PILOT-SCALE SEMI-PASSIVE TREATMENT OF ARD − EVALUATION OF TREATMENT PRODUCTS FOR DOWNSTREAM USE

Report to the
Water Research Commission

by

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EXECUTIVE SUMMARY

BACKGROUND

The project aimed to evaluate, at pilot scale, an integrated semi-passive system for the treatment of acid rock drainage (ARD). Specifically, the system is designed to treat water originating from diffuse sources, such as waste rock dumps, coal discards, tailings impoundments and low-volume discharges. The technology is not aimed at treating high-volume discharges that are actively pumped from underground basins.

The project draws on recent research that aimed to address three of the primary constraints that have prevented the more widespread implementation of technologies based on biological sulphate reduction. These are the high cost of the organic electron donor, the retention of biomass within the sulphate reducing unit and the management of the sulphide product.

Recent research, support by the Water Research Commission (Projects K5/2109, K5/2110 and K5/2392), demonstrated that the modified linear flow channel reactor (LFCR) was capable of supporting efficient sulphate reduction using a “passive” reactor configuration. The inclusion of carbon microfibers to enhance biomass retention allowed volumetric sulphate reduction rates in excess of those achieved in traditional stirred tank reactors to be maintained, particularly at low hydraulic retention times. Further development resulted in a hybrid LFCR capable of supporting simultaneous sulphate reduction, in the anaerobic bulk liquid zone, and partial sulphide oxidation, in the floating sulphur biofilm.

The hybrid LFCR system has been optimised at laboratory scale using defined media, with lactate and acetate as the carbon source and electron donor. The fundamental research at laboratory scale is ongoing (WRC research project K5/2392), with the current focus on assessing the effect of temperature on system performance.

The preceding research demonstrated that good sulphate reduction rates could be achieved in a passive system, through effective biomass retention, and that the resulting sulphide could be managed through partial oxidation to elemental sulphur within the floating sulphur biofilm. A method for effective biomass harvesting has been developed and the impact of repeated cycles of biofilm harvesting on system performance has been quantified.

While challenges associated with biomass retention and sulphide management have been overcome, the provision of a cost-effective electron donor capable of supporting high rates of sulphate reduction remains an obstacle. Laboratory scale research suggested that effluent from the anaerobic digestion of algae could be a viable electron donor, but subsequent research using a range of other complex carbon sources (manure, grass and other algal species) were less encouraging. Analysis of the data showed that hydrolysis of the complex carbon source was relatively poor at the pH required for effective anaerobic digestion, suggesting that a dedicated hydrolysis reactor, operated at a lower pH, may be a better option.

The primary aim of this project is to commission the pilot plant and monitor its performance for a period of 18-24 months. The first reactor will be dedicated to biomass pre-treatment, with the partially treated (pH 3-4) mine water contacted with the biomass source (initially grass cuttings). Laboratory data has indicated that this will result in further neutralisation of the water and the partial hydrolysis of the grass, followed by fermentation of the soluble products to volatile fatty acids (VFAs). The effluent from this reactor will pass into the second reactor, operated as a hybrid LFCR, where sulphate reduction and sulphide oxidation will occur. A second hybrid LFCR will be available to treat the effluent from the first, if required.

A significant component of this project is the characterisation of the products (treated water and biosulphur), with the aim of evaluating them for potential value addition. Specifically, the intention is to assess whether the treated water is safe to use for small-scale agriculture and whether the biosulphur product has value as a fertiliser component.

Finally, if the characterisation of the treated water indicates that further polishing is required a pilot scale constructed wetland will be included in the integrated process.

AIMS

The initial aims of the project were as follows:

1. To operate a semi-passive plant for the remediation of ARD for a period of two years.
2. To characterise the resulting water quality from the primary integrated system. If additional polishing is required to assess the efficiency of a constructed wetland to achieve this.
3. To evaluate the potential of using the treated water for agricultural purposes − evaluate the risk of accumulation of toxic components.
4. To evaluate the potential to use the sulphur product from the integrated process as a fertiliser component.

However, once it became clear that some of the early assumptions regarding the performance of complex organic carbon sources, particularly in terms of the hydrolysis and release of soluble carbon, were unrealistic and the pilot system under these conditions was not viable the focus shifted to illustrating the magnitude of the challenge that would need to be overcome.

METHODOLOGY

The first part of the research involved assessing the performance of the 2 000 l hybrid-LFCR on-site treating circumneutral mine water from the pit. The SRB culture was scaled up, using acetate as the electron donor and carbon source, to a 1 000 l volume, which was used to inoculate the hybrid-LFCR. Sulphide oxidation, sulphate reduction and floating biofilm formation were monitored to assess performance.

The intended second phase was the evaluation of the integrated system, with the first reactor operating as an accelerated hydrolysis and pre-treatment unit to achieve some neutralisation and residual metal removal, while hydrolysis of the grass cuttings and subsequent acidogenesis occurred. This was delayed for a number of reasons and during this time laboratory-based research made it clear that the proposed integrated system would not be viable.

The laboratory based research focussed on assessing the potential for accelerated hydrolysis of grass cuttings in 500 ml and 1 l reactors, testing the proposed integrated reactor system and small-scale tests using partially treated mine water from the pilot site.

The intended plant growth trials could not be performed, initially due to a shortage of bio-sulphur. An alternative source of bio-sulphur was found, but Covid restrictions prevented laboratory access. Some laboratory research was possible to test activity of sulphur oxidising microbes and assess rates of sulphur solubilisation. This provided an indication of the rate at which sulphate could be made available if the bio-sulphur was used as a soil conditioner.

Some preliminary laboratory studies were conducted to assess the potential for microbial hydrolysis of low-grade coal as an alternative carbon source.

Based on the laboratory research and the challenges associated with carbon source provision a modelling exercise was conducted, working back from the target of reducing sulphate concentration from 2 000 mg/l to 500 mg/l for a feed of 2 000 l/d (2 day HRT), to determine the amount of different substrates required.

RESULTS AND DISCUSSION

The initial evaluation of the 2 000 l reactor at the first pilot site showed that the hybrid-LFCR could be scaled up, but did highlight a number of performance limitations. The system failed when the initial sulphide concentration was too low to support the formation of a complete biofilm, confirming observations made at laboratory scale.

The reactor was successful when the initial sulphide concentration was 180 mg/l, with a complete biofilm forming within 24 hours and active sulphate reduction occurring. The average VSRR was 180 mg/l.d, which was approximately 60% of the rate observed under optimal conditions in the laboratory, most likely as a result of lower temperature. The rate was however, less than 40% of the rate achieved in the laboratory scale reactor

at a 2-day HRT under optimal conditions using lactate as the electron donor. Therefore, the idea of operating a pilot system at a 2-day HRT on acetate or the end products of fermentation of a complex carbon source is unrealistic.

The laboratory-based research showed there was release of soluble COD from grass cuttings, with rapid release of up to 55 mg COD per g of biomass over the first 24 hours. This was most likely not due to microbial fermentation. The slower release of soluble COD over subsequent days could be accounted for by microbial-mediated hydrolysis. Therefore, in practice, the substrate would need to be replenished regularly, or even daily.

Small-scale tests showed that near complete sulphate reduction and iron removal could be achieved from actual mine water in a reactor using grass cuttings as the carbon source. The experiment was performed in batch and required over 30 days to reduce the sulphate concentration from 2 600 mg/l to less than 100 mg/l, a VSRR of 76 mg/l.d.

While the plant growth trials could not be completed, the solubilisation of bio-sulphur from the biofilm by sulphur-oxidising microbes was demonstrated, confirming the potential for using the bio-sulphur as a soil conditioning agent.

KEY CHALLENGES

The evaluation of the pilot scale hybrid-LFCR for partial sulphide oxidation was successful. This unit has potential for integration into passive or semi-passive systems to treat mining impacted water. However, the key challenge remains the consistent provision of sufficient electron donor and organic carbon to sustain sulphate reduction and sulphide oxidation. This could not be overcome during this study, even at laboratory scale, so evaluation of the integrated reactor system was not possible.

The magnitude of the challenge can best be understood by way of an example. In order to reduce the sulphate concentration of a relatively low volume discharge (2 000 l/day) from 2 000 mg/l to an acceptable 500 mg/l requires the reduction of 3 kg of sulphate per day. Assuming an optimal COD to sulphate ratio of 0.7, this equates to a requirement of 2.1 kg of suitable COD per day.

Data generated on the use of pasture grass to support biological sulphate reduction suggested 3.7 g of partially dried (2 day old) grass was required to reduce 1 g of sulphate. Using the example above, this would equate to 11.1 kg of grass per day. *Erogrostis curvula* is the most popular pasture grass in the summer rainfall areas of South Africa and yields between 8 and 20 t/ha annually. Therefore, at least one fifth of a hectare of pasture would be required to produce enough substrate to treat the 2 kl/d discharge. However, this ignores degradation kinetics, which are slow for grass. Data from this study show that just over 55 mg of soluble COD/g grass is released in the first 24 hours, after which the rate slows considerably. Using this COD liberation rate and the optimum COD:sulphate ratio, the requirement for grass becomes 38.2 kg/d or almost 14 t/year.

The situation is further complicated by the fact that much of the laboratory-based research was conducted using readily available carbon sources (lactate and acetate) under controlled conditions. Parallel research confirmed the significant impact of low temperature on sulphate reduction rates.

An additional challenge associated with complex substrates is that only a fraction of the available COD is readily available, so to prevent the system becoming severely constrained by the rate of hydrolysis (as is common in packed bed reactors) the turnover of fresh biomass needs to be high. This creates a problem of how to dispose of the partially degraded substrate. In a mine water treatment scenario the biomass is likely to be associated with potentially toxic metals, which have been adsorbed or precipitated onto the biomass, so could be considered a hazardous waste.

CONCLUSIONS AND RECOMMENDATIONS

The research was not able successfully address the primary aims of project, namely the continuous operation of the integrated pilot-scale system for a period of two years and the evaluation of the bio-sulphur product as a soil conditioning agent using plant growth trials.

Despite this, the project has generated data that will be valuable going forward. The LFCR was shown to be effective for partial sulphide oxidation, with the formation of a harvestable floating biofilm. It could be applied downstream of an effective sulphate reducing reactor or used to treat other sulphide containing effluents.

The hybrid system has potential if a suitable carbon source and electron donor are available. This would most likely need to be in liquid form.

An important cautionary principle to emerge from the research was the need to critically evaluate data generated during laboratory scale research. This is typically generated under controlled conditions, selected to optimise performance. Both scale- and variable environmental factors are likely to have very significant impacts on the performances of biological systems, more so than physical or chemical processes where the impacts of these factors are more likely to be predictable and easier to simulate.

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ACRONYMS & ABBREVIATIONS

AD	Anaerobic digestion
ALD	Anoxic limestone drain
AMD	Acid mine drainage
ARD	Acid rock drainage
BSR	Bacterial sulphate reduction
COD	Chemical oxygen demand
CSTR	Continuously stirred tank reactor
HDS	High density sludge
HLPC	High performance liquid chromatography
HRT	Hydraulic retention time
LFCR	Linear flow channel reactor
RO	Reverse osmosis
SEM	Scanning electron microscopy
SRB	Sulphate reducing bacteria
VFA	Volatile fatty acid
VSLR	Volumetric sulphate loading rate
VSRR	Volumetric sulphate reduction rate

CHAPTER 1: LITERATURE REVIEW

1.1 INTRODUCTION

The project aims to evaluate, at pilot scale, an integrated semi-passive system for the treatment of acid rock drainage (ARD). Specifically, the system is designed to treat water originating from diffuse sources, such as waste rock dumps, coal discards, tailings impoundments and low-volume discharges. The technology is not aimed at treating high-volume discharges that are actively pumped from underground basins.

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Based on the success of the laboratory scale research a pilot scale system, consisting of three 2 000 l reactors, was developed and tested on neutral mine water with elevated sulphate concentrations at a mine site in the Free State. Sulphate reduction, sulphide oxidation and biofilm formation were demonstrated in batch phase, but the system could not be tested in continuous mode due to changes in water quality and ultimately the decision by the mine owners to sell the mine. As a consequence the pilot plant was relocated to an active mine site near eMalahleni in Mpumalanga.

While challenges associated with biomass retention and sulphide management have been overcome, the provision of a cost-effective electron donor capable of supporting high rates of sulphate reduction remains an obstacle. Laboratory scale research suggested that effluent from the anaerobic digestion of algae could be a viable electron donor, but subsequent research using a range of other complex carbon sources (manure, grass and other algal species) were less encouraging. Analysis of the data showed that hydrolysis of the complex carbon source was relatively poor at the pH required for effective anaerobic digestion, suggesting that a dedicated hydrolysis reactor, operated at a lower pH, may be a better option.

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1.2 MINING IMPACTED WATER

Acid rock drainage is essentially caused by the exposure of sulphidic minerals to both oxygen and water as a consequence of mining and processing of metal ores and coal (Johnson and Hallberg, 2005). The sulphide minerals may be exposed as tailings or waste rock, ore stockpiles or in operating and abandoned mine workings. Acid rock drainage can be generated abiotically, through chemical weathering, but the presence of iron and sulphur oxidising microorganisms can increase the kinetics of the process up to a thousand-fold. The reactions involved are detailed below (Equations 1-4) (Akcil and Koldas, 2006).

$$FeS_2 + \frac{7}{2}\,O_2 + H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \qquad \text{Equation 1}$$

$$Fe^{2+} + \frac{1}{4}O_2 + H^+ \rightarrow Fe^{3+} + \frac{1}{2}\,H_2O \qquad \text{Equation 2}$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \qquad \text{Equation 3}$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{3+} + 2SO_4^{2-} + 16H^+ \qquad \text{Equation 4}$$

Pyrite (FeS_2) is the most abundant sulphide mineral and is the primary mineral responsible for ARD generation. The process is initiated due to weathering and oxidation (Equation 1) at a neutral pH. The first reaction is abiotic. The reaction described by Equation 2 may be abiotic, but occurs slowly under acidic conditions in the absence of catalytic microorganisms. The generation of ARD is significantly enhanced when the second reaction is catalysed by aerobic iron-oxidising bacteria such as *Acidithiobacillus ferrooxidans, Leptospirillum ferroxidans* and *Leptospirillum ferriphilum* (Zagury *et al*., 2007; Johnson and Hallberg, 2003).

These particular bacteria are characterised as being acidophilic, aerobic chemoautotrophic species which are most active between pH 1.0 and pH 3.5. The iron-oxidisers are capable of increasing the rate of Fe^{2+} oxidation (Equation 2) by several orders of magnitude (Gazea *et al*., 1996). Ferric iron has limited solubility and if the pH is higher than pH 2.3-3.5, it precipitates as oxyhydroxide, releasing H^+ and therefore lowers the pH as per Equation 3 (Zagury *et al*., 2007). The oxyhydroxide precipitate gives water a red-orange colour, which is a common characteristic of ARD discharge.

In addition to the oxidative reactions, the ferric ions may react with more pyrite as per Equation 4, producing more ferrous iron to drive Equation 2. In the presence of sufficient dissolved oxygen, a continuous cycle is maintained (Johnson and Hallberg, 2003). The process becomes self-sustaining as the pH continues to decrease, as more ferric iron will remain in solution to chemically attack the pyrite.

A second group of microorganisms, capable of oxidising reduced sulphur species, are typically found in these environments and contribute to ARD formation. This group, which includes *Acidithiobacillus*

thiooxidans and *At. caldus*, utilises reduced sulphur species as the electron donor to produce sulphate and protons. The proton acidity contributes to the low pH which typically characterises AMD. A consequence of the low pH is the dissolution of acid-labile minerals, leading to the further release of heavy metals and ions contributing to salinity.

The closure of deep-level mines poses a particularly serious threat in terms of uncontrolled AMD discharges. These workings typically intersect the water table, requiring active dewatering during operation (Adams *et al.*, 2000). Upon cessation of mining activities the dewatering is typically stopped, allowing groundwater rebound to occur (Scott, 1995; Younger, 1997). During this process previously dewatered voids gradually fill with water until a surface overflow point is encountered. Rebound not only results in a repositioning of the water table and surface discharges, but can also have a profound effect on the water quality. During dewatering water passes through the workings along discrete flow-paths which are well washed and as such any soluble minerals are flushed from them. When the workings are left to flood all the void spaces come into contact with water. Regions that have been previously unsaturated are likely to be encrusted with “acid generating salts” (iron hydroxyl-sulphates formed by partial oxidation of pyrite under unsaturated conditions), that rapidly dissolve liberating mineral and proton acidity as well as sulphate (Younger, 1997). This is termed vestigial acidity and results in a highly polluted “first flush” scenario, where active treatment will typically be required. The rate of depletion of vestigial acidity is primarily controlled by hydraulic factors and a number of models have been proposed to predict this (Younger, 2000). In contrast, juvenile acidity arises from the continued oxidation of sulphide minerals as a consequence of seasonal fluctuations in the water table or percolation through waste rock and tailings impoundments. Theoretically, juvenile acidity can persist until all the exposed sulphides have been depleted, which may take tens to hundreds of years. These effluents are less heavily polluted and are more amenable to passive treatment.

Not all mining impacted water presents as an acidic discharge. Where AMD comes into contact with material with a strong acid neutralising capacity, such dolomitic or calcareous formations the acidity may be neutralised prior to discharge, along with the precipitate of a significant proportion of the previously mobilised metals. The discharges are termed neutral or circum-neutral. While the metal load has been substantially reduced the salinity, particularly with respect to sulphate may still be high.

1.2.1 Current management practices

A variety of technologies have been developed for the treatment of AMD and ARD. The established methods are based on oxidation, neutralisation, precipitation and sedimentation. The oxidation converts iron and aluminium to their less soluble oxidised 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 sulphate 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 sulphate reduction.

1.2.1.1 Chemical treatment

The most commonly used chemical treatment method is the addition of an alkaline material to raise the pH, in conjunction with aeration, to accelerate the rate of chemical oxidation of ferrous iron. The most common reagents are lime ($Ca(OH)_2$), slaked lime, calcium carbonate ($CaCO_3$), sodium carbonate (Na_2CO_3) or sodium hydroxide (NaOH). However, each compound varies in cost and effectiveness and therefore the preferred agents are generally lime or calcium oxide, due to economic concerns (Johnson and Hallberg, 2005). Liming as a treatment process is effective in the removal of sulphate to the saturation level of gypsum ($CaSO_4.2H_2O$) and neutralisation of acidity (Equations 5 and 6) as well as the precipitation of dissolved metals as metal hydroxides. However, the resulting sludge (gypsum and metal hydroxides) is voluminous and unstable at a low pH, leading to resolubilisation of the metal hydroxides (reverse of Equation 7) (Lorax International, 2003). The major disadvantage of these treatment processes are the production and disposal of the sludge and the high cost of chemicals (Lorax International, 2003; Johnson and Hallberg, 2005).

$$Ca(OH)_2 + H_2SO_4 \rightarrow CaSO_4.2H_2O(s) \quad \text{Equation 5}$$

$$CaCO_3 + H_2O + H_2SO_4 \rightarrow CaSO_4.2H_2O + CO_2 \quad \text{Equation 6}$$

$$Ca(OH)_2 + H_2SO_4 + Me \rightarrow 2H^+ + CaSO_4 + Me(OH)_2 \quad \text{Equation 7}$$

The high density sludge (HDS) process represents a technological advancement over conventional chemical technologies. It makes use of iron oxide seeds, which are added to the neutralisation reactor. The seeds promote secondary nucleation which results in the deposition of new precipitate on the existing particles. This enhances the precipitation process and leads to the formation of a denser, more granular sludge which significantly enhances the solid-liquid separation efficiency (Loewenthal *et al*., 2001; Hove *et al.*, 2009). The HDS process has been selected as the initial step for the management of AMD from the dewatering of the Witwatersrand basins.

