



# The Effect of Temperature on the Kinetics of Sulphate Reduction and Sulphide Oxidation in an Integrated Semi-Passive Bioprocess for Remediating Acid Rock Drainage<sup>©</sup>

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## Abstract

A hybrid linear flow channel reactor (LFCR), capable of simultaneous sulphate reduction and partial sulphide oxidation leading to elemental sulphur recovery, was developed and optimised for the semi-passive treatment of acid rock drainage. Under field conditions, seasonal temperatures vary substantially, impacting the sulphate reduction and sulphide oxidation kinetics and therefore the overall performance. Decrease in temperature across the range 10 to 30°C resulted in decreased volumetric sulphate reduction rates, sulphate conversion and sulphur biofilm recovered. Acetate proved an efficient, alternative carbon source to lactate. This work will contribute toward development and implementation of the integrated process for ARD treatment.

**Keywords:** semi-passive bioprocess, biological sulphate reduction, partial sulphide oxidation

## Introduction

The generation and discharge of acid rock drainage (ARD) as a result of mining activities in South Africa (SA) has significant implications on the receiving ecosystems (McCarthy, 2011). ARD is generally characterised as acidic water containing high concentrations of sulphate and heavy metals. The long-term environmental and socio-economic effects associated with ARD discharge in SA have been further exacerbated by the prolonged drought. This has resulted in the need for the development of new and efficient ARD treatment technologies.

In SA, the primary focus on remediation of ARD-contaminated water has been on high volume discharges, using established active technologies. Mostly overlooked, the continuous ARD discharge from diffuse sources (waste rock dumps and discard heaps), associated with coal mining, has substantial impact on the environment, due to the large number of sites and their geographic distribution over rural areas. Under these circumstances traditional passive treatments (wetlands) may be used; these require less maintenance and have lower operating costs. However, typical

passive systems are governed by slow, unpredictable kinetics and require extended hydraulic residence times (Skousen *et al.*, 2017), necessitating large land areas. Semi-passive ARD treatment systems present an attractive alternative for addressing these low-flow sources, with lower capital and operational costs than active systems and better control and greater predictability than conventional passive systems. This has led to the development of an integrated semi-passive process, based on a hybrid linear flow channel reactor (LFCR) which enables simultaneous sulphate reduction and partial oxidation of sulphide to elemental sulphur (van Hille *et al.* 2016 and Marais *et al.*, 2017). The operation of the integrated process relies on the formation of niche environments within the LFCR, 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) in the bulk volume reduce sulphate, in the presence of a suitable electron donor, to sulphide. The sulphide is partially re-oxidised by sulphur oxidising bacteria (SOB) under oxygen limiting conditions at the air-liquid interface, forming a floating sulphur biofilm.



Previous studies by van Hille *et al.* (2016) and Marais *et al.* (2017) have reported on the initial proof of concept, development and characterisation of the integrated process under different operating parameters, such as the effect of HRT, electron donor and reactor size. The findings from the initial development have resulted in the commissioning of the process at pilot scale. This has driven the need for further investigation to address key challenges that are expected at pilot scale. These include the upscaling of the process from lab to demonstration scale (aspect ratio and operating reactor volume), the use of a cost effective electron donor, and the effect of seasonal temperature fluctuation on the performance of the process. The potential use of acetate as an alternative carbon source to lactate in the integrated process as well as the effect of reactor geometry has been tested previously as a function of hydraulic residence time (Marais *et al.*, 2017). Further development of the process requires investigation into the effects of temperature on these parameters.

The effect of temperature fluctuations on system performance is likely to be a key challenge during the larger scale implementation of the integrated process. The effect of temperature on sulphate reduction and sulphide oxidation has been described previously for the separate systems under different reactor configurations, including continuously stirred-tank reactor (CSTR) (Moosa *et al.*, 2005; Buisman *et al.*, 2010), fluidised bed reactor (FBR) (Sahinkaya *et al.*, 2007) anaerobic side-stream reactor (Ferrentino *et al.*, 2017), and expanded granular sludge bed reactor (EGSB) (Sposob *et al.*, 2017). However, the effects of temperature on the integrated process has not yet been reported. Microbial activity in response to temperature is characterised by upper and lower limits of temperature for growth (Ferrentino *et al.*, 2017). Most SRB and SOB have been characterised as mesophilic bacteria where their active temperature range is between 10 and 50°C with an optimum temperature at 30°C (Greiben *et al.* 2002 and Tang *et al.*, 2009).

In this study the primary focus was to simulate a range of temperatures that a typical passive wastewater process would be exposed to, particularly in a SA environment.

The research evaluated the effect of temperature on the performance of the integrated process. Additionally, key objectives that ran in parallel were to assess the effect of reactor geometry on system performance as well as the potential of acetate as an alternative carbon source to lactate.

