulphur product (van Hille et al. 2016). In the LFCR, niche en-

vironments are formed, partitioning a distinct aerobic zone at the air liquid interface and an anaerobic zone within the bulk volume of the reactor. The sulphate reducing bacteria (SRB) under anaerobic conditions in the bulk volume reduce sulphate in the presence of a suitable electron donor to sulphide. The sulphide is partially re-oxidised by SOB under oxygen limiting conditions at the air/liquid interface, forming a floating sulphur biofilm.

The effects of various parameters on BSR such as sulphate concentration, temperature, pH, electron donor availability and type, inhibitory effects of metal and sulphide concentration, as well as the use of carrier matrices have been reported with the aim of improving the stability and reliability of these treatments (Elliott et al. 1998; Tsukamoto & Miller. 1999; Moosa et al. 2002; Utgikar et al. 2003; Moosa et al. 2005; Baskaran & Nemati 2006). When introduced as perturbations, the severity of these effects depends on their type, magnitude, duration and frequency. The correct regulation and maintenance of these parameters is therefore essential for optimal process efficiency. Key challenges expected for implementation of the upscaling of integrated process from lab to commercial scale are the selection of a suitable carbon source, the attainment of suitable hydraulic residence time (HRT) and resilience to its fluctuation.

This paper addresses the effect of operating conditions and substrate on the performance of an integrated semi-passive system. Its main objectives are to assess the effect of HRT and reactor volume on system performance and to assess the potential of acetate as alternative carbon source to lactate. It also introduces microbial ecology and community dynamics aspects of the system.

## Methodology

### Microbial cultures and reactor operation

The SRB mixed microbial community was obtained from Prof. Rose at Rhodes University, and has been maintained at the University of Cape Town (UCT) over an extended period on modified Postagate B medium (van Hille and Mooruth 2013). The sulphide oxidising bacteria (SOB) culture was obtained from van Hille, UCT (van Hille and Mooruth 2013). The reactors were operated at a feed sulphate concentration of 1000 mg/L supplemented with lactate or acetate to maintain a chemical oxygen demand (COD) to sulphate ratio of 0.7. The reactors were run at 30 °C and neutral pH.

### Linear Flow Channel Reactor (LFCR)

Two geometrically similar lab-scale Perspex LFCRs (2 L and 8 L), fitted with carbon fibres for biomass retention, were tested. The 8 L reactor simulated the dimensions of the pilot plant under study. The channel reactor was relatively well mixed with limited turbulent mixing. The 2 L LFCR is fully described by van Hille et al. (2016).

Modifications introduced include the reorientation of the carbon microfibers for enhanced biomass retention and the inclusion of a heat exchanger (4 mm ID) for temperature control. Intact colonized carbon microfibers and floating sulphur biofilm were fixed in paraformal-

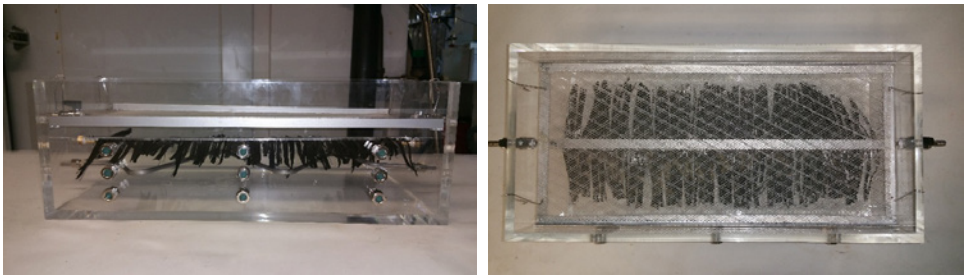


***Figure 1**: Images illustrating the LFCR design a) side view b) top view of the 8 L LFCR prior to inoculation fitted with strips of carbon microfibers, heat-exchange coil and harvesting mesh plate.*

dehyde or glutaraldehyde for fluorescence in situ hybridisation (FISH) and scanning electron microscopy (SEM) analysis, respectively.

### Analytical methods

Dissolved sulphide was quantified using the colorimetric N,N-dimethyl-p- phenylenediamine method (APHA 2005). Residual sulphate concentrations were measured using the barium sulphate method (APHA 2005). Volatile fatty acids (VFAs) analysis was conducted to quantify the concentration of lactic, acetic, and propionic acids in the feed and reactor samples. The concentration of each VFA was determined using HPLC on a Waters Breeze 2 HPLC system equipped with a Bio-Rad Aminex HPX-87H column and a UV (210 nm wavelength) detector (van Hille and Mooruth 2013). The pH analysis was conducted on a Cyberscan 2500 micro pH meter. Redox potential was measured using a Metrohm pH lab 827 redox meter.