Mine water that has been partially treated by lime dosing or the HDS process is typically near neutral in pH and has a substantially reduced metal load, although some elements such as aluminium and manganese require the pH to be elevated to closer to pH 10 for effective removal. The partially treated water remains saturated, or close to saturated with respect to gypsum. As such, it cannot be discharged to the aquatic environment without additional treatment due to the high salinity.

1.2.1.2 Membrane technology

The two commercial technologies which utilise membranes in mine water treatment, are reverse osmosis and electrodialysis. Electrodialysis involves an electric potential being utilised to move dissolved ions through a selectively permeable membrane. Similarly, reverse osmosis involves the forceful movement of water through a semi-permeable membrane (which excludes all but pure water) via high-pressure pumps.

The favoured approach to deal with gypsiferous mine water is to treat if further using reverse osmosis (RO), which is capable of generating potable water. While effective, RO carries high capital and operating costs and is energy intensive. An alternate approach, which has been the subject of some research in South Africa, is to use the gypsiferous water for agriculture. This will be discussed in greater detail later.

Reverse osmosis is a very flexible technology in that it can treat numerous types of wastewater. However, the membrane may be severely affected by fouling, depending on the quality of the feed water (Lorax International, 2003). Reverse osmosis has emerged as the technology of choice for second stage treatment of AMD in South Africa, following an initial neutralisation and precipitation step, and has been employed at the eMalahleni Water Reclamation Plant for several years, achieving very high water recoveries (Günther and Naidu, 2008). Despite the high water recoveries, a substantial

amount of hypersaline brine is produced which needs to be managed. This, together with the lime and energy requirements means the process remains costly. Eutectic freeze crystallisation is being considered as an option to assist with brine management (Nathoo *et al*., 2009).

1.2.1.3 Biological sulphate reduction

Biological sulphate reduction (BSR) has been extensively researched and proponents suggest it has the potential to be a more economical alternative to the costly physical and chemical processes described above, both to treat raw ARD or partially treated, gypsiferous water. This technology is essentially dependent on the ability of anaerobic sulphate reducing bacteria (SRB) to utilise sulphate as their terminal electron acceptor. Most SRB are heterotrophic and require an organic carbon source (volatile fatty acid or short chain alcohol) as the electron donor. A small number of autotrophic species exist that are able to utilise hydrogen as the electron donor and fix carbon dioxide. Sulphate reduction may be assimilatory, where the sulphide is incorporated in sulphur-containing amino acids, or dissimilatory, where the sulphide is released to the external medium. The latter process forms the basis of AMD remediation processes as it is not directly linked to biomass growth. A generalised reaction for dissimilatory sulphate reduction is shown below (Zagury et al., 2007; Oyekola, 2008).

$$2CH_2O + SO_4^{2-} \rightarrow 2HCO_3^- + H_2S \qquad \text{Equation 8}$$

Whilst the sulphate is reduced to sulphide there is the simultaneous generation of alkalinity, predominantly as bicarbonate (HCO_3^-). From an ARD treatment perspective the alkalinity acts to neutralise the acidity while the sulphide is available for the precipitation of metals as metal sulphides (Johnson and Hallberg, 2005). Metal sulphides are particularly insoluble, even at relatively low pH values and produce more compact precipitates than hydroxide equivalents. Theoretically, sulphide precipitation is a highly effective method to reduce heavy metal concentrations to insignificant levels and thermodynamic data suggest that individual metal sulphides can be sequentially precipitated by adjusting the pH (Hammack *et al*., 1993). However, the extremely high supersaturation induced by the low solubility promotes primary nucleation, resulting in the precipitation of a large number of very small (< 0.2 µm) particles, complicating downstream separation (Mokone *et al.,* 2010).

Sulphide is a toxic, corrosive and malodorous compound which needs to be removed from the treated effluent prior to ultimate discharge. While metal sulphide precipitation can potentially remove a portion of the sulphide, the fact that most ARD is derived from pyrite (FeS_2) means that if the sulphate reduction process is more than 50% efficient there will always be residual sulphide. In reality the residual sulphide is normally significantly higher as the majority of the iron is removed prior to the sulphate reduction step. One attractive option for the management of residual sulphide is the partial oxidation to elemental sulphur, which can be recovered as a value adding product. This has been achieved in an active process (Janssen et al., 1995) and more recently in a passive system (Van Hille and Mooruth, 2011; Molwantwa and Rose, 2013).

The most significant constraint working against the more widespread application of technologies based on BSR is the provision of a cost effective carbon source and electron donor. Where these types of technologies have been applied the electron donor has typically been a low molecular weight alcohol (methanol or ethanol), while much on the laboratory scale research has been conducted using volatile fatty acids, such as lactate, as the electron donor. At full scale, the constant provision of these electron donors is typically not economically viable, particularly for the remediation of mining impacted water.

Complex or waste organic carbon sources, such as sewage sludge, manure and residual biomass are potentially attractive alternatives, but the key challenge remains optimising the hydrolysis and subsequent acidogenic reactions to provide required electron donor for sustained periods of time. These types of biomass normally have a proportion of the organic carbon that is readily labile so short-

term performance is encouraging, but once this is exhausted the system becomes rate limited by the hydrolysis of the cellulosic and lignocellulosic fractions.

1.2.1.4 Semi-passive systems

Traditional passive systems, such as anoxic limestone drains, permeable reactive barriers and natural and constructed wetlands have been used extensively in Europe and North America. 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). As a consequence the application of passive systems tends to be limited to low volume, relatively benign wastewaters, typical of the juvenile acidity phase of AMD and to ARD from diffuse sources or at end-of-pipe from certain processes.

In South Africa many of the sites impacted by mine water demand interventions that are not readily achievable using tradition passive systems, specifically with respect to the reaction kinetics and ability to adjust to changes in flow rate and compositions. As a consequence much research has been directed toward the development of semi-passive or managed passive systems. Early research was led by Pulles Howard and de Lange (PHD) and Golder Associates Africa (GAA), with contributions from a number of academic institutions, particularly Rhodes University, the University of Cape Town and the University of Pretoria and has addressed the problem at both a fundamental science and more applied pilot plant level (Pulles and Heath, 2009).

The programme addressed two primary challenges, the low sulphate reduction efficiencies reported for systems developed abroad and significant inhibition of existing systems at pH levels below pH 4.5.

The development of the IMPI process began in 1995, with the aim of developing a system that could achieve high rates of sulphate reduction over a sustained period, utilising lignocellulosic material as the source of electron donor. The hydrolysis of lignocellulose was identified as the rate limiting step. The long-term reactor studies that were undertaken as part of the programme allowed the characterisation of five distinct phases of sulphate reduction in passive systems (Coetser *et al*., 2005; Molwantwa *et al*., 2010). These were a lag phase (90-150 days), where the microbial community became established and adapted to the environment, a high performance phase (< 8 months), during which high rates of sulphate reduction were maintained, followed by a crash phase and finally a sustained phase, where relatively low rates of sulphate reduction were observed. Eventually, sulphate reduction ceased once all the hydrolysable lignocellulose had been consumed. Pulles and Heath (2009) suggested that many published studies were not conducted for long enough to reach the crash phase, resulting in optimistic projections of longer-term performance.

The initial target of the research was to develop a system capable of sustained sulphate reduction of over 600 $mM/m^3.d$, considered the threshold for economic viability. Based on the understanding that hydrolysis of lignocellulose was rate limiting, research was conducted to investigate the potential of pre-treatment, using white rot fungi to break down the lignin matrix. The technical feasibility of the system was demonstrated, but unfavourable economics led to the termination of the research.

The second approach, pursued in collaboration with Rhodes University, focussed on understanding the mechanisms of lignocellulose hydrolysis. The research suggested that the degradation of lignocellulose was enhanced under sulphidogenic conditions (Roman, 2004) and that the provision of some readily usable organic carbon (molasses) could significantly reduce the effect of the crash phase and enhance the level of sulphate reduction in phase 4 (sustained phase). These insights led to the development of the patented degrading packed bed reactor (DPBR), which formed the basis of the IMPI process (Figure 1). The DPBR has an optimised packing configuration, with layers of different carbon sources designed to ensure efficient performance. The upper part of the reactor is responsible for removing dissolved oxygen and generating sulphide and alkalinity, which the lower part of the reactor is the site of

accelerated lignocelluose degradation and volatile fatty acid production. To ensure efficient operation the influent is typically supplemented with molasses (0.05-0.1%) as a readily utilisable carbon source (Coetser *et al*., 2005).

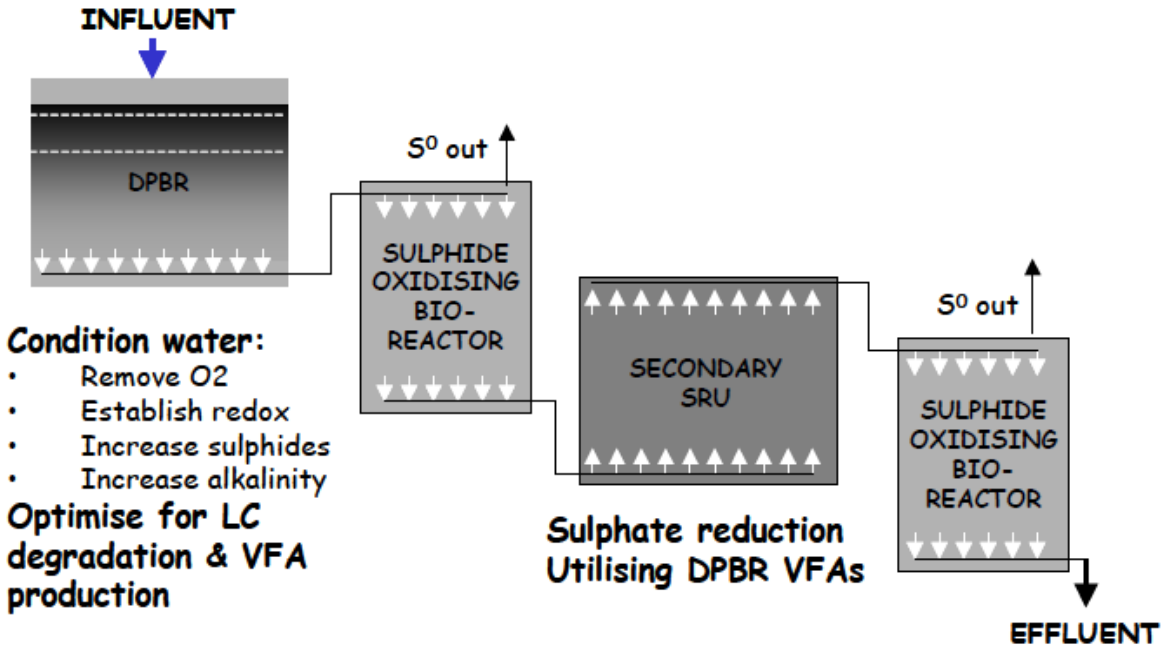

Figure 1: Schematic representation of the IMPI process (Pulles and Heath, 2009)

The overall configuration typically consisted of two sulphate reducing units, the DPBR and a secondary sulphate reduction unit, and two biological sulphide oxidising units which are operated under conditions that promote the partial oxidation of sulphide to elemental sulphur, rather than the complete oxidation back to sulphate. The sulphide oxidation units were not supplied with organic carbon.

A long term (4 years) study conducted at Vryheid Coronation Colliery (VCC) showed stable performance, with sulphate reduction rates significantly higher than the 600 mM/m^3.d target. Performance improved over the summer months, confirming the impact of temperature on sulphate reduction rate. More recent experiences at the VCC site have identified problems with the long term hydraulics of the DPBR, with compaction of particularly the manure layer negatively affecting the hydraulic permeability through the bed.

A full scale system, designed to treat 200 m^3 of mine water, was constructed at the Middelburg mine in Mpumalanga. The system contained a novel sulphide oxidation reactor, the linear flow channel reactor (LFCR) which made use of a floating sulphur biofilm to achieve partial oxidation of sulphide. The system was plagued by a number of construction and operational issues, as well as challenges with the LFCR and performance did not meet expectations. A detailed study into the LFCR was conducted at the University of Cape Town, leading to further optimisation in design and operating parameters (Van Hille and Mooruth, 2013).

The research program at the University of Cape Town has continued, with the focus shifting to evaluating strategies to enhance the rates of sulphate reduction, primarily through improved biomass retention. The LFCR, previously used exclusively for sulphide oxidation, has been evaluated for sulphate reduction, using suspended carbon microfibers to provide a large surface area for microbial attachment. Initial proof of concept experiments were successful, with volumetric sulphate reduction rates of up to 45 mg/l.h achieved at a hydraulic retention time of 12 hours, a 20% improvement on tradition stirred tank reactors despite no active mixing. When the reactors were decommissioned the true extent of biomass accumulation was observed (Figure 2).

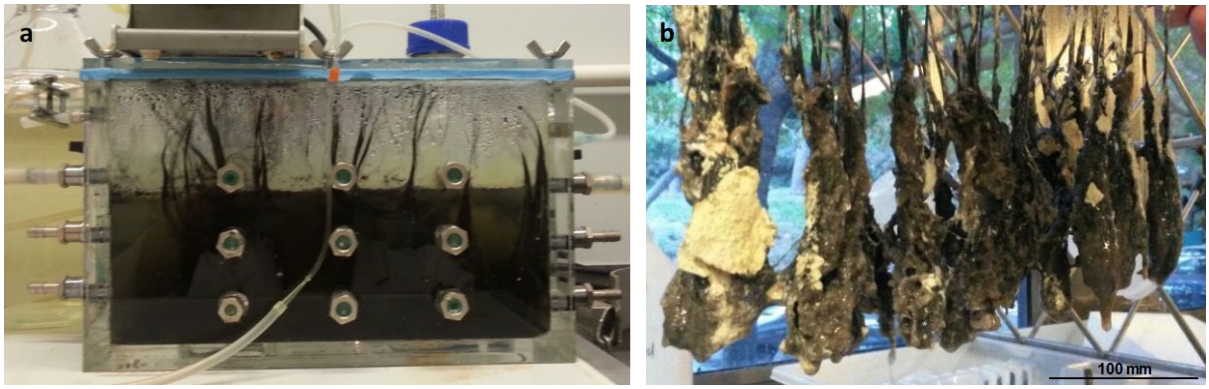

Figure 2: Images showing the application of the LFCR for sulphate reduction: (a) reactor configuration and (b) biomass accumulation on the carbon fibres

During the operation of the LFCR for sulphate reduction it became apparent that a small amount of oxygen still entered the reactor, leading to the development of a sulphur biofilm at the air-liquid interface. This observation, coupled with previous hydrodynamic studies that illustrated the absence of turbulent mixing in the LFCR, prompted a series of experiments to evaluate the potential of supporting simultaneous sulphate reduction and sulphide oxidation within a single reactor unit, open to the atmosphere. These experiments proved particularly successful, with further improvement of the VSSR to 62.5 mg/l.h at a 12 hour HRT. The laboratory scale hybrid LFCR has been used as the basis for the design of the three 2 000 l reactors that are being evaluated at pilot scale during this project.

1.3 MINING IMPACTED WATER FOR AGRICULTURE

In South Africa, important coal reserves in Mpumalanga underlie one of the most important agricultural regions of the country, which is particularly significant as only a relatively small portion of the country can be viewed as having high agricultural potential. South African coal reserves tend to be associated with pyritic formations, so exploitation of the reserves carry a high risk of generating acidic, mining impacted water. As described above, the most widely applied method of treating raw mine water is primary neutralisation with lime or hydrated lime. A significant portion of the sulphate in the water is precipitated as gypsum during this process, resulting in an effluent where the major ions in solution are Ca^{2+} and SO_4^{2-}. Further treatment of this gypsiferous mine water is possible, using techniques such as RO and this is being applied at several locations, albeit at significant cost and with the generation of large volumes of hypersaline brines that require further management.

Lime treated acid mine water is used for dust suppression on site, but substantial volumes remain in storage impoundments. The potential for using this partially treated water for agricultural purposes has been explored for over 30 years (Jovanovic *et al*., 1998), but concerns surrounding soil salinisation have confined this to research and pilot scale.

An early trial is described in the 1998 paper by Javonovic and co-workers, where a range of potential crop species was evaluated over a three year period (1993/94 to 1995/96). A total of 20 species, divided into four broad categories, were grown on small plots under three different irrigation regimes (wet, medium and dry). The study experienced some challenges associated with depth of root penetration and subsequent nutritional problems, but these were attributed to soil acidity and compaction, rather than salinity. Good results were achieved for irrigated soybean, millet, cowpeas and winter cereal crops. The authors speculated that if the subsoil quality challenges could be overcome that maize cultivation could be economically attractive.

Encouragingly, no evidence of foliar injury, which has been reported for crops irrigated with saline water high in sodium and chloride, was recorded. From a soil chemistry perspective, an increase in soil

salinity was observed in the plots irrigated with lime-treated mine water, but the levels were below that of gypsum saturated water. This suggested that a significant amount of salinity was removed through gypsum precipitation in the soil, which could be linked to high transpiration rates. For this reason, crops that maintain a large transpiring canopy for as long as possible are preferred.

Pasture grass, such as Tall Fescue (*Festuca arundinacea*) is often used in the initial stages of rehabilitating following pit closure. Pasture-fed beef has been reported to have preferential qualities over grain-feed beef, with respect to fatty acid composition and overall animal health. The possibility of using gypsiferous mine water to irrigate rehabilitated land covered in pasture grass for grazing is therefore an attractive option. The effect of saline water on grass seed germination was investigated as part of a Coaltech supported project (Truter *et al*., 2011). The study deviated somewhat from the approach used by most researchers, who rely almost exclusively on NaCl to prepare saline solutions, by using the more appropriate $CaSO_4$ to prepare solutions between 100 and 1 000 $mS.m^{-1}$. In addition, actual mine water with a salinity of 435 $mS.m^{-1}$ was tested. The study tested 21 species of grass using the International Seed Testing Authority guidelines.

The results showed a very diverse response, with Kikuyu (*Pennisetum clandestinum*) and *Lolium multiflorum* not showing significant effects. Germination efficiency remained stable above 90% across the entire range. Tall Fescue presented encouraging results, with germination efficiency remaining above 80% below a salinity of 500 $mS.m^{-1}$, after which it declined significantly as a function of increasing salinity. Almost half the species tested showed germination efficiencies below 50% when the mine water from the pilot site was used.

Four of the species most commonly used on rehabilitated mine sites were additionally tested using pot trials, rather than the ISTA procedure, using soil from the coal mine site. The germination % was significantly lower than that obtained using the standard protocol, with three of the species (*Cynodon dactylon, Eragrostis curvula* and *Festuca arundinacea*) achieving between 50 and 60% on the mine water, while the fourth (*Digiteria eriantha*) recorded less than 20%, even with distilled water (Truter *et al*., 2011).