## Material and Methods

### *Microbial cultures and reactor operation*

The sulphate reducing mixed microbial community has been maintained at the University of Cape Town (UCT) on modified Postgate B medium (van Hille *et al.*, 2013; Marais *et al.*, 2017). The sulphide oxidising bacteria (SOB) culture was obtained from van Hille, UCT (van Hille *et al.*, 2013). The reactors were operated continuously at a defined hydraulic residence time (HRT) with a feed sulphate concentration of 1000 mg/L and supplemented with either lactate or acetate to maintain a chemical oxygen demand (COD) to sulphate ratio of 0.7.

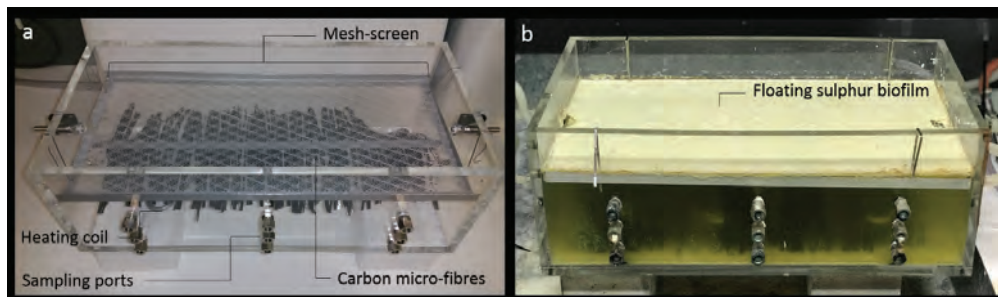
### *Linear Flow Channel Reactor (LFCR)*

Three lab-scale Perspex LFCRs (2 and 8L) were operated throughout the study. The 8L reactor variation simulated the relative dimensions of the pilot scale reactors. The reactor is distinctly different, compared to the 2L LFCR design, in aspect ratio. The 2L LFCR is fully detailed by van Hille *et al.* (2016). The standard hybrid LFCR configuration (Fig. 1) includes carbon microfibers as support matrices for enhanced biomass retention, a heat exchanger (4 mm ID) for temperature control, sampling ports along the front of the reactor and a mesh screen to harvest the floating S biofilm.

### *Analytical methods*

Dissolved sulphide was quantified using the colorimetric N,N-dimethyl-p-phenylenediamine method (APHA 2005). Residual sulphate concentrations were measured by the barium sulphate method (APHA 2005). Volatile fatty acid (VFA) 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 with a Bio-Rad Aminex HPX-87H column and a UV (210 nm wavelength)





**Figure 1:** Images illustrating the 8L LFCR a) design prior to inoculation fitted with strips of carbon micro-fibers, heat-exchange coil, harvesting mesh screen and sampling ports b) the inoculated LFCR with a well-developed sulphur biofilm at the surface.

detector (van Hille *et al.*, 2013). 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.

#### *Floating sulphur biofilm collapse and harvesting*

The floating sulphur biofilm (FSB) is not attached to a solid surface; instead it develops at the air-liquid interface (surface) 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 (Mooruth, 2013). The FSB was collapsed by physically disrupting the biofilm and collecting settled fragments onto the submerged mesh-screen (termed collapse). The sulphur product was recovered by removing the mesh-screen and collecting accumulated biofilm (termed harvesting). The biofilm was dried at 80°C and weighed.

#### *Effect of temperature on the integrated process*

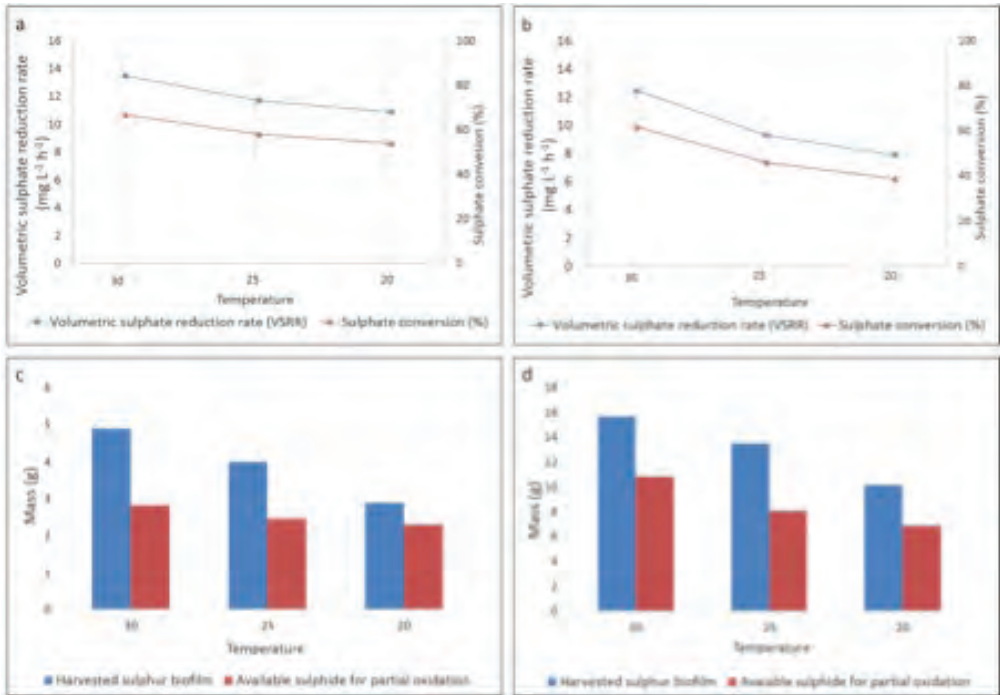
This study evaluated the effects of temperature on the integrated system across temperatures of 10, 15, 20, 25 and 30°C. The temperature was controlled by passing either heated or cooled liquid through the submerged heat exchanger. The study began by gradually adapting the reactor to a 2 d HRT, previously shown to be optimum (Marais *et al.*, 2017) at 30°C, after which the temperature was reduced stepwise to 10°C. The system was run for a total of 12 HRTs at each temperature, with a biofilm collapse after 6 HRTs