### Floating sulphur biofilm collapse and harvesting

The floating sulphur biofilm (FSB) is not attached to a solid surface, instead develops at the air-liquid interface of the bulk fluid relying on surface tension for support. The biofilm "scaffold" consists of extracellular polymeric substances (EPS). This imparts structural integrity and retains the biomass and elemental sulphur. The FSB was collapsed by physically disrupting the biofilm with a spatula and allowing the fragments to settle on the mesh-plate (termed collapse). Following disruption, the biofilm reformed to cover the entire surface of the reactor within 24 h. The sulphur product could be recovered by removing the mesh-plate and collecting the accumulated biofilm (termed harvesting). The biofilm was dried at 37°C and weighed.

### Results and discussion

The reactors were inoculated with an active SRB culture and operated at a 4 day HRT. Fig. 1 illustrates the system performance and operation of the 2 L LFCR fed lactate. Experiment 1 and 2 were operated for 12 and 29 days respectively prior to collapse, evaluating the effect of biofilm collapse on system performance over time. Initially, a thin biofilm formed on the surface of the LFCRs, providing a barrier to oxygen mass transfer and creating the necessary redox and microenvironment partial sulphide oxidation to elemental sulphur.

Sulphate reduction, in the anaerobic bulk fluid, caused the dissolved sulphide concentration to increase steadily, from 50 to 220 mg/L and 260 mg/L for experiment 1 and 2, achieving sulphate conversion efficiencies of 47 and 61% for experiment 1 and 2, respectively (Fig. 1). This confirmed effective biological sulphate reduction and the establishment of an active SRB community.

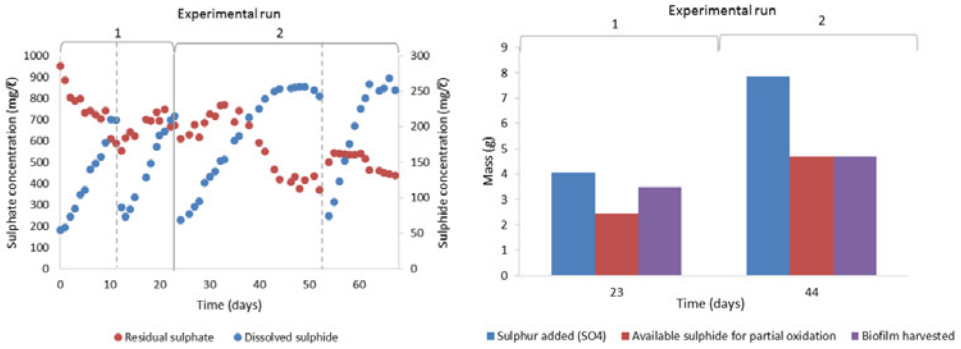
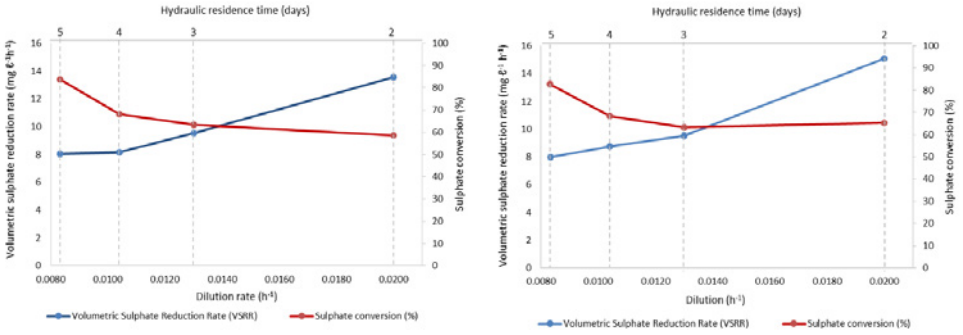



***Figure 1**: Integrated LFCR performance on lactate a) BSR performance showing residual sulphate and dissolved sulphide concentration as a function of time, vertical lines indicate biofilm collapse (dashed) and harvest (solid), b) partial sulphide oxidation via floating sulphur biofilm showing the total amount of sulphur added in the form of sulphate over the duration of the experiment, sulphur in g in the form of dissolved sulphide available for partial oxidation, and total mass of biofilm recovered.*

The system maintained a high sulphide concentration until biofilm collapse or harvesting. Disruption to the biofilm resulted in a rapid decrease in dissolved sulphide concentration, due to re-oxidation following the removal of the barrier to oxygen mass transfer. The re-oxidation was only partial, indicating the complete consumption of oxygen entering the system. The bulk fluid remained anaerobic and sulphate reduction was not affected. As the biofilm reformed, retarding oxygen mass transfer, the sulphide concentration increased again. This cycle could be repeated numerous times.

