# Passive treatment of Acid mine drainage via a linear flow channel reactor utilising a floating sulphur biofilm

Neehal Mooruth

*Centre for Bioprocess Engineering Research (CeBER), Department of Chemical Engineering, University of Cape Town, Private Bag X3, Rondebosch, 7701, South Africa, neehal.mooruth@uct.ac.za*

**Abstract** Remediation of acid mine drainage (AMD) was performed within a linear flow channel reactor (LFCR) via a floating sulphur biofilm (FSB). A full sulphur species material balance was conducted in order to characterize the sulphide oxidation. The reactor was able to achieve an 82%, 96% and 93% conversion of sulphide with a hydraulic residence time of 2, 4 and 5 days respectively. 92%, 24.8% and 55.5% of the converted sulphide reported to the biofilm as sulphur for the 2, 4 and 5 day residence times respectively. Biofilm analysis and various factors affecting growth were also identified.

**Key Words** Acid mine drainage, Floating biofilm, Passive treatment, Residence time

## Introduction

South Africa has been classified as a water stressed country and as a result, water has been recognised as an important strategic resource. Furthermore the Department of Water Affairs predicted that the demand for potable water would exceed supply by 2020. Acid mine drainage (AMD) has been identified as a major threat to South Africa's water resources, particularly in the gold and coal mining regions of the country. A number of treatment technologies have been developed for the remediation of AMD. The most commonly used chemical treatment method is the addition of an alkaline material such as slaked lime ($CaCO_3$) or sodium hydroxide (NaOH). However, chemical treatment is uneconomical and results in the production of sludge as gypsum and metal hydroxides (Johnson and Hallberg, 2005). On the other hand a passive treatment system involves very little chemicals or energy and is significantly cheaper.

The Integrated Managed Passive (IMPI) process developed by Pulles, Howard and de Lange, in association with Rhodes University, is a semi-passive process. The process utilises several degrading packed bed reactors (DPBRs) to reduce the sulphate load via biological sulphate reduction (BSR). Thereafter, the sulphide effluent is oxidized within a linear flow channel reactor via a floating sulphur biofilm (FSB) to elemental sulphur.

A FSB is available to form on the surface of the water due to the surface tension of the water. Furthermore the air-liquid interface is the ideal substrate as there is sufficient nutrients and oxygen for the aerobic sulphide oxidising bacteria (SOB).

## Sulphide Oxidation

Sulphides are essentially comprised of three chemical species $H_2S$, $S_x^{2-}$ and $HS^-$. The hydrogen sulphide that will be dealt with within the experimental system will predominantly occur in an aqueous or dissociated state ($HS^-$). Due to the complex redox conditions within the reactor especially within the biofilm, microbial and abiotic sulphide oxidation processes can occur simultaneously.

In order to promote the production of elemental sulphur within the biofilm, the ratio of sulphide to oxygen must be maintained at 2:1 or greater (Buisman *et al.*, 1990). Additionally it will ensure the minimisation of unwanted products such as sulphate, thiosulphate and colloidal sulphur. Under oxygen limited conditions sulphur is the major product of bacterial sulphide oxidation; while sulphate is predominantly formed under high redox oxygen rich environments (Janssen *et al.*, 1995, 1997). A fully developed biofilm ensures the correct oxygen ratio is maintained by impeding the oxygen mass transfer from the headspace into the reactor.

$$2\,HS^- + O_2 \rightarrow 2\,S^0 + 2\,OH^- \quad (1)$$

If the biofilm is not fully developed and this ratio is not maintained then the major product would be sulphate as the sulphide oxidising bacteria (SOB) derive more energy from the complete oxidation to sulphate (Equation 2). Moreover, if the sulphide loading is low and no reduced sulphur compounds are available then the oxidation of sulphur would be preferred by SOB (Equation 3).