and a biofilm harvest at the end of each run. The sulphur content of the harvested biofilm was determined by elemental analysis. It was hypothesised that a decrease in operational temperature will result in a decrease in overall system performance.

### **Results and discussion**

Results from the study, shown in Fig. 2, reveal that the decrease in operating temperature across both 2L and 8L LFCR configurations resulted in a decrease in volumetric sulphate reduction rate (VSRR) (2L: 13.48 - 10.88 mg L<sup>-1</sup> h<sup>-1</sup>; 8L: 12.46 - 7.86 mg L<sup>-1</sup> h<sup>-1</sup>) and sulphate conversion efficiency (2L: 66.73 to 53.86 %; 8L: 61.68 to 38.91 %) on decreasing temperature over the range 30 to 10°C. As expected, the highest VSRR and sulphate conversion output was achieved at 30°C. Studies by Greben *et al.* (2002) and Ferrentino *et al.* (2017) reported that biological sulphate reduction was relatively stable under temperature perturbations between 20 – 15°C which was found to only account for 3 and 13% decrease in specific sulphate reduction rate, respectively. Similar conclusions can be drawn from the current study where a 5°C reduction in operational temperature from 25 to 20°C resulted in a 7 and 15% decrease in VSRR in the 2L and 8L LFCR respectively. Based on these findings the 2L LFCR outperformed the 8L LFCR, achieving higher VSRR and sulphate conversion throughout the study. Additionally, the 2L LFCR was less sensitive to temperature perturbation compared to the 8L LFCR, which may be a result of higher relative biomass retention in the 2L LFCR, a result of longer system operation.





**Figure 2:** Effect of residence time on system performance showing volumetric sulphate reduction rate and sulphate conversion efficiency as a function of temperature a) 2L lactate fed LFCR, b) 8L lactate fed LFCR, and partial sulphide oxidation via floating sulphur biofilm showing the mass of biofilm recovered and the total sulphur (grams) in the form of dissolved sulphide available for partial oxidation c) 2L lactate fed LFCR d) 8L lactate fed LFCR.

Due to the nature of temperature regulation through a submerged heat exchanger, a slight variation in temperature across the reactor was observed. The air-liquid interface, where sulphide oxidation occurs, was exposed to the controlled temperature from below and ambient temperature from above. Thus, the effect of temperature may have had less impact on the performance of the sulphide oxidation component compared to the sulphate reduction efficiency.

The partial oxidation of sulphide to elemental sulphur occurs under oxygen limiting conditions. This can be achieved through limiting the supply of oxidising agent (oxygen or nitrate) or high concentrations of sulphide in the medium. Most active sulphide removal processes promote partial sulphide oxidation by creating oxygen limiting conditions that require fine process control (DO levels). However, the integrated process relies on the sulphide generated through sulphate reduction in the bulk volume of the reactor and the

oxygen diffusion barrier created by the biofilm to maintain oxygen limiting conditions within the biofilm. Results from the current study (Fig. 2c and d) showed a decrease in sulphur biofilm recovery (2L: 4.9 – 2.9 g; 8L: 15.7 – 10.2 g) and available sulphide-S for partial oxidation (2L: 2.84 – 2.29 g; 8L: 10.85 – 6.85 g) as temperature decreased from 30 to 20°C. This was consistent across both 2L and 8L lactate-fed LFCR configurations. Studies by Sposob et al. (2017) and Xu et al. (2016) investigated the effect of temperature on the removal of sulphide for elemental sulphur production between 10–25°C. The studies showed that a decrease in temperature caused a decrease in elemental sulphur production. Similar findings in relation to the reduction in recovered biofilm mass strongly suggests that the partial sulphide oxidation in the floating sulphur biofilm was similarly affected by decreasing temperature. The decrease in available sulphide-S was directly proportional to the sulphate reduction activity, as



temperature decreased the availability of the substrate (sulphide) for partial oxidation. Previous studies have shown that the floating sulphur biofilm is predominantly comprised of elemental sulphur (Mooruth, 2013). This suggests that partial oxidation through the floating sulphur biofilm was efficient but was most likely limited by the availability of sulphide, given that the temperature change at the surface may not have been as significant as that within the bulk volume of the reactor.