In addition to germination efficiency the study continued to look at shoot to root ratio and total plant growth over an extended trial period. The four species were grown in seedling trays and watered daily with the appropriate salinity water for the first four weeks and twice per week thereafter. Plants were grown for six weeks before harvesting began. Once harvesting started three cells in the tray were harvested for analysis on each of five consecutive weeks. The results showed that shoot mass increased faster than root mass for all species, but could not be sustained unless there was a significant increase in root mass after week 8. Previous research (Dai *et al*., 2009) had indicated that high salinity reduces the efficiency of nutrient absorption so plants increased root mass as an adaptation strategy. Of the species tested in the trial Tall Fescue showed the greatest ability to adapt to increased salinity under saline stress and performed best when irrigated with gypsiferous mine water.

The research indicates that soil can act as an effective salt sink, through the precipitation of gypsum in particular. This may have additional benefits with respect to the provision of sulphur in a form that can be assimilated by plants. This is described in greater detail below.

1.4 SULPHUR

Sulphur makes up approximately 0.07% of the earth's crust and occurs in three primary forms, elemental sulphur, sulphides and evaporates. Elemental sulphur is found in many parts of the world, with the majority derived from sulphate reduction, although some deposits relating to volcanic sublimation exist. Elemental sulphur formed through the reaction between gypsum and hydrogen sulphide, with the resulting product typically trapped within limestone beds.

Sedimentary sulphide deposits, primarily the iron sulphides pyrite (FeS_2) and pyrrhotite ($Fe_{(1-x)}S$), are widespread and originated from the reduction of sulphate in seawater. While iron sulphides are most common, a number of other base metal sulphides occur. Several of these, such as the copper sulphides chalcocite, covellite and chalcopyrite, the lead sulphide galena, the nickel sulphide pentlandite and the mercury sulphide cinnabar are commercially exploited.

The third form, evaporates, are typically found in arid regions and originated from the evaporation of sweater. As the process progressed calcium carbonate was the first mineral to crystallise, followed by substantial quantities of calcium sulphate, initially as gypsum ($CaSO_4.2H_2O$) and then the more stable anhydrite ($CaSO_4$). When the seawater had been reduced to 10% of the original volume, halite (NaCl) crystallisation started. Finally, when the volume had been reduced to 1% of the original magnesium and potassium salts crystallised. Calcium sulphate is the most abundant of the evaporates, but sulphate salts of other minerals such as magnesium (Epsom salts), barium (barite), strontium (celestite) and sodium (glauberite) also exist in these systems.

1.4.1 Extraction and utilisation of sulphur minerals

Elemental sulphur, initially from volcanic deposits, has been mined for over 2 000 years, with archaeological evidence from the Middle East, Taiwan and Italy to support this. Sulphur played an important role in the overall industrialisation of Europe and as a key component of gunpowder played an important role in geopolitical history of the world.

The development of the Frasch process in 1894 revolutionised sulphur mining, allowing sulphur deposits in salt domes to be commercially exploited. Superheated water (330˚C) is injected into the formations through wells and highly pure molten sulphur is pumped to the surface. The Frasch process was the only economically viable method of sulphur extraction for almost a century, until the development of the Claus process. The last Frasch mine in the US closed in 2 000, but the process is still used in Poland and Mexico. The Claus process was first patented in 1883, but has been applied widely since the late 1970s as a method of desulphurising natural gas. Natural gas can contain up to 28% H_2S. The process occurs in two steps, the first being the oxidation of some hydrogen sulphide to sulphur dioxide, followed by the further reaction of sulphur dioxide with more hydrogen sulphide to form elemental sulphur. Today, the vast majority of elemental sulphur is produced in this way.

The United States is the primary producer of sulphur, accounting for almost 15% of the approximately 70 million tons produced in 2016. Other significant producers include China, Canada, Russia, Saudi Arabia, the UAE and Kazakhstan.

The global market analysis for sulphur shows that since 2014 supply has exceeded demand, with a projected excess of almost four million tons in 2017.

1.4.2 Applications of sulphur

The majority of elemental sulphur (>80%) is used in the production of sulphuric acid for industrial applications, primarily the processing of phosphate ores for fertiliser production and the leaching of base metal oxides and nickel laterites. Other important applications of sulphur-based chemicals are the production of carbon disulphide for cellophane and rayon manufacture, the vulcanisation of rubber, bleaching of paper and the preservation of fruit and fruit-based products (sulphites).

Gypsum is extensively used in the manufacture of Portland cement and as a component in agricultural fertiliser mixtures. The importance of sulphur as a fertiliser component and the relevance to this project will be discussed in greater detail later.

1.4.3 Biochemistry of sulphur

Sulphur is one of the key macronutrients required for life. It is minor constituent of fats, body fluids, and skeletal minerals. Sulphur is a key component in most proteins as it is found in the amino acids methionine and cysteine. Sulphur-sulphur interactions are important in determining protein tertiary structure.

1.4.3.1 Role of sulphur in the origin of life

The question of how life on earth originated has been the focus of much speculation and research, with the abiotic synthesis of important biomolecules an important stage in developing the hypothesis. Much of the research has focused on the synthesis of amino acids under a methane, hydrogen and ammonia atmosphere and the hypothesis that RNA catalysed the most important biochemical reactions, such as protein synthesis. More recently, the potential role of reduced sulphur has been explored (Domagal-Goldman *et al*., 2011).

The potential role of sulphur compounds was further highlighted with the discovery of deep-sea hydrothermal vents, specifically the potential of iron and nickel sulphides to catalyse the synthesis of keto acids, such as pyruvate and the assembly of complex amino acids (e.g. phenylalanine).

1.4.3.2 Sulphur biomolecules

During the evolution of living organisms in an anoxic environment sulphur, as hydrogen sulphide, could readily be incorporated into amino acids (cysteine and methionine) and then into proteins, with the thiolate (RS^-) or thioether groups acting as a ligand for transition metals important in functional metalloproteins.

The situation has become more complex for modern organisms, where most operate in an aerobic environment. Sulphur typically enters the cell in the form of sulphate and this has to be reduced internally before it can be incorporated into biomolecules. A summary of the scheme for the uptake of sulphate and subsequent assimilation is presented below (Figure 3).

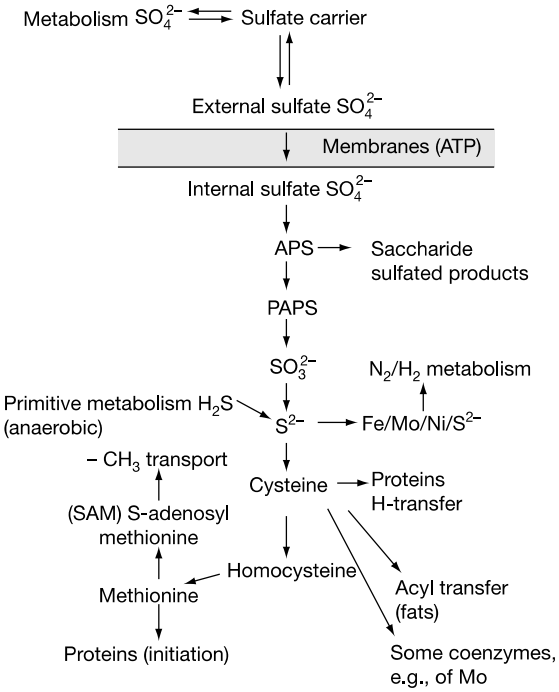

Figure 3: Uptake and incorporation of sulphate into cells (Frausto da Silva and Williams, 2001)

Glutathione is also involved in scavenging free radicals, which are capable of damaging nucleic acids. There is research indicating a correlation between aging rate and the concentration of glutathione in intracellular fluids.

The biochemical importance of sulphur compounds means that these molecules have a significant impact on the productivity and yield of many agriculturally important plant and animal species. The impacts of sulphur deficiency have been recorded for a number of cereal crops, rapeseed (Blake-Kalff *et al.,* 2001) and sheep, where low dietary sulphur intake significantly reduced wool growth (White *et al.,* 2001).

1.4.4 Sulphur in soils and agriculture

Sulphur exists in soils in both organic and inorganic forms, with the majority typically associated with organic compounds. Inorganic sulphur, as sulphate, is the form that is readily taken up by plants (Figure 4). It can exist in solution, adsorbed to solid particles or as insoluble salts such as gypsum.

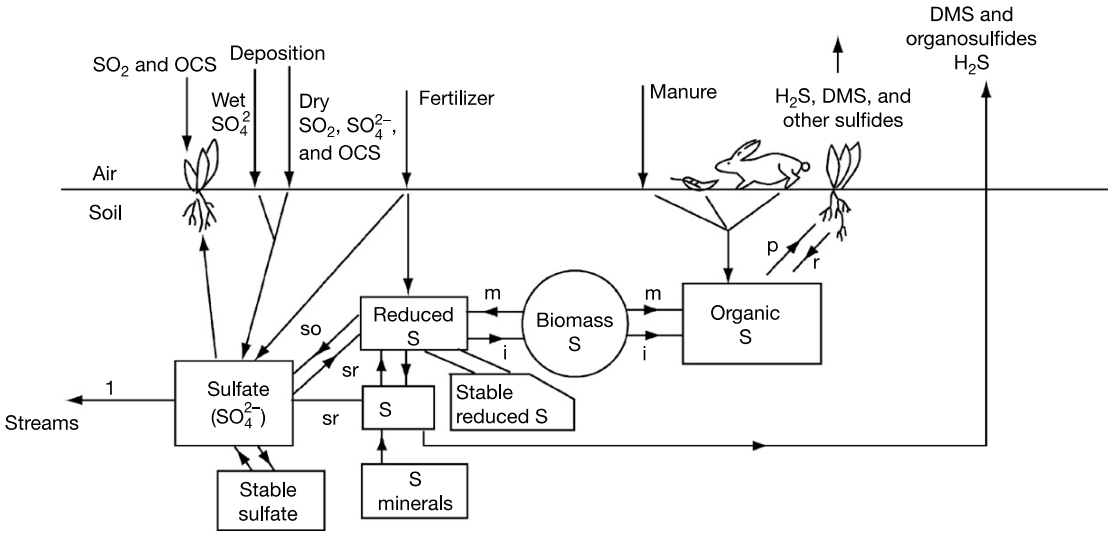

Figure 4: The sulphur cycle in the soil. Abbreviations: (i) immobilisation, (m) mineralisation, (p) plant uptake, (r) root exudation and turnover, (so) oxidation, (l) leaching and (sr) reduction (Havlin *et al.,* 1999)

Sulphate in the soil can be of atmospheric origin, primarily from reactions involving sulphur dioxide. Historically, volcanic activity was the main source of atmospheric SO_2, but since the industrial revolution the combustion of fossil fuels has contributed significantly to SO_2 emissions. A move toward renewable energy sources and improvements in emissions reduction technologies is reducing anthropogenic emissions, which are expected to peak around 2020 then decline significantly (Figure 5)

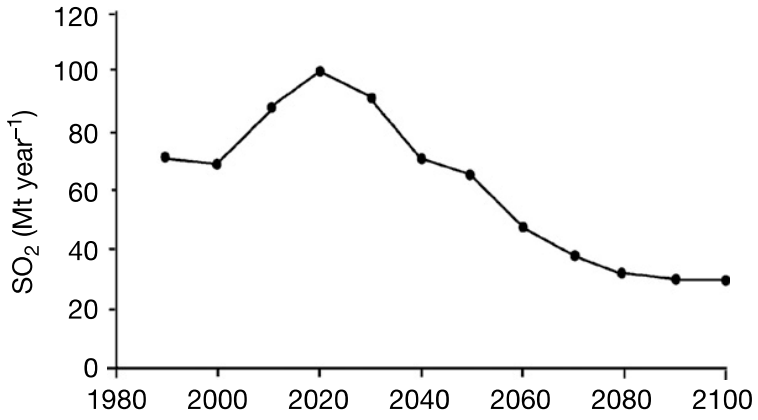

Figure 5: Predicted sulphur emissions from anthropogenic sources (http://sres.ciesin.org)

The dissolution of SO_2 is enhanced under alkaline conditions, so seawater represents an important sink. In addition, SO_2 can be converted to plant-available sulphate by dry-deposition, gas exchange across vegetative surfaces, or wet deposition through rain or other forms of precipitation. In addition to atmospheric sulphur, other sources of sulphate in soil include added fertiliser and biologically mineralised organic sulphur.

As mentioned above, the majority of sulphur in calcareous and non-calcareous soils is associated with organic compounds. The ratio of C/N/S is typically in the region of 120/10/1.4, with the N/S ratio usually between 6 and 8. Around 50% of the organic sulphur is present as esters or ethers (C-O-S linkages) and is classified as HI-reducible (hydroiodic acid). The second class, C-bonded sulphur is predominantly found in the amino acids cysteine and methionine. Finally, residual sulphur is associated with humic and fulvic acids, in both reduced and oxidised forms.

The sulphur retained in soils is subject to a range of biotic processes (Figure 4), resulting in continuous degradation and re-polymerisation. Mineralisation and immobilisation of S in soils is a dynamic process. The retention of sulphate in soils by adsorption depends on a number of factors, including pH, sulphate concentration, presence of other ions and the nature of the colloidal system. Sulphate adsorbs relatively weakly, so sulphate leaching, even from soils with a high capacity to retain sulphate, can be substantial. Sulphur can be leached as both sulphate or dissolved organic sulphur, but the latter accounts for less than 20% (Zhao and McGrath, 1994). Sulphur leaching rates depend on the factors listed above and studies using lysimeters and analysis of river catchments have estimated rates between 1 and 60 kg S per hectare (Erikson, 2009).

Over the last 10-15 years, changes in legislation and agricultural practices have significantly reduced the sulphur addition to soils, particularly in Europe. These include reductions in SO_2 emissions, the shift to low-S fertilisers, the low S return with manure and a reduction in the use of sulphur containing fungicides (Ercoli *et al*., 2012; Zhao *et al*., 2006). This has led to widespread sulphur deficiency.

A study by Bailey and co-workers (2001) used sulphur balance data from sites in Northern Ireland under grassland cultivation over a 50 year period (1940-1990) to assess changes in organic carbon storage. They used wet and dry sulphur deposition data as well as sulphur imported in fertilisers and animal feeds against sulphur export in agricultural products (beef, pork, mutton, poultry, milk, eggs, potatoes, apples and mushrooms) and leach data based on river monitoring. The results showed that the land had first acted as a sink and then a source of sulphur, with the reserves that were built up between 1940 and 1965 being totally depleted by the mid 1980s. They concluded that the negative sulphur budgets from the mid 1970s could be attributed to net mineralisation of soil organic matter. The study provided evidence that a decline in rainfall and sulphur fertiliser addition since the mid 1960s precipitated the breakdown in soil organic matter in order to unlock organic sulphur. Therefore, sulphur deficiency has a concomitant negative impact on organic carbon content.

1.4.4.1 Sulphur in South African soils

Historically, sulphur from the atmosphere, fertiliser and the soil sink have been sufficient to meet the demands for growing maize and other crops in South Africa. However, over recent years a shift from the use of sulphur containing fertilisers to highly concentrated NPK fertilisers, coupled with increasingly stringent restrictions on SO_2 emissions has created a sulphur deficit in places, consistent with earlier experiences in Europe.

A number of factors influence the rate at which sulphur is removed from agricultural soils. These include the physical nature of the soil, the organic content, patterns of rainfall and irrigation and the yield of crops cultivated in the soil. In the summer rainfall regions of South Africa, rain typically occurs as intensive thunderstorms, which can increase the rate of sulphur leaching from the soils (Van Biljon *et al*., 2004). Sulphur depletion is more likely to occur in sandy textures soils with a low organic carbon content as these soils tend to have a smaller organic sulphur pool as well as a reduced capacity to mineralise organic sulphur. In addition, well-draining sandy soils are more susceptible to sulphate leaching.

The sulphur status of agricultural soil is often used to predict the sulphur requirements ahead of developing a program for fertiliser addition. Typically, three indices are used to determine the sulphur status. Two of these, the sulphur concentration in plant tissue and the N:S ratio in the tissue are determined by analysing crops from the previous year, with the concentration of extractible sulphate the third component of the index. There has been some criticism of the methods, as well as some inconsistency with respect to the leach solution employed.

Prior to 1995, there had been no co-ordinated assessment on the threshold value for sulphur in soils in the maize producing regions of South Africa. A study by Van Biljon and associates (2004) was

performed to assess the threshold levels, below which active sulphur addition would be required to maintain yields. Their analysis determined a threshold level of approximately 9 mg S per kg of soil. The resulting database included over 140 000 samples and found that over 40% contained S levels below the threshold. Sulphur deficiency was most widespread in the western Free State and North West provinces, there 72 and 67% of the samples were shown to be deficient.

1.4.4.2 Consequences of sulphur deficient soils for agriculture

Sulphur deficiency affects crop yields and quality by decreasing the proportion of sulphur containing amino acids in the plant material. The lower concentrations of these amino acids reduce the nutritional quality of the crops and can have a negative impact on the properties of grains used for baking.

Sulphur deficiency symptoms typically resemble those of nitrogen deficiency, with the leaves become pale-yellow or light-green. Unlike nitrogen, sulphur-deficiency symptoms appear first on the younger leaves and persist even after nitrogen application. I n cotton, tobacco and citrus some of the older leaves are affected first.

Plants deficient in sulphur are small and spindly with short and slender stalks. Growth is typically retarded and maturity in cereals is delayed. In legumes, nodule formation may be negatively affected and nitrogen-fixation reduced. Forage crops contain an undesirably wide N:S ratio and thus have lower nutritive value.

Studies on the effect of sulphur addition on the growth and metabolism of sugar beet (Thomas *et al*., 2003) showed the addition of 25 kg per ha of sulphur to sulphur deficient soils resulted in a 25% increase together with a significant increase in root and shoot dry matter. In addition, quality was improved through a reduction in the alpha-amino nitrogen concentration. Application of additional sulphur to high S-status sites had no effect on growth and metabolism.

1.4.5 Sulphur containing fertilisers

There are more than 20 types of commercially available sulphur containing fertilisers, broadly divided into four categories. These are fertilisers containing sulphate, those containing elemental sulphur, a combination of the two and liquid sulphur fertilisers.

1.4.5.1 Sulphate containing fertilisers

Sulphate-containing fertilisers account for the majority of sulphur applied to agricultural land. These materials provide S primarily as a component of multi-nutrient fertilisers as sulphate, which is immediately available for plant uptake. The most readily available and popular sources are ammonium sulphate (AS), single superphosphate (SSP), potassium sulphate and potassium and magnesium sulphate. Ammonium sulphate is one of the oldest nitrogen and sulphur containing fertilisers and remains popular. Most of the AS is generated as a by-product from the production of caprolactam. Ammonium sulphate is readily soluble in water (744 g/l at 20˚C), so is highly susceptible to leaching. In addition, it is not suitable for acidic soils.

Gypsum is another sulphate containing mineral that has been used as a fertiliser. It contains around 13% sulphur as plant available sulphate. Historically, gypsum has been used in the production of ground nuts as the additional calcium is beneficial during seed pod formation.

1.4.5.2 Sulphur containing fertilisers

Elemental sulphur has been used as a soil conditioner to increase acidity and assist in the reclamation of sodic soils for a number of years, but is becoming increasingly popular as a source of sulphur for fertiliser.

To be effective the elemental sulphur needs to be oxidised to sulphate by sulphur oxidising soil microbes. The rate of oxidation depends on a number of factors, such as particle size, rate and method of application, microbial activity and environmental conditions. The application of fine powder below the surface enhances the rate of oxidation and when correctly applied to soil with an active microbial community sulphate availability can be similar to that achieved with direct sulphate addition. Repeated addition of sulphur fertilisers is likely to enrich the soil with sulphur oxidising species, increasing the effect.