$$HS^- + 2\,O_2 \rightarrow 2\,SO_4^{2-} + H^+ \quad (2)$$

$$2\,S^0 + 3\,O_2 \rightarrow 2\,SO_4^{2-} + 2\,H^+ \quad (3)$$

## Materials and Methods

### *Experimental Reactor Configuration*

The experimental setup consisted of two degrading packed bed reactor (DPBR) columns, and three purpose built LFCRs. The DPBRs were fed a syn-

thetic partially treated AMD feed from the bottom in order to minimise channeling and promote reactor stability. The feed rate was 2.9L/day (2g $SO_4^{2-}$/L), which, was also supplemented with molasses (1.5g/L) to promote biological sulphate reduction. The packing within the column consisted of organics such as hay, bagasse, wood chips, sewage sludge and plastic beads. The columns were inoculated with a mixed consortium of bacteria in order to perform the task of biological sulphate reduction as well as sulphide oxidation in the LFCRs.

The LFCRs were designed according to the following dimensions: (0.15 × 0.1 × 2.5m) with 15 sampling ports, across three levels, on the front wall of the reactor (figure 1). Each port was fitted with a rubber septum (GC injection septum) and samples were withdrawn using a 100 mm hypodermic needle. Samples (2 ml) were collected on a daily basis, along with influent and effluent samples.

### *Experimental conditions*

The LFCR's were run under different sulphide loading conditions realised by the different sulphide and sulphate concentrations in the feed tanks. The DPBR effluent fed the feed tanks. All the channels were operated at ambient conditions (25 °C, 1bar). The predominant bacterial species within the LFCR were identified as *Beggiatoa spp, Chromatium Okenii, Klebsiella spp, Pseudemonas spp, Spirochaete spp.* and *Thiomonas Intermedia K12*. The following conditions (table 1) were set at the start of the experiments within each of the linear flow channel reactors.

### *Sample Analyses*

The samples were analysed for pH, aqueous sulphide concentration, colloidal sulphur, sulphate, polysulphides and volatile fatty acids (VFAs). The aqueous sulphide analyses were performed on a UV-Vis spectrophotometer (Cline, 1969). The sulphur and polysulphide analyses performed as described by (Mockel, 1984) and (Kamyshny *et al.*, 2004, 2006) respectively on a Thermo Scientific HPLC Spectrasystem. A reversed phase C18 column was utilised. The sulphate analysis was performed on a Waters 717plus system with a conductivity detector and Waters IC-PAK HR Anion column (4.6 × 75mm). Scanning Electron Microscopy and Energy-Dispersive X-Ray spectroscopy (SEM-EDX) were performed on the harvested floating biofilms.

***Table 1*** *Experimental conditions within the LFCRs.*

| Run No. | Air F low Rate (L/Day) | Volumetric F eed F low Rate (L/day) | Sulphide Loading Rate (mM/day) | Hydraulic Residence Time (Days) | Run Time (Days) | Abiotic System | pH |
|---|---|---|---|---|---|---|---|
| 1 | 48 | 5 | 2.04 | 5 | 19 | ☐ | 7.5 |
| 2 | 48 | 5 | 6.27 | 5 | 10 | ✓ | 11.6 |
| 3 | 48 | 6.25 | 5.37 | 4 | 16 | ☐ | 7.8 |
| 4 | 48 | 6.25 | 4.75 | 4 | 16 | ☐ | 7.7 |
| 5 | 48 | 12 | 4.85 | 2 | 12 | ☐ | 7.5 |
| 6 | 48 | 12 | 5.06 | 2 | 12 | ✓ | 7.0 |