A parallel study assessed the effect of temperature on the use of acetate as an alternative electron donor to lactate. Results (Tab. 1) revealed the similar performance in sulphate reduction obtained through the use of either electron donor at 30 and 25°C. The decrease in temperature (30 -20°C) resulted in the decrease in VSRR (lactate-fed: 13.48 to 10.88 mg L<sup>-1</sup> h<sup>-1</sup>; acetate-fed: 12.36 to 7.18 mg L<sup>-1</sup> h<sup>-1</sup>) and sulphate conversion (lactate-fed: 67 to 54 %; acetate-fed: 61 to 36 %). The lactate-fed LFCR proved more efficient at 20°C and was capable of maintaining sulphate conversion >50 %. The sulphate reduction conversion in the acetate-fed LFCR was significantly reduced to 36 % at 20°C. A previous study by Marais et al. (2017) assessed the effect of hydraulic residence time on the integrated process and revealed that after exposure to perturbations (HRT and biofilm collapse), a lactate-fed system recovered rapidly with negligible effect on VSRR while an acetate-fed LFCR was more sensitive and required longer periods to recover. This is most likely attributed to the lower growth rate of acetate oxidisers (doubling time 10-16 h) compared to that of lactate oxidisers (doubling time

3-10 h) (Celis et al., 2013).

At 30°C, all the lactate was utilised via partial oxidation by SRB, resulting in the accumulation of acetate. This contributed to relatively high residual COD measured in the effluent (results not shown). In the 2L lactate-fed LFCR, the decrease in temperature to 20°C resulted in an increase in residual lactate, an indication of incomplete carbon source utilisation and reduced microbial activity. Similarly, an increase in residual acetate was observed within the 2L acetate-fed LFCR. This revealed that temperature had a direct effect on the consumption of both carbon sources.

The amount of biofilm recovered as temperature decreased was inconsistent across the lactate-fed and acetate-fed LFCR. During the operation of the acetate-fed LFCR at 25°C, premature collapse and incomplete formation of the sulphur biofilm was observed, a result of tearing and spontaneous collapse. This resulted the regeneration of the biofilm outside of the studies' parameters of inducing collapse after every 6 HRTs. Hence, a greater mass of biofilm was recovered (20 and 25°C) from the acetate-fed LFCR than the lactate-fed LFCR where biofilm collapse was controlled. At 20°C biofilm formation was affected and could not maintain its structure, often disintegrating or prematurely collapsing. This could reflect the effect of temperature on the microbial community responsible for EPS production that form part of the floating sulphur biofilm. It may also indicate that the reduced SRB activity did not generate sufficient sulphide to sustain the development of a structurally sound biofilm.

**Table 1.** Effect of carbon source on VSRR and sulphate conversion efficiency.

Carbon source	Temperature (°C)	Sulphate loading rate (mg L <sup>-1</sup> h <sup>-1</sup> )	Volumetric sulphate reduction rate (mg L <sup>-1</sup> h <sup>-1</sup> )	Sulphate conversion (%)	Sulphur biofilm recovered (g)
Lactate	30	20.2	13.48	67	4.9
	25	20.2	11.70	58	4.0
	20	20.2	10.88	54	2.9
Acetate	30	20.2	12.36	61	4.6
	25	20.2	11.50	57	5.0
	20	20.2	7.18	36	3.6





## Conclusions

This study confirmed that temperature plays a critical role in the overall activity of the sulphate reducing and sulphide oxidising components in the integrated process. Based on these findings, the system may require operation at a longer residence time in order to compensate for the loss in performance at lower temperatures. The reduction in performance at low temperature was more pronounced in the acetate-fed system. At 20°C, the significant decrease in biological sulphate reduction and poor biofilm formation affected the stability and robustness of the 2L acetate-fed LFCR. The increased recovery of sulphur biofilm observed as a result of increased biofilm collapse and regeneration, highlights the importance of regulating the sulphur biofilm in order to facilitate optimal sulphate reduction and sulphur recovery in the integrated process. On-going work is currently focused on investigating temperatures below 20°C and to define the critical temperature, beyond which system performance is significantly reduced.

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