More innovative uses of elemental sulphur include the addition of hydrophilic clay, such as bentonite, which absorbs soil moisture, increasing the rate at which the granules dissolve to release the fine grained sulphur. A second innovation is the production of sulphur coated urea (SCU), where sulpur makes up approximately 20% of the mass.

Several researchers have investigated the potential of using particular species of fungi and bacteria to enhance the rate of nutrient release. Ali and Wainwright (1995) noted that the fungus *Phanerochaete chrysosporium* enhance the rate of sulphate and ammonium release from sulphur coated urea granules. More recent research has aimed at seeding sulphur coated urea with bacteria from the genera *Thiobacillus* and *Acidithiobacillus*. These systems require the inoculum to be grown on site and need to overcome the challenges associated with the hydrophobicity of elemental sulphur to ensure effective coating of the urea.

The biosulphur produced in the floating biofilm of the system being piloted in this project overcomes both these challenges, by generating a product that is less hydrophobic, as a result of the organic carbon and is pre-seeded with active sulphur oxidising organisms.

1.4.5.3 Sulphur management is agricultural soils

Optimising sulphur uptake and reducing sulphur leaching are important goals for agricultural management. These can be achieved in number of ways, including synchronising the addition of sulphur to the periods of highest uptake and reducing the mineralisation of organic sulphur during periods of low plant uptake.

Recent research (Fox *et al.*, 2016) on the addition of biochar to soils used to cultivate barley found that in addition to significantly increasing the yields the abundance of bacterial species responsible for phosphorous cycling (*Brevundimonas* sp) and desulfurising sulfonates (*Arthrobacter* sp and *Cupriavadis* sp) increase over 100 fold. Augmentation with 1 and 2% biochar resulted in a 5-fold increase in seed numbers per head and a 40% increase in individual seed mass.

Bioaugmentation, with or without sulphur addition, has been shown to have significant benefits on soil quality and crop performance as a result of improved mobilisation of sulphur, phosphorous and other nutrients. A study by Mohamed and co-workers (2014) investigated the effect of dual inoculation with mycorrhizal fungi and selected strains of *Thiobacilli* on the growth of maize and onions in sandy soils. The results showed that dual inoculation resulted in significant increases in the amount of N, P, K and S in the plant rhizosphere after 60 and 90 days. In addition, the acidity generated as a result of sulphur oxidation increased the mobility of other key nutrients, such as Mg, Al, Mn and K.

1.5 PROJECT AIMS

The aims addressed in the project were as follows:

1. To operate a semi-passive plant for the remediation of ARD for a period of two years.
2. To characterise the resulting water quality from the primary integrated system. If additional polishing is required to assess the efficiency of a constructed wetland to achieve this.
3. To evaluate the potential of using the treated water for agricultural purposes − evaluate the risk of accumulation of toxic components.
4. To evaluate the potential to use the sulphur product from the integrated process as a fertiliser component.

CHAPTER 2: MATERIALS AND METHODS

2.1 INTRODUCTION

This chapter presents an overview of the microbial cultures used in this research, a description of the individual reactor units and the detail of the routine analyses performed. The specific experimental programme relating to each of the sets of experiments is described in detail in the relevant chapters.

2.2 MICROBIAL CULTURES

2.2.1 Sulphate reducing bacteria (SRB) stock culture

The mixed SRB 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. Previously, the stock culture has been used to generate cultures adapted to ethanol and acetate.

2.2.2 Sulphide oxidising bacteria (SOB) culture

The SOB culture was obtained from previous studies (Van Hille and Mooruth, 2013) on sulphide oxidation conducted within CeBER. The culture was initially selected for by feeding a channel reactor with overflow from a laboratory-scale sulphate reducing column, packed with lignocellulosic material and inoculated with a mixture of the SRB culture described above, rumen fluid obtained from an abattoir and the indigenous community associated with the material used to pack the column (wood chips, grass, primary sewage sludge and leaf mulch). The two cultures were mixed together to inoculate the reactor.

2.3 REACTOR UNITS

2.3.1 Laboratory scale hybrid linear flow channel reactors

2.3.1.1 2 l reactors

The standard hybrid LFCR, with an operating volume of 2 l, developed for the original proof of concept study was used for some of the laboratory work in this project (Figure 6). It was constructed from Perspex (11 mm thickness) and had internal dimensions of 250 mm (l) x 10 mm (w) x 15 mm (h). The front facing side of the reactor was fitted with six sampling ports, allowing the bulk reactor volume to be monitored across the length and at different heights. The reactor design was based on the original 25 l LFCR described by (Mooruth, 2013). The hybrid LFCR was fitted with a plastic strip (10 mm wide) holding carbon microfibers as a microbial support matrix and a heat exchanger (4 mm ID) for temperature control. A harvesting screen, made of plastic mesh fixed to an aluminium frame, was designed to lie 5 mm below the liquid surface to facilitate biofilm harvesting. Reactor feed was pumped in continuously through the uppermost inlet port on the left side of the reactor while the effluent flowed from the equivalent exit port on the right side of the reactor.

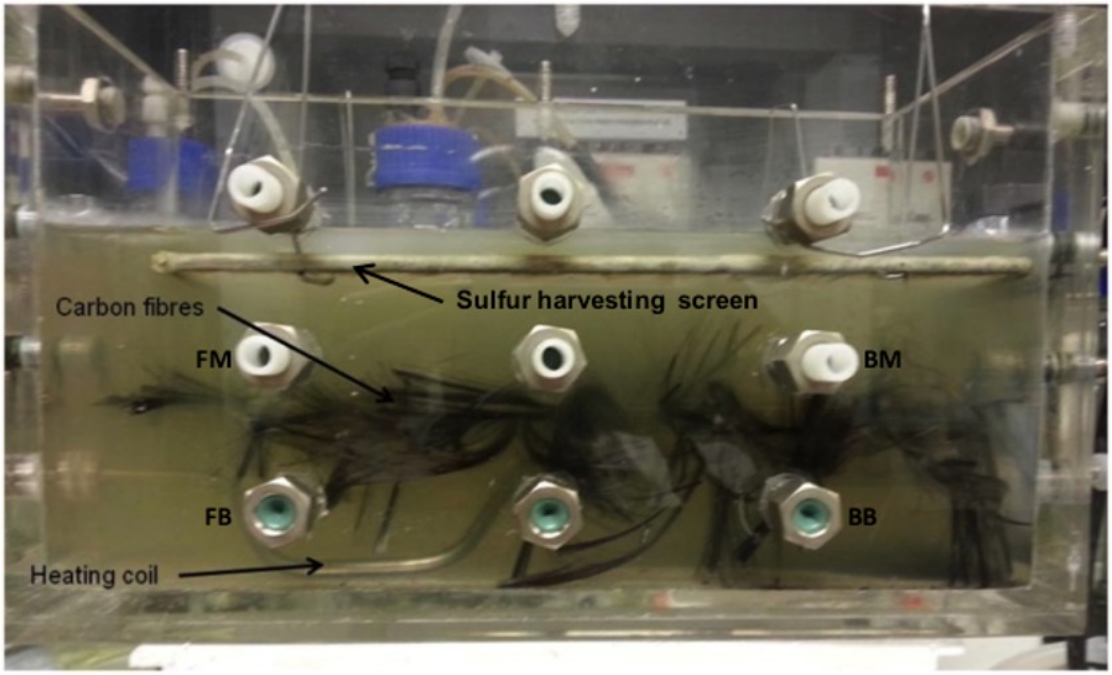

Figure 6: Photograph of the hybrid linear flow channel reactor showing the position of the heating coil, carbon fibres, sulphur harvesting screen and the location of the sampling ports (FM, FB, BM, and BB)

2.3.1.2 8 l reactors

The ultimate intention of the research was to evaluate the system at pilot scale, with a 1 000-fold increase in reactor volume. Initially, the dimensions of the pilot scale LFCR were based on scaling up the original hybrid LFCR. However, following the decision to construct the pilot scale LFCRs out of a transparent material and the constraints this imposed, it was decided to construct slightly larger laboratory scale reactors with relative dimensions similar to the pilot scale units. The reactors were constructed from Plexiglass (10 mm), with internal dimensions of 450 mm (l) x 200 mm (w) x 150 mm (h). Operating at liquid height of 100 mm the working volume would be approximately 8 l. The location of the sample ports is shown in Figure 7.

The left and right-side walls each contained three tapped (1/8" BSP) holes (Figure 8). The uppermost holes were used as the feed and effluent ports, while the middle and lower holes were used as attachment points for rod supporting the carbon fibres and the inlet and outlet points for the heat exchanger respectively.

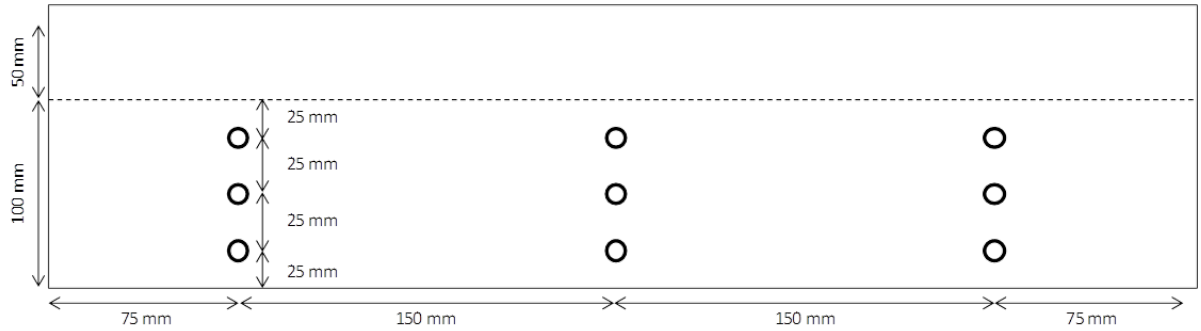

Figure 7: Schematic diagram of the new LFCR showing positions of the sample ports in the front wall.

The sulphur harvesting screen was help in position, just below the liquid level, using wire hooks (Figure 9).

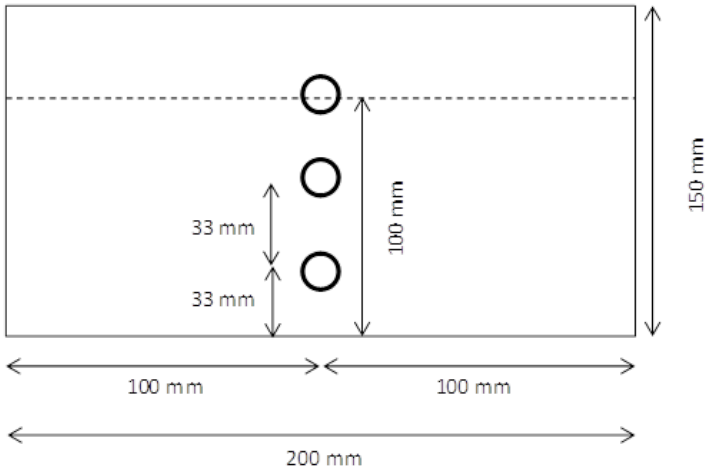

Figure 8: Schematic representation of the left and right side walls of the new LFCR showing location of feed/effluent port as well as the positioning of the carbon fibre support rod and heat exchange pipe.

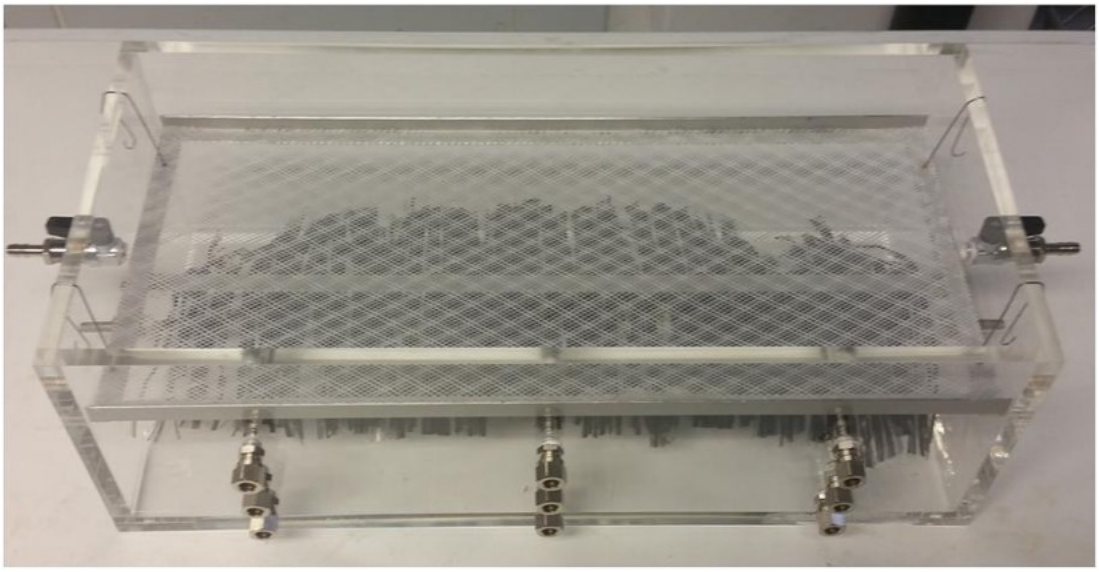

Figure 9: Photograph of the new LFCR prior to inoculation

2.3.2 Pilot scale hybrid linear flow channel reactors

The pilot scale channel reactors were constructed of Plexiglass (15 mm thickness). The maximum dimensions of standard Plexiglass sheets is 3 050 mm x 2 050 mm, which constrained the length of the channel to an internal length of 3 000 mm. Longer sheeting required specialised fabrication, which would significantly increase the cost. To maintain the desired volume of 2 025 l, at a working depth on 500 mm, the width needed to be increased from 1 230 mm in the original design to 1 350 mm. A schematic diagram showing the overall dimensions is presented in Figure 10.

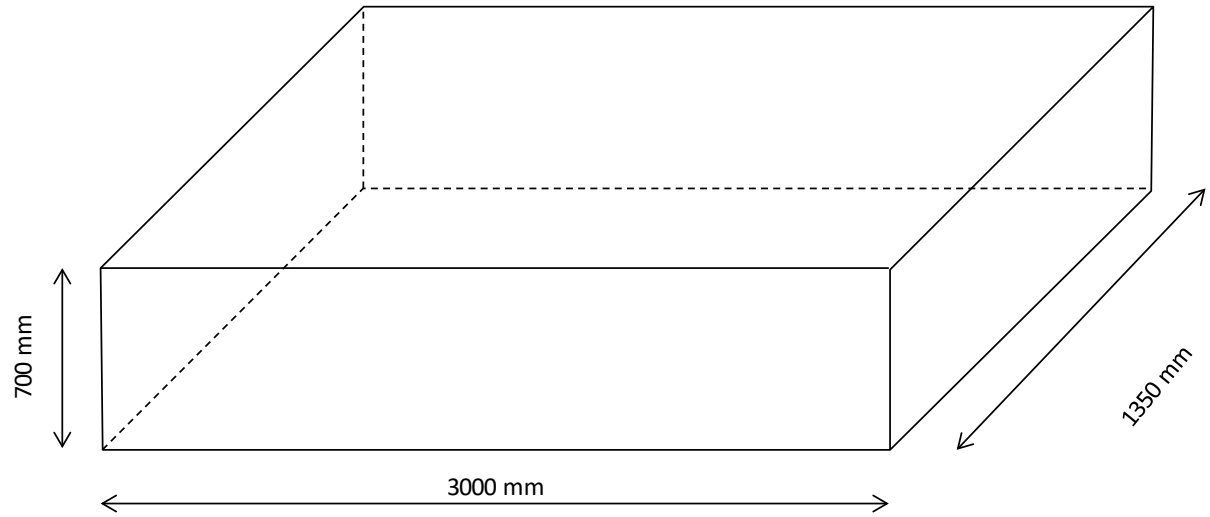

Figure 10: Overall dimensions of the channel reactor. Measurements reflect internal values

The advantages of constructing the reactors from Plexiglass include that the reactor internals (carbon fibres and harvesting screen) will be clearly visible and it will be possible to include sampling ports similar to those in the laboratory scale reactor. The front wall of the reactor was fitted with 15 potential sampling points (Figure 11).

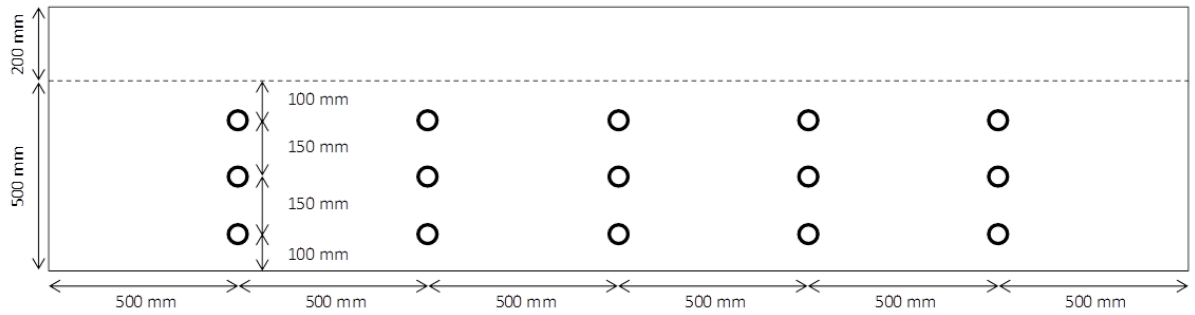

Figure 11: Schematic representation of the front wall of the pilot scale reactor showing the location of sample ports

The left and right side walls contained a single port (30 mm diameter) for fresh feed and effluent outflow (Figure 12).

The reactor contained three parallel beams, manufactured from two pieces of aluminium angle between which the carbon fibres were held. The fibres extended approximately 200 mm from each side of the beam. The beams rested on a 10 mm wide Plexiglass ledge fixed on the inside of the left and right walls at a height of 240 mm above the base. The sulphur harvesting screen was constructed of aluminium square tubing which created a frame that held the same plastic mesh as shown in the laboratory scale reactor (Figure 9).

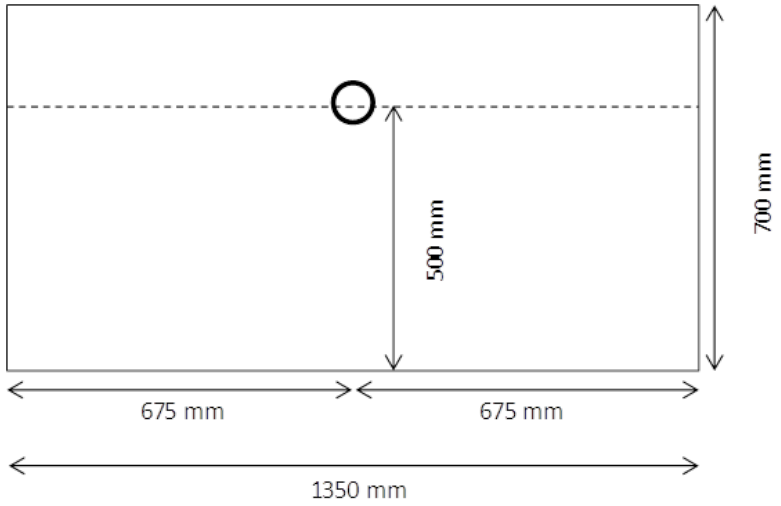

Figure 12: Schematic representation of the left and right walls of the reactor showing the location of the feed/effluent port

The constructed pilot scale LFCR, fitted with the carbon fibre beams and harvesting screen and loaded into the support stand at the pilot site is shown in Figure 13. Each of the three reactors is supported by a custom designed and constructed stand to provide additional structural support when the reactors are filled. The stand height varies from 120 to 60 cm, to the base of the reactor, to support gravity flow between the reactors connected in series.