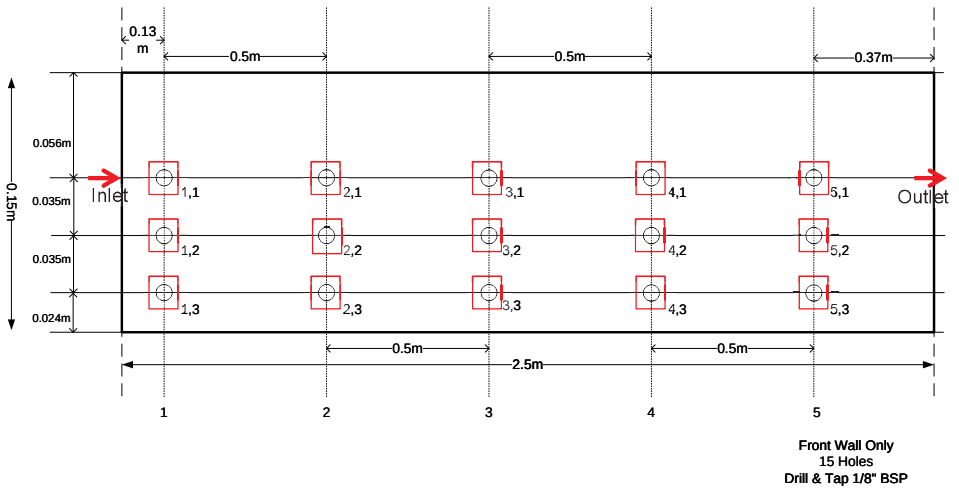


***Figure 1*** *Schematic diagram of Linear Flow Channel Reactor front wall with sampling ports.*

## Results

Various experiments were conducted in order to ascertain the effect of residence time on the performance of the LFCR. Run 1 was operated as a biotic system and was fed sulphide effluent from the DPBR. The average inlet sulphide concentration loading was 2.04 mM/day with a sulphide conversion of 93.02%. 36.05% of incoming sulphide was converted to unwanted products viz. sulphate (9.55%) and colloidal sulphur (26.5%). Colloidal sulphur is difficult to harvest as compared to sulphur formed within the biofilm. Colloidal sulphur also promotes the production of unwanted polysulphides. These were formed as a result of poor biofilm development. The biofilm contained a large amount of inorganic materials such as gypsum and calcium carbonate (43.12 wt%).

The performance of the LFCR in experimental run 3 was significantly better as a more stable and complex biofilm developed with a greater organic content. The biofilm was significantly thicker due to the initial dose of sodium acetate. It was identified that the LFCRs were carbon substrate limited due to the low concentration of VFAs leaving the DPBRs. A higher sulphide conversion of 96.1% was achieved with 24.8% of the converted sulphide reporting to the biofilm as sulphur. 40.63% of the influent sulphide was converted to unwanted products, sulphate (32.89%) and colloidal sulphur (7.74%). The remaining sulphide was converted to sulphur within the biofilm. However, due to the extremely low flow rate the biofilm that formed was not structurally sturdy even though it was 200 mm thick. The poor structural integrity is also visible in figure 2a as the biofilm is broken and not well defined on the underside. Hence, it was unable to hold all the produced sulphur within the structural matrix of the biofilm, as a result the biofilm collapsed. A large proportion of the sulphur (34.9%) fell to the reactor floor.

Experimental run 4 was executed concurrently however, the biofilm was initially grown at a volumetric flow rate of 12.5 L/day and thereafter the flow rate decreased to 6.25 L/day at the start of run 4. The LFCR performance was lower due to the biofilm being thinner in nature and more brittle. However, it was more structurally robust and therefore did not collapse hence the amount of sulphur solids on the reactor floor was significantly less. 35.35% of the converted sulphide was converted to sulphur within the biofilm. However, due to the extremely long residence time the remaining sulphide was converted to unwanted products, sulphate and sulphur solids. Furthermore the colloidal sulphur was converted to sulphate due to the thin nature of the biofilm (50 µm) and longer residence time. Figure 2b clearly shows the biofilm has a greater structural integrity, is more compact and has well defined edges.