From Fig. 1b, it was shown that collapsing and harvesting the biofilm more frequently (experiment 1) allowed for higher biofilm recovery than running the system for longer periods of time (experiment 2). As the FSB matures over time, oxygen mass transfer across the biofilm becomes limiting, significantly reducing the rate of sulphide oxidation with concomitant accumulation of dissolved sulphide in the reactor. The time between collapse and harvesting events affects the composition of the biofilm, with the ratio of elemental sulphur to organic material shifting. A study by Mooruth et al. (2013) revealed the proportional relationship of FSB content (sulphur and organic material) as a function of HRT. A decrease in HRT led to an increase in the relative proportion of elemental sulphur while the organic composition decreased. Data in Fig. 1a indicated that longer periods between FSB collapse and harvesting (experiment 2) promoted higher sulphate conversion. This suggests that the integrated system be further optimized to maximize sulphate removal with minimal sacrifice in sulphide oxidation through controlled management of FSB inter-collapsing and harvesting regimes.

***Figure 2**: Effect of residence time on system performance showing volumetric sulphate reduction rate and sulphate conversion efficiency as a function of dilution rate a) 2L lactate fed LFCR, B) 8L lactate fed LFCR.*




The effect of HRT (5 to 2 days) was studied in a 2 L and 8 L LFCR using lactate as a sole carbon source (Fig. 2). A stable FSB and steady state was achieved at each HRT. Negligible difference was observed in system performance in terms of VSRR and sulphate conversion efficiency with LFCR scale. An increase in volumetric sulphate reduction rate (VSRR) (2 L: 8.05 – 13.56 mg L-1 h-1; 8 L: 7.96 – 15.11 mg L-1 h-1) and decrease in sulphate conversion efficiency (2 L: 84 – 59%; 8 L: 83 – 65%) was observed as the HRT was reduced from 5 to 2 days. The highest VSRR output was exhibited under a 2 day HRT at 12.8 and 15.1 mg L-1 h-1 with the highest sulphate conversion efficiency obtained at a 5 day HRT accounting for 84 and 83 % for the 2 L and 8 L LFCR respectively.

The outcome confirms that HRT plays a critical role in the overall microbial activity. At a shorter HRT the system did not allow adequate reaction time to reach high conversion efficiency based on the available bacterial activity; however, it promoted faster VSRRs. The data suggest that the maximum VSRR may be further increased with a lower HRT, albeit at the cost of conversion efficiency. In systems without adequate biomass retention, operating the system below an HRT of 1 day resulted in system failure as a consequence of increased proliferation of fermentative microorganisms, reduced sulphate conversion efficiency and cell washout (Oyekola et al. 2009). Based on the compromise between rate and conversion, the choice of HRT should consider the desired water quality and treatment rate.

Lactate as a sole carbon source was more efficient than acetate (Tab. 1). Maximum sulphate conversion efficiencies obtained in a 2 L LFCR configuration for lactate and acetate were 84 and 62 % at a VSRR of 8.05 and 5.90 mg L- 1h- 1 respectively. The lactate was fully utilized through incomplete oxidation to acetate which accumulated; this contributed to relatively high residual COD measured in the effluent (results not shown).

***Table 1**: The effect of carbon source and linear flow channel reactor (LFCR) configuration on volumetric sulphate reduction rate (VSRR) and sulphate conversion efficiency.*

| Reactor configuration | Carbon source | Sulphate loading rate (mg $L^{-1}$ $h^{-1}$) | Volumetric sulphate reduction rate (mg $L^{-1}$ $h^{-1}$) | Sulphate conversion efficiency (%) |
|---|---|---|---|---|
| 2 L LFCR | Acetate | 9.63 | 5.90 | 62 |
| 2 L LFCR | Lactate | 9.63 | 8.05 | 84 |
| 8 L LFCR* | Lactate | 9.63 | 7.97 | 83 |

*Different aspect ratio to simulate pilot plant specifications

Acetate as a sole carbon source showed 73% utilization through complete oxidation with low residual COD in the effluent. The acetate-fed reactor required a long start up period (±40 days) while both 2 and 8 L lactate-fed LFCRs were stable within ±20 days. The low VSSR and long start up period with acetate may be attributed to the lower growth rate of complete oxidizers (doubling time 10-16 h) in contrast to incomplete oxidisers (doubling time 3-10 h) (Celis et al. 2013). The lactate-fed SRBs, catalysing incomplete oxidation of the carbon source are more robust, evident during collapse and harvesting of the FSB (Fig. 1a) (Celis et al., 2013). Following perturbation, the lactate system recovered quickly with negligible effect on VSRR. In contrast, the acetate-fed LFCR was more sensitive to biofilm collapse and harvesting, showing decreased VSRR and a longer recovery period. Hence, carbon source selection impacts effluent quality and performance, robustness and cost of ARD treatment.