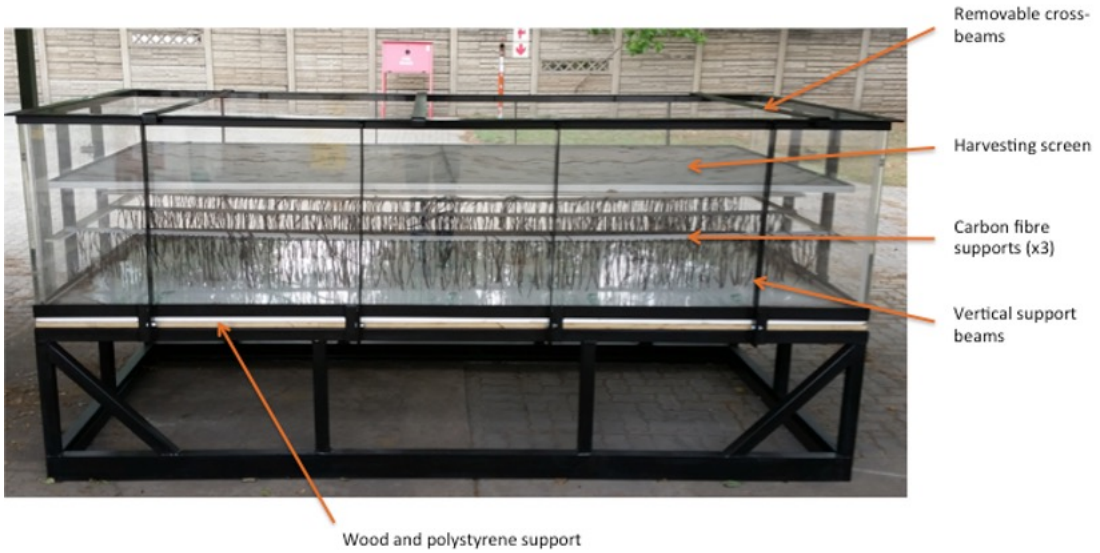

Figure 13: Photograph showing a single hybrid reactor unit and steel support frame

2.4 ANALYTICAL METHODS

2.4.1 pH and redox potential

The solution pH was measured using a Cyberscan 2500 micro pH meter, fitted with an XS microprobe (6 mm). The meter was calibrated daily using pH 4.0 and pH 7.0 buffers. Redox potential was measured using a Metrohm pH lab 827 redox meter.

2.4.2 Sulphide

Aqueous sulphide was quantified using the colorimetric DMPD method (APHA, 2005). The principle of the method is reaction of aqueous sulphide with N,N-dimethyl-p-phenylenediamine (DMPD), catalysed by ferric ions, to produce methylene blue. An appropriate volume of sample (10-4 800 µl) is added to 200 µl of 1% zinc acetate. The volume is made up to 5 ml with deoxygenated water, after which 500 µl of 0.4% N,N-dimethyl-p-phenylene diamine (in 6 M HCl) and 500 µl of 1.6% ferric chloride (in 6 M HCl) are added. The sample is mixed well and left to react for a minimum of 5 minutes after which the absorbance is read at 670 nm and the concentration determined relative to a standard curve. The assay has a maximum detection limit of just over 1 mg/l so significant dilution is required. This is typically achieved by using a small volume (20-50 µl) of sample.

2.4.3 Sulphate

Dissolved sulphate concentrations were measured using the barium sulphate method. Samples (2 ml) were centrifuged at 14 000 x *g* for 5 minutes to remove particulate matter. An appropriate volume of supernatant was diluted with deionised water to a final volume of 5 ml, to which 0.25 ml of conditioning reagent (50 ml glycerol, 30 ml 32% HCl, 100 ml absolute ethanol, 75 g NaCl, 300 ml deionised water) was added. A volume (10 µl) of saturated $BaCl_2$ solution was added and the contents of the tube were mixed by vortexing for 15 seconds. The barium reacts with any sulphate present in the sample to produce insoluble $BaSO_4$. The turbidity resulting from the $BaSO_4$ precipitate was measured at 420 nm using a Shimadzu UV spectrophotometer and quantified against a standard curve (0-100 mg/l).

2.4.4 Anions by ion chromatography

Anion (fluoride, chloride, nitrate, sulphate, thiosulphate and phosphate) concentrations was determined by ion chromatography on a Thermo Scientific DIONEX ICS-1600 system equipped with an IonPac AG16 anion column, a 10 µl injection loop and a conductivity detector with suppression. A 22 mM NaOH solution was used as the mobile phase at a flow rate of 1 ml/min. Standard solutions of the respective sodium salts were used to prepare mixed ion standards (100-500 mg/l) and fresh standard curves were generated for each analytical run. Data were analysed using the Chromeleon software package.

2.4.5 Soluble, solid and total COD

All COD measurements were carried out using the Merck reagent test protocol for high (1500-1 0000 mg/l) and low (10-150 mg/l) concentrations. The method is based on the oxidation of the sample with a hot sulphuric acid solution containing potassium dichromate, with silver sulphate as the catalyst. The chloride is masked with mercury sulphate. The concentration of unconsumed yellow $Cr_2O_7^{2-}$ ions or green Cr_3^{+} ions is then determined photometrically and used to quantify oxygen demand. The reactions were performed in glass COD tubes. For the high concentration assay, 2.2 ml of COD reagent A and 1.8 ml of COD reagent B were added to the tube. The sample (1 ml) was added to the reagents in the tube and the contents mixed using a vortex mixer. A blank was prepared using 1 ml of deionised water instead of the sample. For the low concentration range 0.3 ml of reagent A, 2.3 ml of reagent B and 3 ml of sample were used. The tubes were heated at 150°C for 120 minutes in a heating block, then allowed to cool to room temperature. The absorbance was measured using a spectrophotometer at 610 nm. To quantify the COD concentrations, standard solutions were prepared using potassium hydrogen phthalate and data used to prepare standard curves.

2.4.6 Volatile fatty acids (VFAs)

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 present in all digesters over the duration of digestion. 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 M H_2SO_4 at a flow rate of 0.6 ml/min. The pressure in the column did not exceed 2 000 psi. 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.

CHAPTER 3: EVALUATION OF THE SYSTEM AT PILOT SCALE

3.1 INTRODUCTION

A number of potential pilot sites were investigated in collaboration with Anglo Coal, the industry partner in the research, with a colliery in the Free State selected. The mine produced high sulphate, circum-neutral that most closely resembled the synthetic media used in the preceding laboratory scale research.

The initial intention was to utilise an abandoned wastewater treatment facility on the mine property and modify the existing concrete channels to create the hybrid reactors. However, issues relating to access, security and infrastructure to house the field laboratory resulted in the site being deemed unsuitable. As a consequence, the hybrid reactor units were redesigned, resulting in the 2 000 l Plexiglass reactors. These allowed for observation of the bulk volume and the individual units could be moved, if required.

The reactors were located at the Environmental Offices site, outside the boundary of the working mine, for ease of access. Mine water from the pit was delivered to the site via a 4 km pipeline and was stored in four 10 000 l tanks on site.

3.2 BUILD UP OF SULPHATE REDUCING BACTERIA CULTURE

The sulphate reducing culture originated from laboratory stock cultures maintained in the Centre for Bioprocess Engineering Research (CeBER) at the University of Cape Town. The stock culture was initially maintained on modified Postgate B medium, but was adapted to sodium acetate, rather than lactate, once the inoculm scale up started.

Inoculum build-up was started at laboratory scale in a series of 10 and 15 l glass containers, maintained in batch mode on an acetate based medium with an initial sulphate concentration of 3 500 mg/l. The sulphide concentration was monitored regularly as a measure of SRB activity. The final analysis prior to shipping revealed sulphide concentrations between 680 and 1 170 mg/l. Stock cultures from these reactors were blended into four 25 l plastic drums, such that each drum had a sulphide concentration of approximately 400 mg/l. These drums were transported to the mine site.

The 25 l drums were sampled again at the pilot site, approximately one month after they were packed in Cape Town and revealed additional activity in two of the four drums. The sulphide concentration in these drums increased to 790 and 1 012 mg/l respectively, while the remaining two remained near 400 mg/l. Three of the 25 l drums were used to inoculate two 210 l drums containing raw mine water. The first drum (Inoc 1) was inoculated from 25 l drums 1 and 4, while the second (Inoc 2) was inoculated with 25 l from drum 2. The final drum was maintained as an on-site backup culture. Each 210 l drum was supplemented with 500 g of sodium acetate and 100 g of yeast extract.

The mine water was pumped from the pit to a series of holding tanks on the perimeter of the test site and could be gravity fed into the pilot reactors. The initial analysis of the mine water showed it was alkaline (pH 8.24), with significant dissolved components (EC 7 095 µS/cm) and a sulphate concentration of 3 889 mg/l. Due to the high pH the concentration of iron was negligible so metal precipitation in the reactor was expected to be minimal.

The SRB cultures were monitored by the MSc student during the weekly site visits. The first drum showed limited activity in the month preceding the inoculation of the channel, with no significant change in sulphate or sulphide concentration (Figure 14). The second drum was substantially more active,

showing almost complete sulphate reduction over the four-week period. The sulphide concentration increased from 256 mg/l at inoculation to 741 mg/l.

Despite the lack of activity in one of the drums the decision was taken to inoculate the channel reactor as planned.

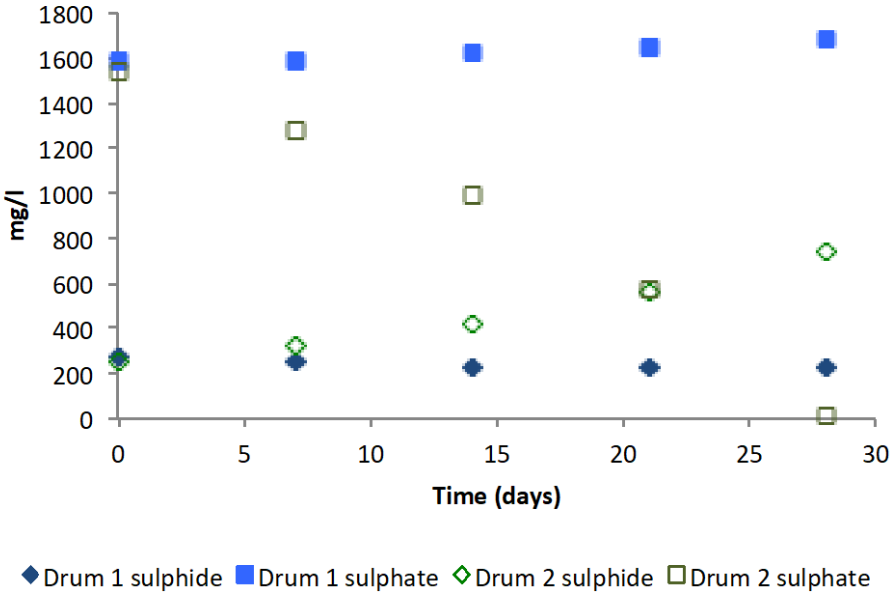

Figure 14: Sulphide and sulphate concentrations measured in the 210 l drums preceding the inoculation of the 2 000 l channel

3.3 EVALUATION OF THE PILOT SCALE CHANNEL REACTOR

The first channel was inoculated by blending raw mine water with tap water from site to reduce the sulphate concentration to approximately 2 000 mg/l. The reactor was set up to use acetate as the carbon source and electron donor, at a COD:SO_4^{2-} ratio of 0.7. Therefore, for 2 g/l sulphate an acetate concentration of 1.3 g/l was required as 1 g acetate is equivalent to 1.07 g COD. A blend of sodium acetate (1 kg), acetic acid (870 ml) and ammonium acetate (1.452 kg) was dissolved in water and added to the channel. The ammonium acetate provided 50% of the nitrogen provided by the modified Postgate medium that was used in the build-up of the inoculum.

Once the reactor was filled to close to the level control port 140 l of inoculum from each of the drums was pumped into the channel near the bottom. Based on the sulphide concentration in the two inoculation drums the initial sulphide concentration in the 2 000 l reactor was calculated to be approximately 68 mg/l. This was significantly lower than the 150 mg/l that was originally planned for. The drums were topped up with fresh media to provide inoculum for subsequent experiments.

The reactor was sampled from six of the nine sampling points four hours after inoculation. Based on the hydrodynamic studies performed on the 8 l channel reactors this should have been sufficient for the contents to have mixed fully by diffusion. The first set of data points is presented in Table 1 and shows that there had been almost complete mixing within the reactor, although the sulphide concentrations were lower near the bottom of the reactor.

Table 1: Parameters measured from six of the nine sampling points 4 h after inoculation. F refers to first set of sampling ports, closest to inlet, and B to the third set of ports

	Sample port					
Parameter	F top	F middle	F bottom	B top	B middle	B bottom
T˚C	22.7	21.8	20.3	22.7	21.7	20.2
pH	7.81	7.80	7.80	7.81	7.79	7.77
HS^- (mg/l)	62.1	67.4	53.2	74.0	73.7	52.0
SO_4^{2-} (mg/l)	1720	2513	2240	2220	2173	2260

The site was visited again six days after the inoculation. The channel was covered by a thin biofilm that was almost complete (Figure 15), although there were small areas where there was no barrier to oxygen penetration.

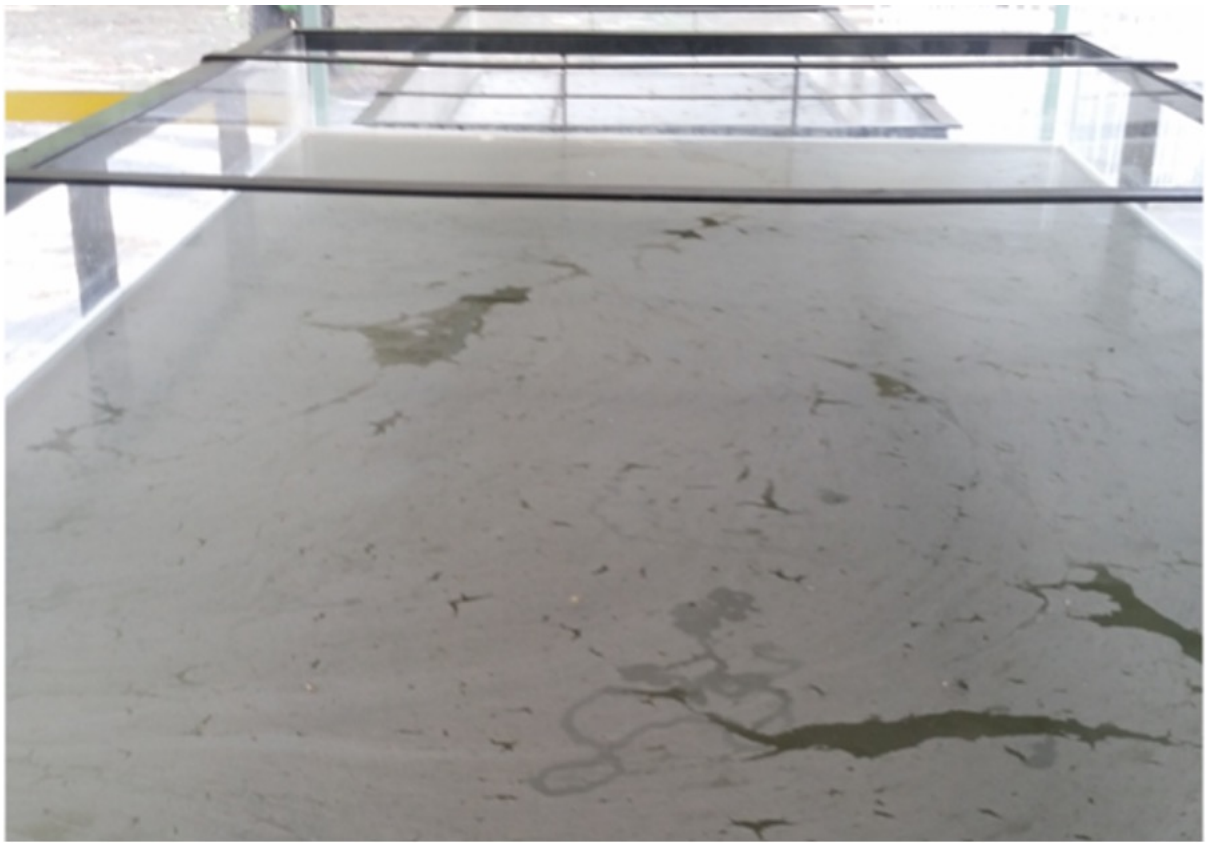

Figure 15: Image showing partial biofilm formation in the reactor six days after inoculation

The reactor was sampled from the same six ports as before and the results are presented in Table 2. The pH in the channel had not changed significantly, with only a slight increase. By contrast, sulphide concentration had decreased by close to 85%, with a mean concentration of just over 10 mg/l across the different ports. The sulphate concentration has also decreased and there were significant differences in the sulphate concentration in different parts on the reactor, with substantially higher sulphate concentrations measured in the samples from the lowest of the ports.

The mean sulphate concentration had decreased from 2188 mg/l to 1669 mg/l, which represented an average volumetric sulphate reduction rate (VSRR) of 86 mg/l.d, which is a fraction of the rates achieved in the laboratory studies.

Table 2: Parameters measured from six of the nine sampling points six days after inoculation. F refers to first set of sampling ports, closest to inlet, and B to the third set of ports

	Sample port					
Parameter	F top	F middle	F bottom	B top	B middle	B bottom
T˚C	28.2	27.4	26.9	28.3	27.5	26.9
pH	7.83	7.89	7.88	7.85	7.89	7.87
HS^- (mg/l)	11.3	12.2	14.9	9.5	9.8	13.4
SO_4^{2-} (mg/l)	1493	1487	1753	1653	1700	1967

The performance of the reactor had deteriorated further by the time it was sampled again seven days later. Rather than getting thicker the biofilm now covered less than 50% of the reactor surface. This was attributed to fragmentation of the incomplete biofilm and some complete oxidation of the elemental sulphur back to sulphate. The soluble sulphide concentrations had decreased to almost zero across the entire reactor volume and there was evidence of algal growth on the walls of the reactor.

The most likely cause of the system failure was that the sulphide concentration in the inoculum was not high enough. The biofilm observed after six days indicated that the culture did contain both the heterotrophs and sulphide oxidisers required for biofilm formation. However, there was not enough sulphide to ensure that the biofilm became thick enough to effectively exclude oxygen and mop up any additional oxygen that entered the reactor, with the consequence that the bulk liquid could not be kept anaerobic. This led to inhibition of the sulphate reducing community, which limited additional sulphide generation. Ultimately the reactor became favourable for colonisation by algae that introduced additional oxygen. The experiment was then terminated and the focus shifted to generating a greater volume of sulphide rich inoculum.

Shortly after the termination of the first channel experiment the mining operation cut through an aquifer that caused the pit to be flooded with fresh water. This resulted in a dramatic improvement in the quality of the water provided to the pilot site. The sulphate concentration fell to 284 mg/l, which was too low to use as a reactor feed.