The efficiency of the LFCR was optimised in experimental run 5. An 82.2% sulphide conversion was attained with 92% of the converted sulphide reporting as solid sulphur in the biofilm. The average inlet sulphide concentration loading was 5.03 mM/day; approximately 2.5 times greater than run 1; furthermore the residence time within the LFCR was reduced to 2 days. The overall operating efficiency of the LFCR was increased three fold due to the formation of a fully developed biofilm (30 mm). Furthermore a smaller proportion of colloidal sulphur was formed due to the lower residence time. The film analysis revealed that it was composed of sulphur (66%) and the remainder as organic carbon (7.6%) and inorganic material. The inorganic material was predominantly phosphate (6.35%), calcium (12.7%) and manganese (6.35%) and occurred on the upper surface of the biofilm (figure 3a). These inorganics had originated from the DPBR effluent as a result of the synthetic AMD feed.

***Table 2*** *Total mole balance on sulphur species formed.*

| Run No. | Sulphide Converted (mmoles) | Sulphide Conversion (%) | Sulphate (mmoles) | Colloidal Sulphur (mmoles) | Sulphur in Biofilm (mmoles) | Polysulphides (mmoles) | Solids – Sulphur (mmoles) |
|---|---|---|---|---|---|---|---|
| 1 | 130.04 | 93.02 | 12.42 | 34.41 | 72.2 | 11.07 | 0 |
| 2 | 77.98 | 24.7 | 4.61 | 0 | - | 33.4 | - |
| 3* | 580.67 | 96.1 | 190.96 | 44.94 | 144.07 | - | 202.8 |
| 4* | 498.27 | 93.3 | 238.35 | (79.31) | 178.0 | - | 83.8 |
| 5* | 572.73 | 82.2 | 33.0 | 6.65 | 527.0 | 4.0 | 0 |
| 6 | 256.76 | 28.76 | 3.0 | - | - | 11.05 | - |

* The reactors were initially dosed with 20g of sodium acetate ($CH_3COONa$) to ensure no carbon substrate limitation

The biofilm analysis of experimental run 3 and 4 were conducted. The biofilm (a) in run 3 was composed of sulphur (43%) and the remainder biomass. Experimental run 4 had sulphur content of 81.3%, inorganic content of 6.9% and the remainder as organic carbon. The biofilm was able to hold a greater proportion of sulphur due to it being more compact, densely packed and having a greater structural integrity (figure 2b). The structural integrity was improved due to the exopolysaccharide (EPS) strand networks (figure 3b)

Experimental run 2 was operated abiotically in order to determine the extent of chemical sulphide oxidation within the biological systems. The average sulphide concentration fed into the channel was 6.27mM/day and was converted to sulphate (6%), polysulphides (42.8%) and the remainder was thiosulphate (51.2%) as no sulphur was formed. No sulphur was formed due to the high pH of 11.6. Experimental run 6 had a similar sulphide conversion of 28.76% with a sulphide loading of 5.06 mM/day. However, the sulphide was converted to sulphate (1.2%), polysulphides (4.3%) and the rest to thiosulphate. Colloidal sulphur was initially observed when the reactor feed was pumped into the greenish solution and this observation resemebled that of the Tyndall effect (Chen and Morris, 1972). The colloidal sulphur was formed due to the feed being buffered to a pH of 7. However, this disappeared due to the sulphur reacting with the sulphide within the reactor and consequently the polysulphide concentration increased (van den Bosch, 2008):