## Conclusion

The research to date has demonstrated the feasibility of the integrated process. Geometry and operating reactor volume showed minimal effect on system performance over the range considered, thus confirming the stability and robustness of the integrated sulphur process on scale up from a 2 L to 8 L LFCR. Scaling up the process to commercial scale has yet to be demonstrated. The findings conclude that 1) the regulation of biofilm collapse and harvesting is critical for establishing high sulphate removal and efficient sulphur recovery; 2) obtaining optimal system operation is characterised by a compromise between VSRR and sulphate removal efficiency; and 3) acetate as a carbon source facilitated complete carbon oxidation resulting in lower residual COD but also a less robust system when exposed to perturbations. Ultimately, this information will be compiled and applied to inform further optimisation of the bioprocess for efficient treatment of contaminated mining wastewater effluents, including a detailed understanding of the response of the microbial community to process perturbations.

**Acknowledgments**

The authors acknowledge the Water Research Commission (WRC), the Department of Science and Technology (DST), and the National Research Foundation (NRF) of South Africa for funding through the South African Research Chair in Bioprocess Engineering (UID 64778) held by STLH. Dr. Huddy is funded through a DST/NRF research career advancement fellowship (UID 91465). Tynan Marais acknowledges studentship support from the NRF.

**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.

Baskaran V, Nemati M (2006) Anaerobic reduction of sulfate in immobilized cell bioreactors, using a microbial culture originated from an oil reservoir. Biochemical Engineering Journal, 314(2): 148–159.

Celis LB, Gallegos-Garcia M, Vidriales G, Razo-Flores E (2013) Rapid start-up of a sulfidogenic biofilm reactor: overcoming low acetate consumption. J Chem Technol Biotechnol 88: 1672–1679.

Elliott P, Ragusa S, Catcheside D (1998) Growth of sulfate-reducing bacteria under acidic conditions in an upflow anaerobic bioreactor as a treatment system for acid mine drainage. Water Research. 32: 3724-3730.

Loy, A., Lehner, A., Lee, N., Adamczyk, J., Meier, H., Ernst, J., Schleifer, K.-H., Wagner, M., 2002. Oligonucleotide Microarray for 16S rRNA Gene-Based Detection of All Recognized Lineages of Sulfate-Reducing Prokaryotes in the Environment. Applied and Environmental Microbiology 68, 5064–5081. doi:10.1128/AEM.68.10.5064-5081.2002

McCarthy TS (2011) The impact of acid mine drainage in South Africa. South African Journal of Science, 107 (5/6): 1-7.

Mooruth N (2013) An investigation towards passive treatment solutions for the oxidation of sulphide and subsequent removal of sulphur from acid mine water. PhD thesis, University of Cape Town, South Africa.

Moosa S, Nemati M, Harrison STL (2002) A kinetic study on anaerobic reduction of sulphate, Part I : Effect of sulphate concentration, Chemical Engineering Science 57, 2773–2780.

Moosa S, Nemati M, Harrison STL (2005) A kinetic study on anaerobic reduction of sulphate, part II: incorporation of temperature effects in the kinetic model. Chemical Engineering Science 60(13): 3517–3524.

Rose P (2013) Long-term sustainability in the management of acid mine drainage wastewaters – development of the Rhodes BioSURE Process, Water SA 39(5): 583–592.

Tsukamoto T, Miller G (1999) Methanol as a carbon source for microbiological treatment of acid mine drainage. Water Research, 33(6), 1365–1370.

Utgikar VP, Tabak HH, Haines JR, Govind R (2003) Quantification of toxic and inhibitory impact of copper and zinc on mixed cultures of sulfate-reducing bacteria. Biotechnology and Bioengineering, 82(3), 306–12pp.

van Hille R, Mooruth N, Marais T, Naidoo N, Moss G, Harrison S, Muhlbauer R (2016) Development of a pilot-scale semi-passive system for the bioremediation of ARD. Proceedings IMWA 2016. 957-964.

Zagury GJ, Kulnieks VI, Neculita CM (2007) Characterization and reactivity assessment of organic substrates for sulphate-reducing bacteria in acid mine drainage treatment. Chemosphere 64: 944–954.