In an attempt to overcome the challenge sodium sulphate salt was sourced from the freeze crystallisation plant at the mine and used to supplement the pit water. Tests on the salt showed that there had been additional absorption of water into the crystals, so it was calculated that 6.7 g of salt would needed to be added per litre to increase the sulphate concentration of the mine water to the desired 2 000 mg/l.

Initial data suggested that the salt was in some way inhibitory to the SRB, possibly as a result of the presence of anti-scaling additives. However, after a period of adaptation sulphate reducing activity recovered.

The sulphate and sulphide concentrations in the 210 l drums were monitored for a number of weeks to confirm active sulphate reduction. When the sulphide concentration in the 210 l drums exceeded 600 mg/l, 75% of the volume from each drum was used to inoculate a 1 000 l JoJo tank that contained 700 l of mine water, supplemented with sodium sulphate to achieve a sulphate concentration of 2 000 mg/l. The three stages of inoculum scale-up, from 25 l to 1 000 l are shown in Figure 16.

Figure 16: Image showing stages of SRB culture build-up, from 25 l drums (back) to 210 l drums (front) and ultimately 1 000 l tank (green tank)

The 1 000 l tank was sampled weekly by withdrawing 5 ml from just below the surface, using a 5 ml autopipette. The sulphide concentration was measured immediately, after which the pH and sulphate were determined. A 2 ml fraction was centrifuged at 14 000 rpm to remove any solids and the supernatant used to determine the soluble COD. The data for the six-week monitoring period are summarised in Figure 17.

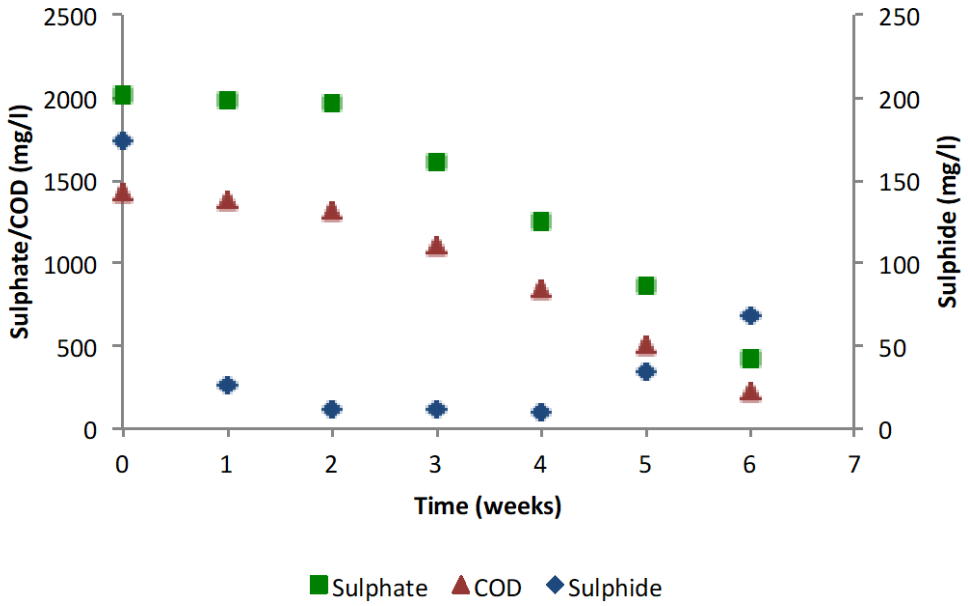

Figure 17: Sulphate, sulphide and COD concentrations measured in the 1 000 l tank during the SRB culture scale-up

The pH in the reactor stayed relatively constant, between pH 8.21 and 8.25 for the duration of the experiment. There was no significant sulphate reduction during the first two weeks, although the soluble

COD did decrease marginally. It is possible that the culture needed to adapt to the increased sodium concentration, due to the supplementation of the mine water with sodium sulphate.

The sulphide concentration did decrease substantially over the first seven days, most likely due to partial and complete oxidation. A biofilm developed over the liquid surface during the first week, which was evidence of the presence of sulphur oxidising species.

Active sulphate reduction was observed after two weeks and proceeded at a relatively constant rate of approximately 56 mg/l.d. The ratio of COD consumed per sulphate reduced ranged between 0.61 and 0.84, which is close to the theoretical yield of 0.7 for acetate as a substrate, indicating that the majority of the COD reduction could be attributed to sulphate reduction. Despite the evidence of sulphate reduction, the measured sulphide concentration did not increase between week 2 and 4 and only marginally for the following two weeks.

Previous work at laboratory scale showed that vertical stratification of sulphide occurred in the LFCR due to lack of active mixing and small differences in density. A sample was taken from the tap at the base of the tank on week six and this showed a sulphide concentration of 1 345 mg/l, suggesting that stratification had occurred in the tank. This was confirmed by taking samples at different depths using a piece of tubing connected to a 50 ml syringe. At this point there was sufficient sulphide in the tank to re-inoculate the channel.

The channel was re-inoculated at the beginning of May, using almost the entire volume of the 1 000 l tank. The volume was made up to 2 000 l using low sulphate mine water that had been supplemented with sodium sulphate, sodium acetate, acetic acid and ammonium acetate, using the same recipe as before. Samples were taken from six of the nine sampling ports every second day for a period of 8 days and analysed for pH, sulphate, sulphide, soluble COD and temperature. The pH data are presented in Figure 18 and show that there was little change in the pH, due to the buffering capacity of the inoculum. There was a small difference in pH with depth, with marginally higher values recorded at the top sampling point. This is most likely due to the release of additional alkalinity associated with the partial oxidation of sulphide. The sulphide data are presented in Figure 19.

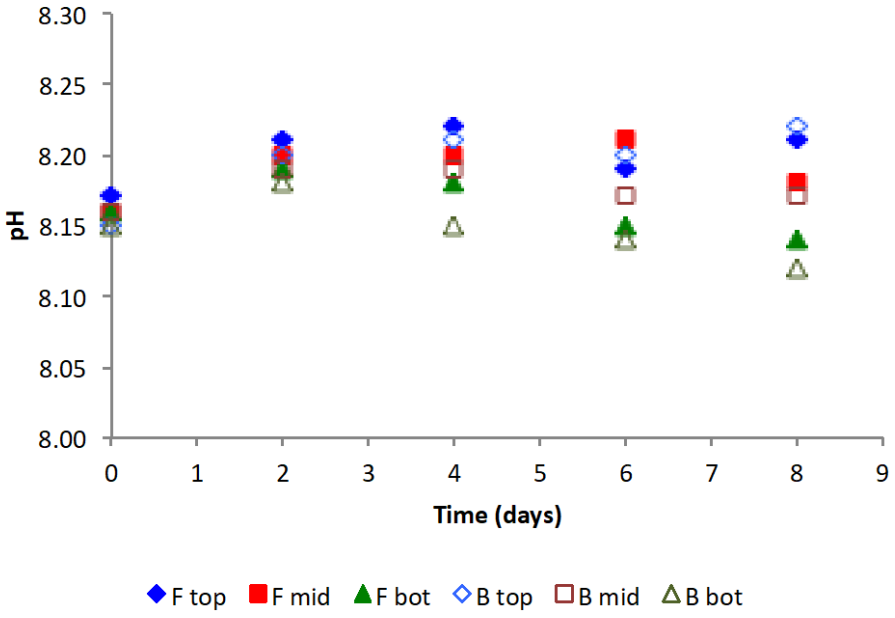

Figure 18: pH measured from six of the nine sampling points during the second channel reactor test

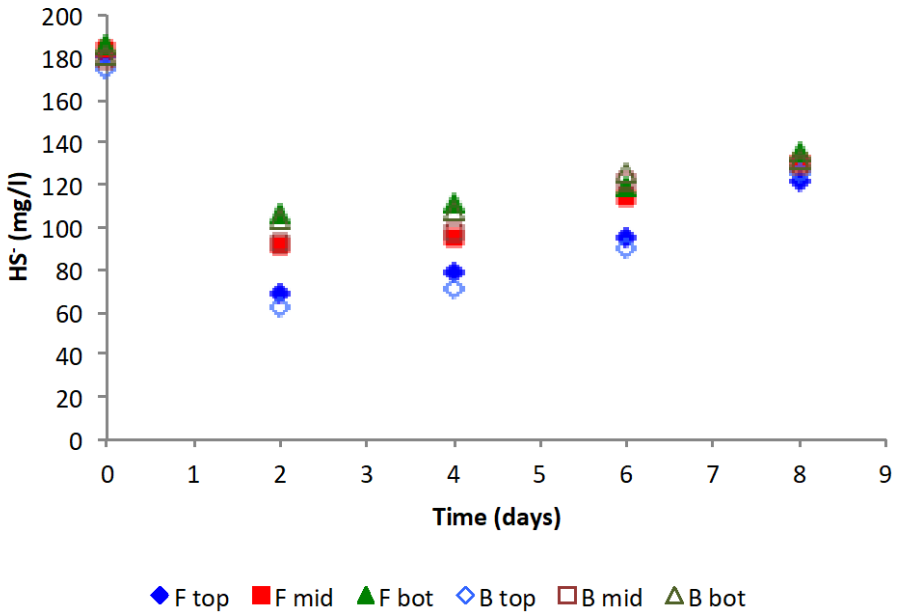

Figure 19: Sulphide measured from six of the nine sampling points during the second channel reactor test

Shortly after inoculation the sulphide concentration in the bulk liquid was approximately 180 mg/l. This fell substantially over the first 48 hours, initially due to oxidation as a result of oxygen mass transfer into the bulk liquid and then due to the conversion to elemental sulphur within the floating biofilm.

Significant stratification was observed during the first six days, with higher sulphide concentrations in the lower part of the reactor due to the lack of active mixing. Once the biofilm had formed and active sulphate reduction started the differences in sulphide between the upper and lower portions of the reactor decreased. In addition, the measured sulphide concentrations increased after day four, indicating that the rate of sulphate reduction in the bulk liquid began to exceed the rate of sulphide oxidation in the biofilm. This is similar to what was observed in the laboratory scale reactors.

The aqueous sulphate data are shown in Figure 20 and indicate that there was period of about four days during which sulphate reduction was limited. This may have been an acclimation period after the inoculation or the fact that before a complete biofilm had formed there would have been more complete oxidation of sulphide to sulphate, which would have masked some of the sulphate reduction.

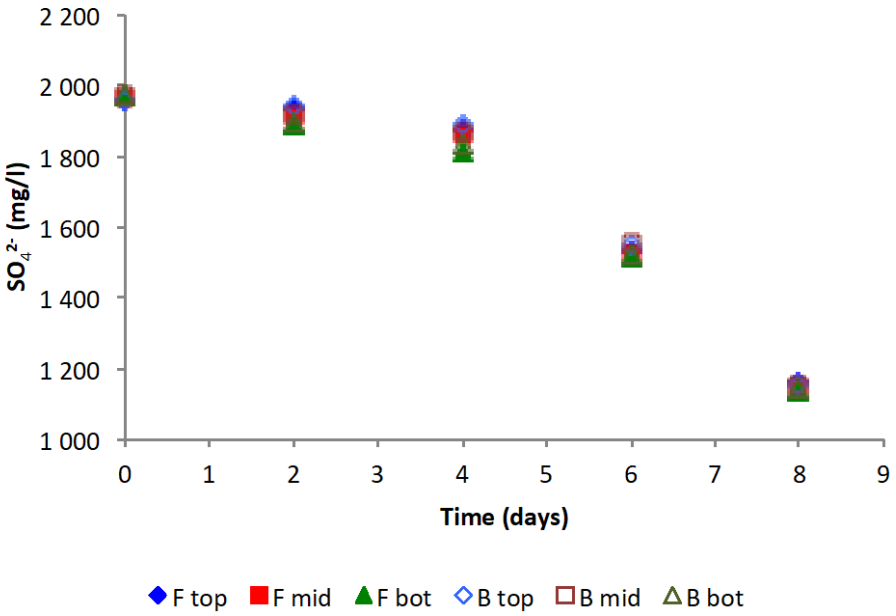

Figure 20: Sulphate measured from six of the nine sampling points during the second channel reactor test

As with the sulphide data, there was some vertical stratification of the sulphate during the first four days, with higher sulphate concentrations recorded in the upper part of the reactor. The difference was relatively small (50-70 mg/l) and was most likely due to some complete oxidation of sulphide to sulphate before complete biofilm formation. The residual sulphide remained above 60 mg/l, suggesting that any oxygen that diffused into the bulk liquid was consumed by sulphide, so inhibition of the SRB due to dissolved oxygen was unlikely.

Once a complete biofilm had formed the measured sulphate concentration began to decrease steadily, between day 4 and day 8, with over 40% of the available sulphate being reduced. The average sulphate reduction rate was around 180 mg/l.d or 7.5 mg/l.h. The rate was almost four times higher than that achieved during the inoculum build-up in the 1 000 l tank, but only about 60% of the rate that had previously been achieved at laboratory scale. More importantly, this was less than 40% of the VSRR achieved in laboratory studies at 30˚C using lactate as an electron donor.

Some of the differences in sulphate reduction rate can be accounted for by temperature. The laboratory scale research, conducted in the 2 l channel reactor, showed that temperature has a significant impact on the sulphate reduction rate, particularly in the acetate-fed system (Figure 21). A maximum rate of around 12.5 mg/l.h was achieved in the 2 l reactor at a controlled temperature of 30˚C. This decreased by over 50% at a temperature of 15˚C.

Temperature measurements in the 2 000 l reactor showed that the liquid temperature was typically between 7-12˚C lower than the air temperature. Water has a high specific heat capacity, so warming the reactor after it has cooled overnight takes a relatively long time. This would be even more significant for a larger reactor and is one of the caveats of extrapolating laboratory data under controlled conditions to larger scale, outdoor reactors.

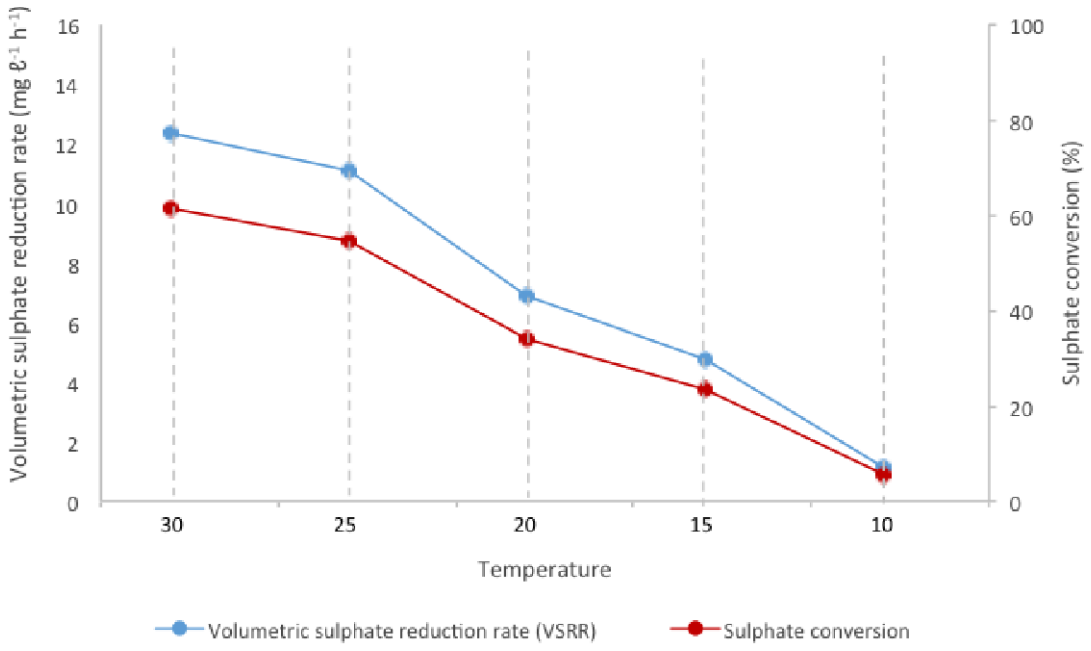

Figure 21: Effect of temperature on the volumetric sulphate reduction rate in 2 l hybrid LFCR maintained on acetate

The soluble COD measured in the reactor (Figure 22) followed a similar trend to the sulphate data, indicating that the majority of the acetate was consumed by SRB. There was some reduction in COD over the first 48 hours, which could be due to aerobic or microaerobic heterotrophs at near the surface.

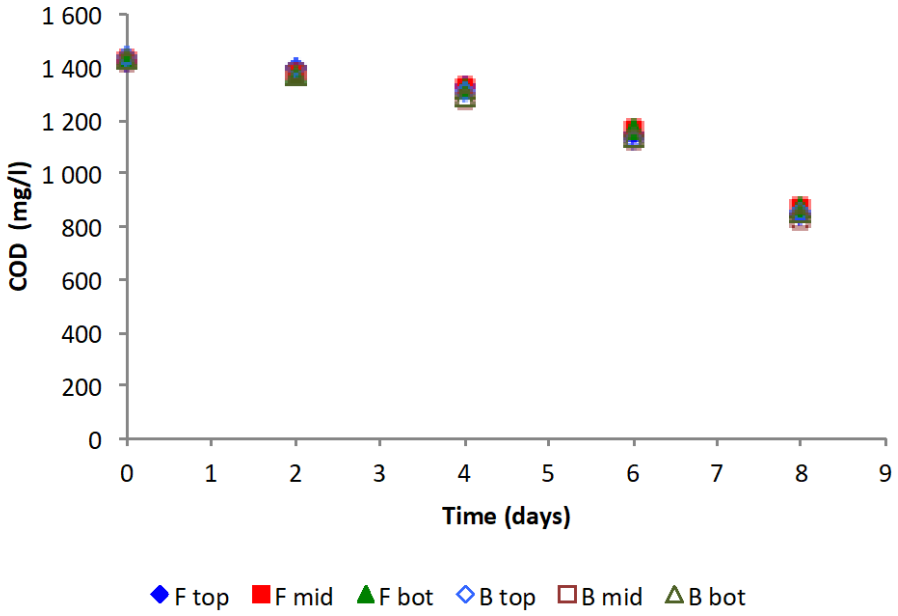

Figure 22: Soluble COD measured from six of the nine sampling points during the second channel reactor test

Previous work has shown that some acetate is required to support the initial formation of the biofilm, so it is likely that some of the COD consumed was converted into extracellular polymeric substances that form the backbone of the floating biofilm.

The formation of a thick, complete biofilm within 48 hours (Figure 23) illustrates the potential of the system to manage sulphide produced by biological sulphate reduction. The fact that the batch operation

showed sulphate reduction rates that were relatively consistent with data from the laboratory scale systems was also encouraging.

Figure 23: Image showing biofilm formation after 48 hours during the second channel reactor test

Unfortunately, due to the changes in the mine water quality and the fact that the ownership of the mine was likely to change the test had to be stopped before the reactor could be evaluated in continuous mode.

The other critical factor that was not evaluated during the initial pilot scale work was the practicality of using a complex carbon source. All work was done using acetate as carbon source.

3.4 RELOCATION OF THE PILOT PLANT AND SUBSEQUENT CHALLNGES

The reactors were transported to the new site, within the boundary of a working coal mine in Mpumalanga. The preferred system configuration is presented in Figure 24.