$$HS^- + (x\text{-}1)\, S^0 \longleftrightarrow S_x^{2-} + H^+ \qquad (4)$$

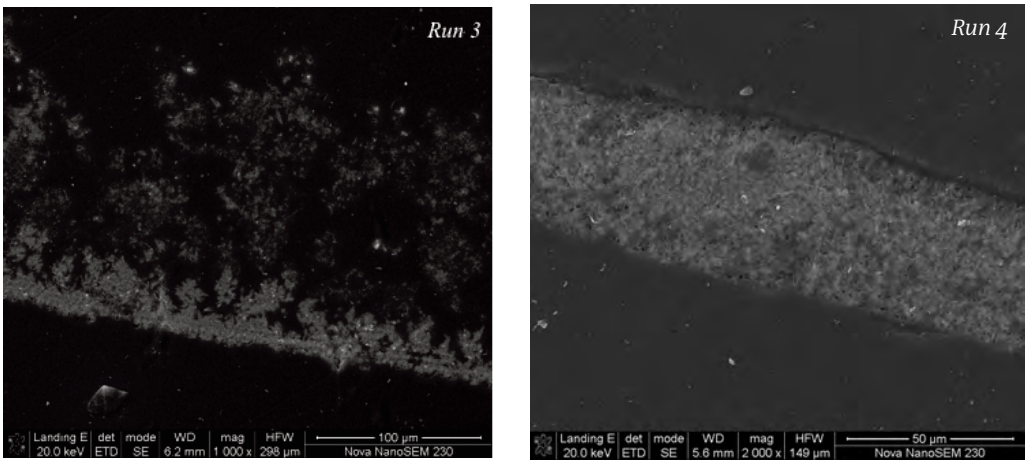


***Figure 2*** *SEM micrograph of floating sulphur biofilm in cross-sectional view of experimental run 3 & 4.*

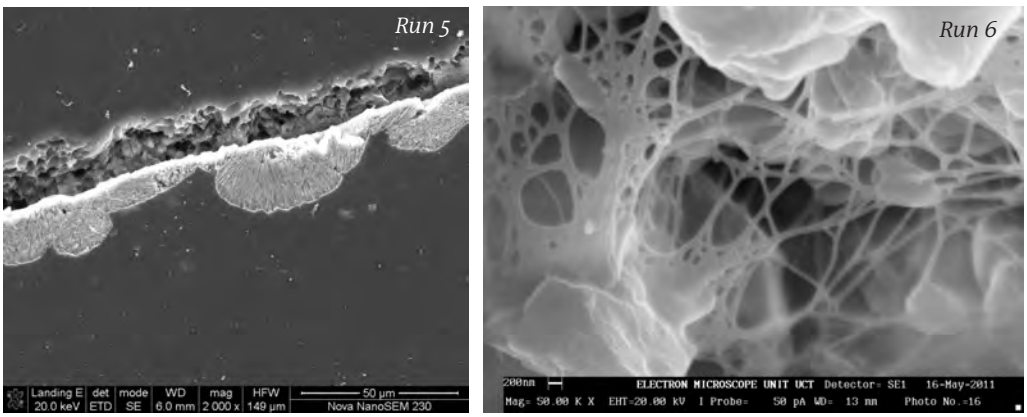


***Figure 3*** *SEM micrograph of floating sulphur biofilm in cross-sectional view (run 5) and SEM micrograph depicting EPS strand network.*

## Conclusions

It was determined that the residence time of the LFCR affects the proportion of the various sulphur species formed. Decreasing the residence time improved the LFCR operating efficiency. Firstly, this was achieved as the colloidal sulphur spent less time within the bulk liquid layer. Therefore the amount of colloidal sulphur converted back to sulphate was decreased. Secondly, the biofilm structural integrity improved with a decrease in the residence time as well as sodium acetate dosing, as the reactor was carbon substrate limited. Lastly, it was determined that a more densely packed and structurally robust biofilm is able to maintain the correct sulphide to oxygen ratio as well as hold the produced sulphur. The extent of chemical sulphide oxidation was determined at pH 11 and 7. Furthermore it was determined that the primary products were thiosulphate and polysulphides. Therefore chemical sulphide oxidation forms a small percentage of overall sulphide oxidation. Further tests will be conducted on decreasing the residence time and varying carbon substrate loading in order to optimize biofilm development and sulphur production.

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