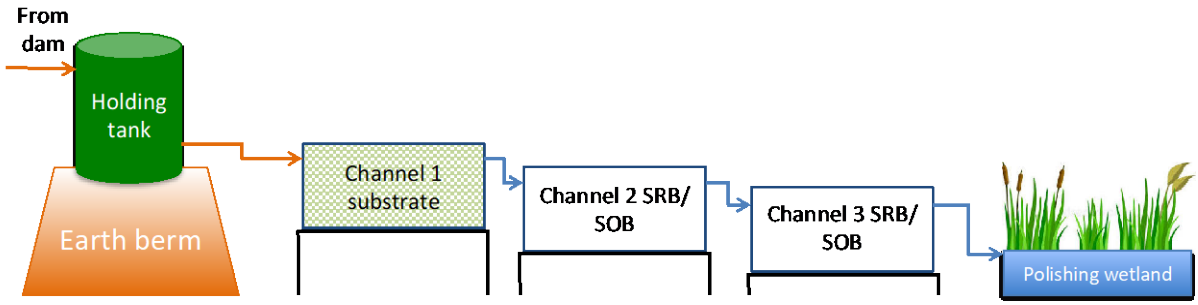

Figure 24: Schematic representation of proposed pilot scale plant at Mpumalanga site

The water to be treated was mine water that had undergone partial treatment by lime addition to increase the pH and reduce the iron load. The partially treated water was stored in a holding dam. The intention was to pump water from the dam into a 10 000 l tank on the earth berm surrounding the dam and then use gravity to feed into the first reactor, with the feed rate controlled by an adjustable valve. The first reactor would be packed with substrate, either grass cutting or sugarcane bagasse. It had been hypothesised that a combination of the residual acidity in the mine water and microbial activity would hydrolyse the substrate, releasing soluble organics into the second reactor, where sulphate

reduction and sulphide oxidation would occur. The third channel would provide space for additional sulphate reduction and the oxidation of any remaining sulphide. If the water required additional treatment to meet the mine's discharge criteria a polishing wetland could be constructed.

The reactors were moved from the mine site in the Free State to the new site near eMalahleni, along with the drums of inoculum and the 1 000 l tank used for inoculum build-up. While the old site was outside the boundary of the active operation, the new site was within the mine boundary. This introduced a number of regulatory requirements relating to health and safety, which delayed progress on site substantially. The process of receiving approval for the mine contractor's pack took over six months. The process was further complicated by the fact that the MSc student assigned to the project received an offer of employment shortly after he had been inducted, requiring a new student to be identified and subsequently undergo the induction process. Full access to the site for the new student was only granted in February 2018.

The second challenge encountered at the new site related to the location allocated to the pilot reactors. The site was adjacent to the lime treatment dam, from which the mine water to be treated was to be drawn. The reactors were placed on compacted earth as the mine was unable to arrange a concrete slab in time. This was suitable for the first four months, during the dry season, but heavy rains during the summer, coupled with coal fines that had been blown into the area created a thick layer of mud that rendered the site inaccessible for several months.

With the support of the environmental officer on site we were able to motivate for the extension of an existing concrete slab, about 200 m from the original location. After several delays the construction of the slab was completed during the winter of 2017. Attempts to relocate the reactors using a forklift were unsuccessful and a hydraulic crane was required to lift them. During this period Mintek had received approval to construct a pilot plant at the same location, which required a crane to deliver the reactor tanks. Relocation of the channel reactors was scheduled to coincide with delivery of the Mintek reactors and finally occurred in September 2017 (Figure 25).

Unfortunately, during the period the reactors were inaccessible they suffered some damage, particularly to the biofilm harvesting screens, which tore under the accumulated mass of the wet coal fines (Figure 26). These were repaired by replacing the mesh and the fines were cleared from the reactors.

In addition, two of the reactors suffered some structural damage during the relocation (Figure 27). This caused the reactors to leak. The damage to the first reactor was relatively minor and the reactor could be fully repaired. The damage to the second reactor was more serious and all attempts to repair were unsuccessful. The reactors were cleaned out and the site was then ready for inoculation (Figure 28).

In addition to the damage to the reactors the project was hampered by the theft of various components, most notably the 1 000 l JoJo tank that was used to grow up the final inoculum and the 2 000 l tank that would have served as the mine water feed tank.

A number of upgrades were still required for the site to become fully functional, including the provision of a fresh water source. The site did have a safety shower and eye wash station located about 50 m from the reactor site and a request was submitted to install an extension to the feed line to provide water to our reactors and the Mintek system.

Figure 25: Image of crane required to transport reactors to the new site

Figure 26: Photograph showing damage to the harvesting screen and accumulation of coal fines in the channel reactor

Figure 27: Photograph showing damage to the channel reactor inflicted during relocation

Figure 28: Photograph showing new location of channel reactors with Mintek pilot plant in the background

During the period that the site was inaccessible and while we waited on the final upgrades to the services on site to be completed experimental work continued at laboratory scale. The outcomes of this research ultimately showed that operating the system using a complex organic carbon source was not viable at this stage, so the pilot scale work was discontinued.

CHAPTER 4: LABORATORY SCALE RESEARCH

4.1 INTRODUCTION

The cost and availability of the electron donor and carbon source is one of the biggest constraints that has prevented the wider application of treatment approaches based on biological sulphate reduction, particularly in the field of ARD remediation. The cost of electron donor (hydrogen, methanol, ethanol) typically represents more than 50% of the operating expenses of a sulphate reducing application (Bijmans *et al*., 2011). Consequently, these technologies have typically been applied at scale (> 1 ton S reduced per day) where there is concurrent value recovery (Zinifex Budel – zinc recovery) or in gas stream desulphurisation (BioDeSOx – China).

The need to find alternative, low cost electron donors has been recognised as a priority. Magowo and co-workers (2020) recently reviewed the co-occurrence of AMD and organic rich industrial and domestic effluents to try and identify potential opportunities for co-treatment using bacterial sulphate reduction. Possible options include Fischer-Tropsch wastewater, sugar cane processing waste and domestic wastewater.

A second alternative that has been the subject of research in South Africa over the past 15 years is pasture grass. Several researchers have investigated grass cuttings as a potential substrate for SRB in ARD treatment systems, with promising results at laboratory scale (Greben *et al.,* 2009; Ramla and Sheridan, 2015; Mulopo, 2016; Burman *et al*., 2020). In most cases, the reactors were loaded with grass cuttings and water quality measured entering and leaving the reactors. Sulphate removal was attributed solely to biological sulphate reduction, rather than any potential physical or chemical reactions such as adsorption or precipitation and there have not been detailed studies on the rate of hydrolysis under anaerobic conditions. The research described in the following section aimed at addressing some of these questions.

4.2 ACCELERATED HYDROLYSIS OF GRASS AND POTENTIAL AS A MEDIUM FOR PRETREATMENT OF ARD

The decision to evaluate grass cuttings as a sustainable substrate was premised on work conducted at Wits that suggested that the acidity in mine water could accelerate the hydrolysis of grass and liberate soluble organics that could be used as the substrate for biological sulphate reduction. This was evaluated at laboratory scale in batch and continuous reactors. The intention of the batch tests was to assess the extent to which weak acid could achieve cellulose hydrolysis at ambient temperatures and to what extent free acidity was consumed in the process. There is a substantial amount of published work on cellulose hydrolysis using weak acid at elevated temperatures (150-270˚C), but very little data on lower temperatures.

Batch tests were conducted in 1 l glass reactors that were loaded with 15 g of fresh grass cuttings. Water, acidified with sulphuric acid, was added to each reactor to make up the volume to 1 l. Reactors were set up across a range of pH values from pH 2 to pH 5, with a control using plain water.

The addition of fresh grass resulted in a change in the colour of the solution phase within the first 24 hours (Figure 29). The colour became increasingly light as the initial acid concentration increased, most likely as a result of a reaction between the free acid and the leached pigment molecules.

The presence of the grass cuttings resulted caused the pH in all the reactors to increase during the first 24 hours. The measured change was most significant in the reactors that started at pH 3 and above, increasing to between pH 5.9 and 6.7 (Figure 30). The reactor that started at pH 2 did not show a

significant increase in pH during the first seven days of the experiment, but by day 33 the pH had increased to above pH 5.5 (Figure 31). The increase could be attributed to biological activity.

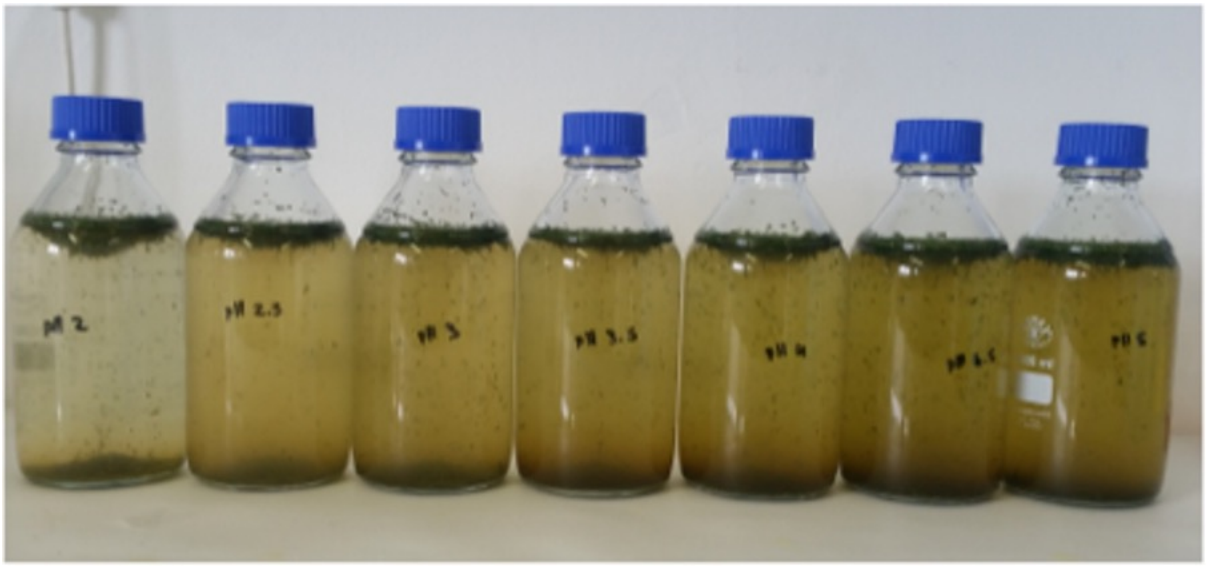

Figure 29: Photograph showing effect of initial pH on the appearance of the liquid phase (24 hours after grass addition)

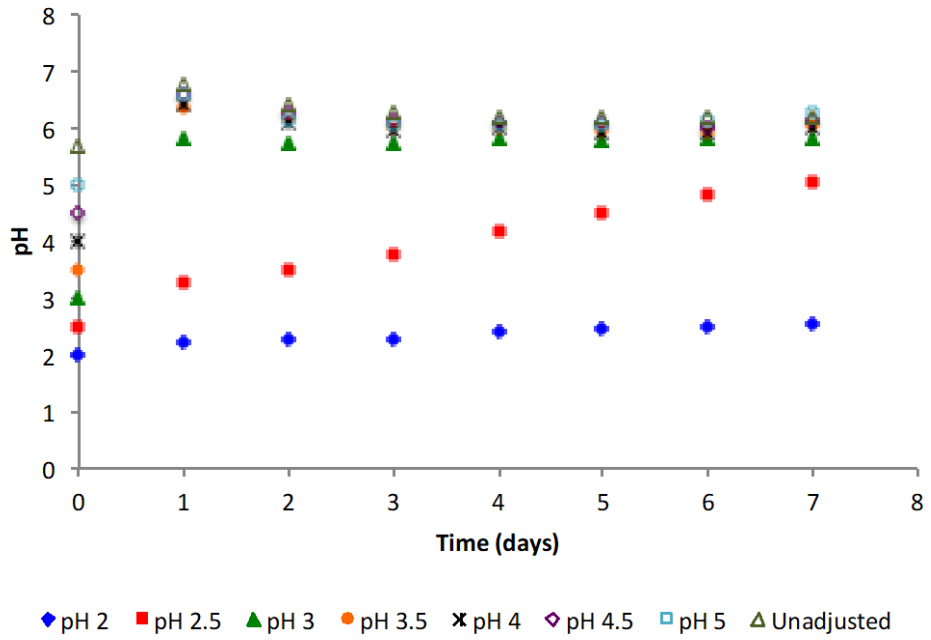

Figure 30: Change in solution pH during the first seven days of the experiment

The reactor that started at pH 2.5 did show a more significant increase during the first 24 hours and the pH continued to climb steadily for the next six days, while the pH in the remaining reactors decrease slightly between 24 and 48 hours before remaining relatively stable. This suggests that the components leached out of the grass, or the hydrolysis products, presented sufficient buffering capacity to consume the free acidity.

While the reactors were not deliberately inoculated, clear evidence of microbial activity became apparent within the first seven days. This could be attributed to microbes that were introduced with the grass cuttings. This was confirmed by light microscopy, which showed a greater number and diversity of bacteria in the higher pH reactors. The presence of the microbes coincided with a substantial

decrease in the redox potential, indicating that the reactors changed from aerobic to anaerobic as the experiment progressed. No significant gas production was observed in the reactors, suggesting that methanogenic archaea were not present in large numbers. When the same grass was added to the methanogenic stock reactor biogas production was observed within the first 24 hours. The low starting pH in reactors could account for the limited biogas formation.

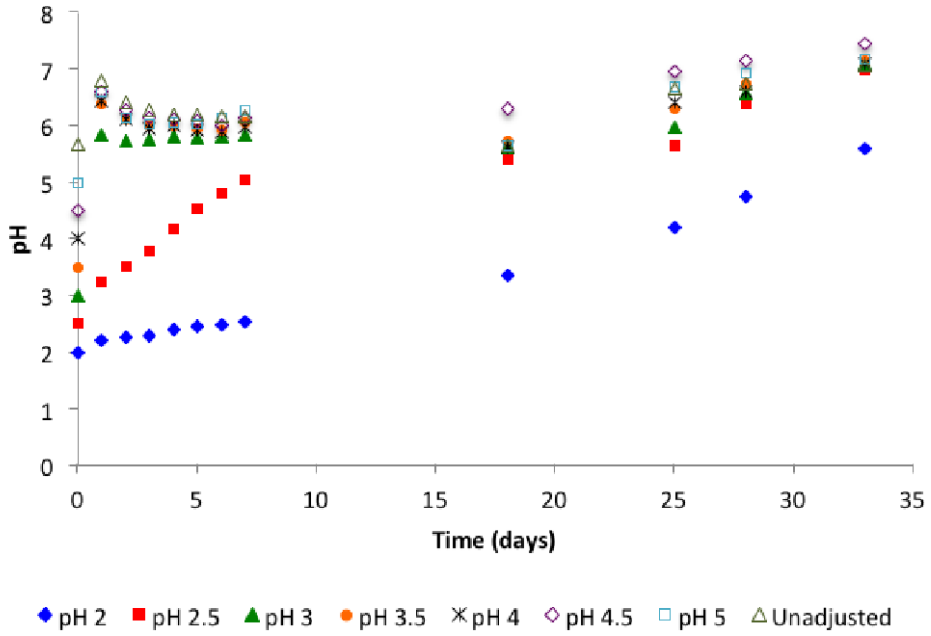

Figure 31: Change in solution pH following contact between fresh grass and dilute sulphuric acid

The results for acidity and alkalinity as a function of time and initial pH are summarised in Table 3. The day 0 data represent the initial acidity as a result of the sulphuric acid added to achieve the desired starting pH. By day 3 it was clear that the addition of the grass had resulted in major changes in the solution chemistry. The pH in the most acidic reactor had increased from pH 2 to pH 2.39, but this had coincided with the consumption of over 250 mg/l of acidity. A smaller change was observed in the pH 2.5 reactor, but for all the other reactors the total acidity had actually increased, suggesting the leaching of organic acids into solution. Between day 3 and day 7 the acidity continued to increase for all the reactors, with the exception of the pH 2 reactor where the acidity decreased further.

By the end of the experiment the pH in all but the most acidic reactor was higher than pH 6, suggesting the presence of alkalinity. This was confirmed by titration, suggesting that the degradation of organic matter and subsequent microbial metabolism had generated alkalinity. Initially the process was abiotic, but after the first few days was likely driven by biological action.

Samples for VFA analysis were taken on day 1,3,5,7,18 and 28. Analysis of the data showed significant changes in the VFA profile as a function of pH and time. The samples taken on day 1 contained very few VFAs with acetate concentrations between 5 and 25 mg/l measured across the pH range and no other VFAs detected. The chromatograms did show a number of large peaks that did not correspond to any of the VFA standards.

Table 3: Acidity and alkalinity values measured for the reactors measured by titration using 0.02 N NaOH (acidity) and 0.01 N H_2SO_4 (alkalinity)

	Acidity (mg/l $CaCO_3$ equivalents)			Alkalinity
Initial pH	Day 0	Day 3	Day 7	Day 28
Unadjusted	0.1	115.0	175.0	362.5
5	1.8	115.0	170.0	312.5
4.5	3.0	105.0	150.0	337.5
4	7.0	130.0	242.5	317.5
3.5	18.0	110.0	165.0	335.0
3	63.0	155.0	212.5	320.0
2.5	223.0	155.0	175.0	237.5
2	816.0	555.0	490.0	15.0

The profile of peaks on the chromatograms changed as the experiment progressed, providing evidence for the metabolism of compounds present following the initial contact between the grass cuttings and the acidified water. Between day 1 and day 7 there was a significant reduction in the area under some of the peaks that did not correspond to the VFA standards. In addition, the acetate concentration increased from 25 to 141 mg/l.

While there was an accumulation of acetate and no real evidence of acetate consumption by methanogens the rate of acetate accumulation was slow and suggested that the solids loading would need to be substantially higher achieve the level of VFA release required to sustain an effective sulphate reduction rate.

4.2.1 Tests on mine water from site

The results from the initial batch tests showed that the addition of grass cuttings was able to increase the pH of acidic solutions, even before any significant microbial activity. The integrated process depends on the activity of sulphate reducing bacteria, so is limited to applications where the pH of feed water is above pH 6 or where the reactor generates sufficient alkalinity during the sulphate reduction process to neutralise the incoming acidity.

The analysis of the partially treated mine water available at the Mpumalanga site showed that the liming process was not particularly effective, with pH fluctuating between pH 3.0 and pH 6.0 and ferrous iron concentrations often exceeding 50 mg/l.

Therefore, pre-treatment of the mine water in a grass reactor could have the dual benefit of consuming acidity and generating soluble organic carbon to sustain the sulphate reduction and sulphide oxidation processes. The retention time in such a pre-treatment reactor would need to be relatively low to keep the reactor size manageable.

Mine water was obtained from the pilot site, after the lime treatment step. Despite the lime addition the water received had a pH of 3.22, which would most likely inhibit the sulphate reducing culture. Batch experiments were set up to contact the mine water with fresh grass at 10 and 20 g/l loadings. The pH was measured regularly over the initial 24 hour period (Figure 32).

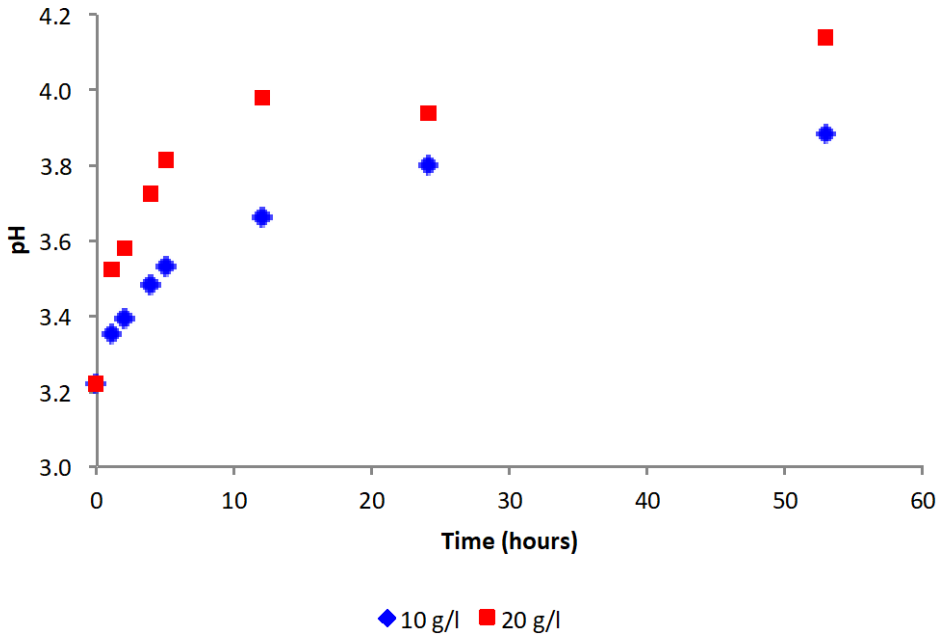

Figure 32: Change in solution pH following addition of increasing mass of fresh grass cuttings to raw mine water

A noticeable increase in pH was observed over the first 12 hours, with the magnitude of the increase a function of the mass loading. In the 20 g/l reactors the pH had increased to just below pH 4. The pH did continue to increase, although more slowly, after the first 12 hours.

The reactors were maintained for a longer period and after about three weeks the reactor loaded with 20 g/l had changed colour (Figure 33) suggesting some sulphate reducing activity and subsequent iron precipitation. No change was observed in the reactor loaded with 10 g/l of grass.

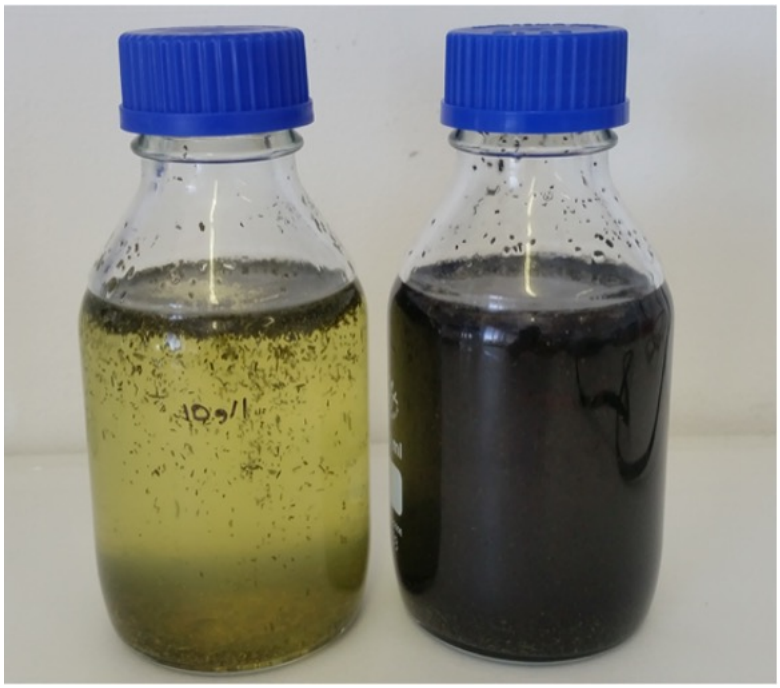

Figure 33: Evidence of sulphide generation following contact of fresh grass cuttings with raw mine water for 20 days

Samples taken on day 25 showed that the pH in the 20 g/l reactor had increased further to pH 5.6 and 20 mg/l of aqueous sulphide was detected, confirming sulphate reducing activity. By day 43 the pH had increased to pH 6.2 and the sulphide concentration to 93 mg/l. The sulphate concentration had decreased from 3273 to 2782 mg/l. Based on the amount of sulphate reduced the expected sulphide concentration would be 163 mg/l, but it was clear that some of the sulphide had reacted with iron in the mine water.

The experiment was extended to over 200 days, with regular monitoring. By day 80 the redox potential had decreased to -400 mV (Figure 34), consistent with an active sulphate reducing culture. The sulphide concentration increased to 180 mg/l, with the reduction of just over 500 mg/l of sulphate (Figure 35).

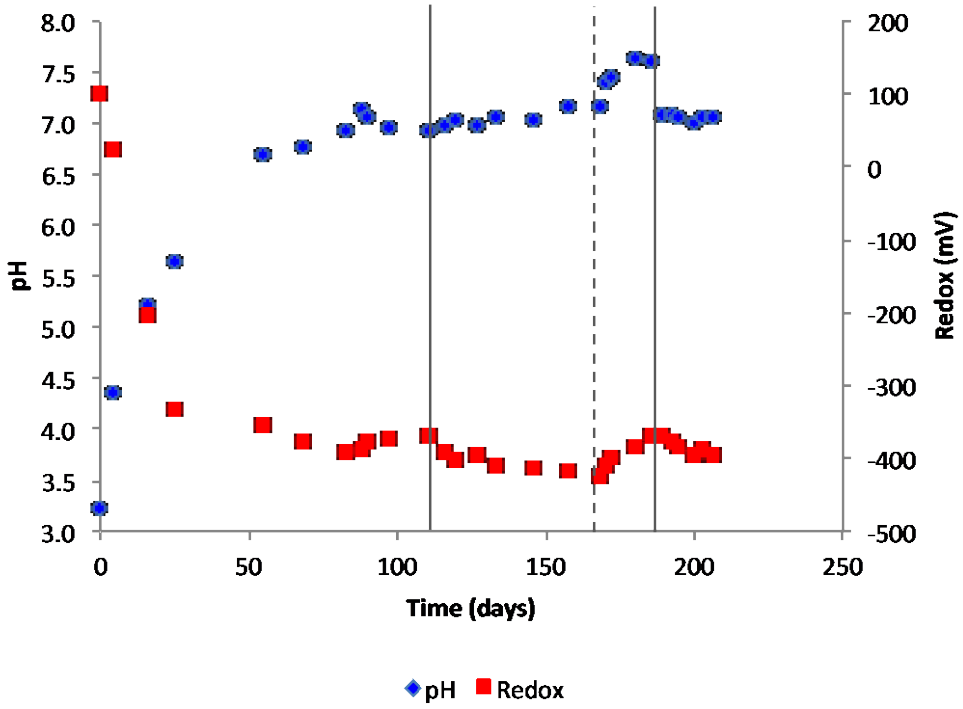

Figure 34: pH and redox potential for experiment contacting 20 g/l grass cuttings with mine water Vertical lines (solid) indicate the addition of 10 g of fresh grass cuttings or (dashed) 500 mg of sulphate

Between day 80 and day 100 the sulphide concentration decreased steadily, falling back to below 30 mg/l and there was an increase in sulphate concentration, indicating re-oxidation of some of the sulphide. This trend suggested that the organic substrate had been exhausted, with autotrophic sulphide oxidation becoming dominant. The absence of VFAs was confirmed by HPLC. Some oxygen was introduced into the system each time it was sampled.

In response to the substrate limitation an additional 10 g of fresh grass cuttings were introduced on day 111, with an almost immediate resumption of sulphate reduction. This phenomenon is similar to that observed in other studies using grass cuttings, where regular or even daily addition of fresh substrate was required (Greben *et al*., 2009).

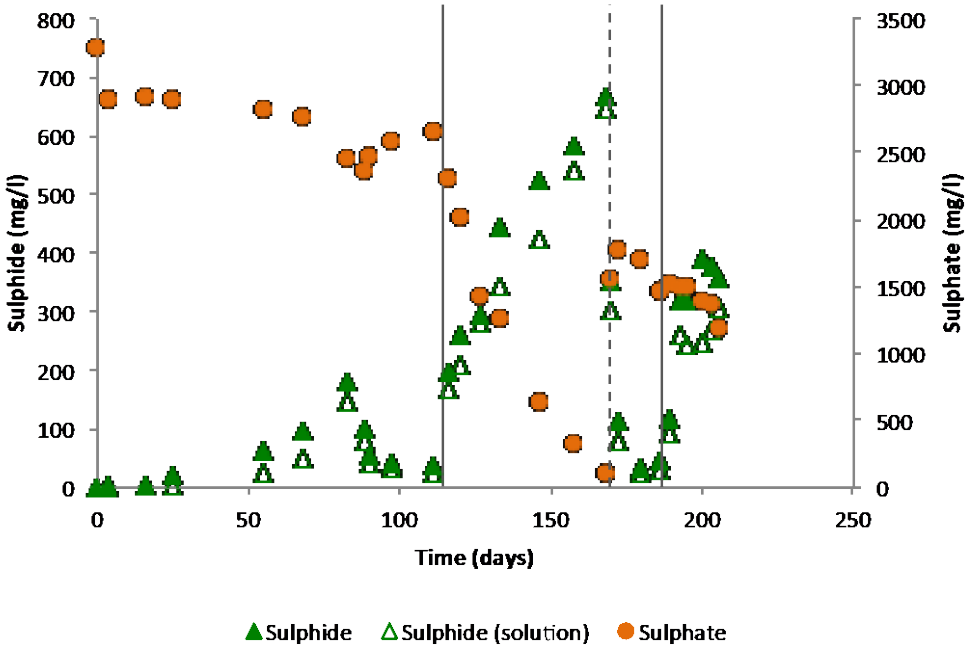

Figure 35: Sulphate and sulphide data for experiment contacting 20 g/l grass cuttings with mine water. Vertical lines (solid) indicate the addition of 10 g of fresh grass cuttings or (dashed) 500 mg of sulphate

Following the resumption of the sulphate reduction it progressed steadily, with a volumetric sulphate reduction rate of 76 mg/l.d. The rate is significantly lower than has been achieved using lactate as a substrate and less than half the rate that was achieved in the pilot reactor on sodium acetate as the substrate but was dependent on the liberation of organic carbon from the grass and its subsequent fermentation to VFAs. Complete sulphate reduction was achieved, albeit over an extended period of time, with sulphide concentrations increasing to around 650 mg/l.

On day 168 a 20% volume of the culture was removed to inoculate a second mine water reactor. The volume was replaced with a sodium sulphate solution, introducing another 500 mg of sulphate, but no additional organic substrate. This perturbation resulted in a rapid decrease in aqueous sulphide, coupled to an increase in pH and redox potential. The most likely cause for this was the introduction of oxygen during the sub-culturing and illustrates the sensitivity of the culture to oxygen, even though the redox potential remained below -370 mV.

Over the following 18 days the culture showed only limited signs of activity. Again, a reduction in organic substrate may have contributed to this, so on day 186 another 10 g of fresh grass cuttings were added, which resulted in an immediate improvement in performance.

By contrast, the reactor inoculated with 10 g/l grass cuttings was not able to elevate the pH above pH 4.3 and did not show any signs of active sulphate reduction. This experiment was terminated after 60 days.

4.2.2 Assessing the potential of a pre-treatment reactor

There were three 2 000 l channel reactors available at the pilot site and the laboratory data suggested that two in series should be sufficient for the sulphate reduction and sulphide oxidation, with the third being available as a pre-treatment option. To assess the potential for this, a laboratory scale unit was set up to treat a blend of real and synthetic mine water.

Within 24 hours a biofilm had started to form on the reactor surface and this biofilm became complete within four days. The biofilm appeared to consist of a number of bacterial and fungal morphologies, with mould-like fungi prominent (Figure 36). The biofilm was sticky, rather than brittle and there was no evidence of sulphur deposition or the crystallisation of salts.

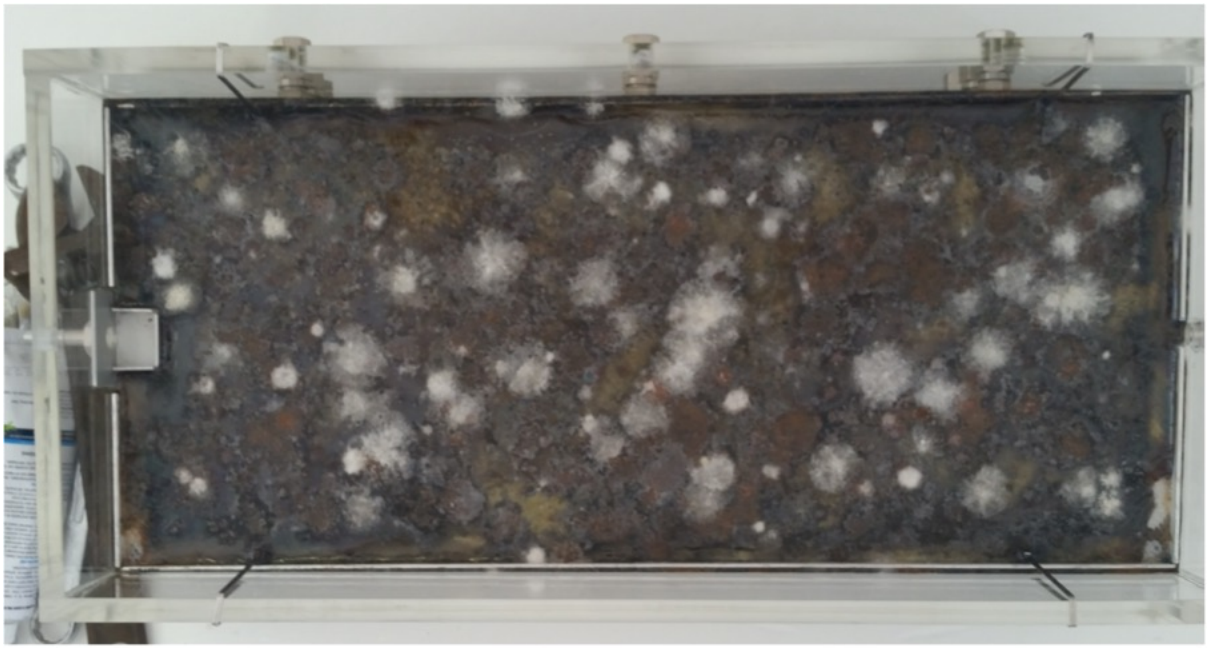

Figure 36: Top view of channel reactor showing establishment of bacterial and fungal biofilm

The reactor was sampled from the middle set of sample ports (top, middle and bottom). The pH and redox potential data (Figure 37) confirmed that there was no significant vertical stratification in the reactor.

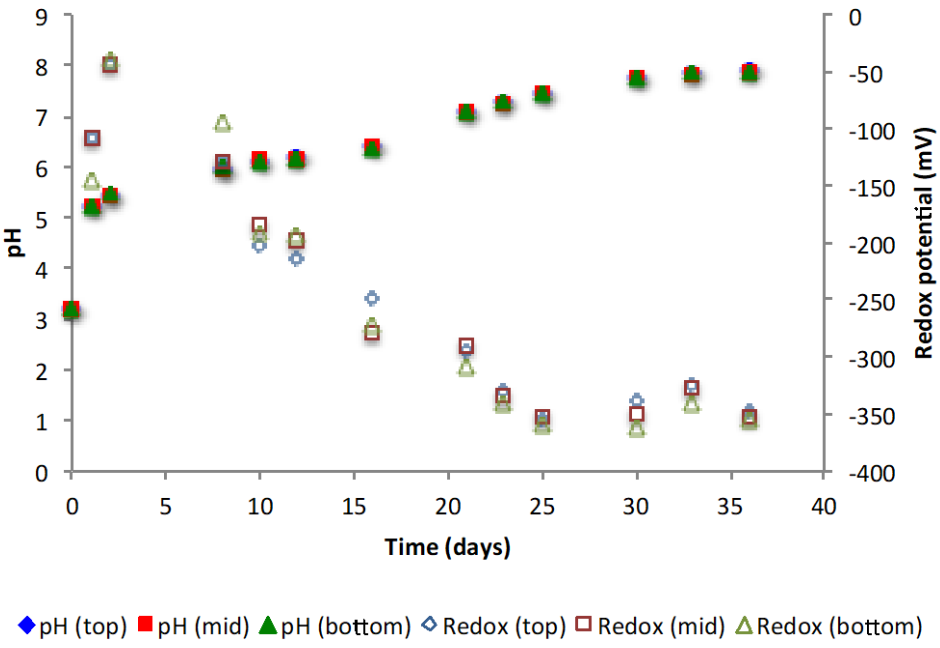

Figure 37: pH and redox potential data for the 8 l channel reactor

The data are consistent with the results from the smaller batch reactors, showing a rapid increase in pH over the first 48 hours, followed by a more gradual increase thereafter, reaching values between pH 7.5 and 7.7 by day 35. The redox potential decreased steadily before stabilising around -350 mV.

There was some evidence for sulphide generation, with the bulk liquid in the reactor showing evidence of iron sulphide precipitation after day 10. The aqueous sulphide analysis (Figure 38) showed the presence of low concentrations of sulphide after day 10, primarily in the non-centrifuged sample, which confirmed the presence of sulphide precipitates. The sulphide concentration did not increase beyond those levels and the concentration in the centrifuged fraction never exceeded 5 mg/l. The sulphate concentration remained stable, confirming the lack of sustained sulphate reduction.

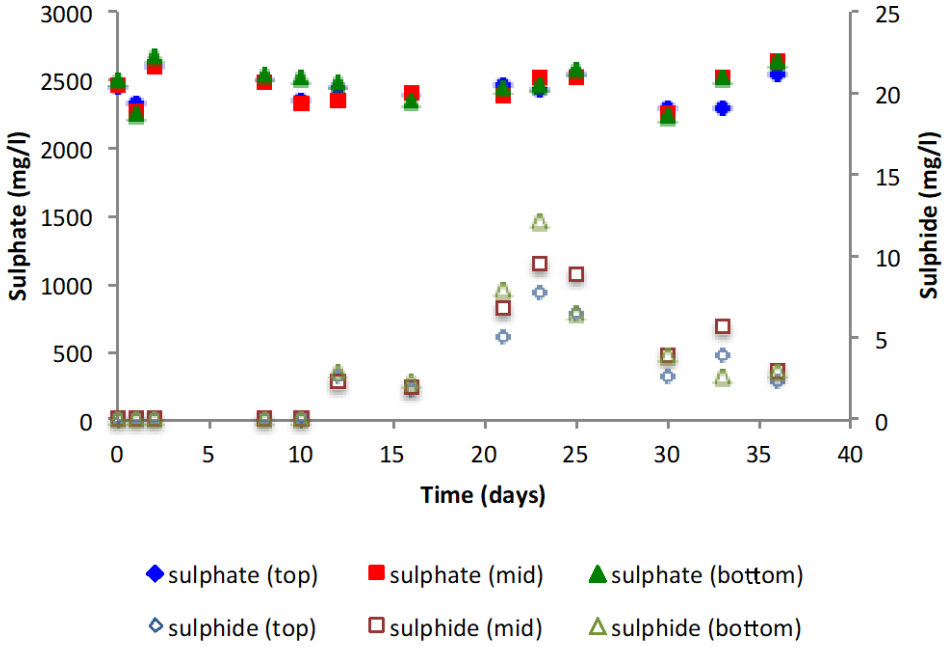

Figure 38: Sulphate and sulphide data for the 8 l channel reactor

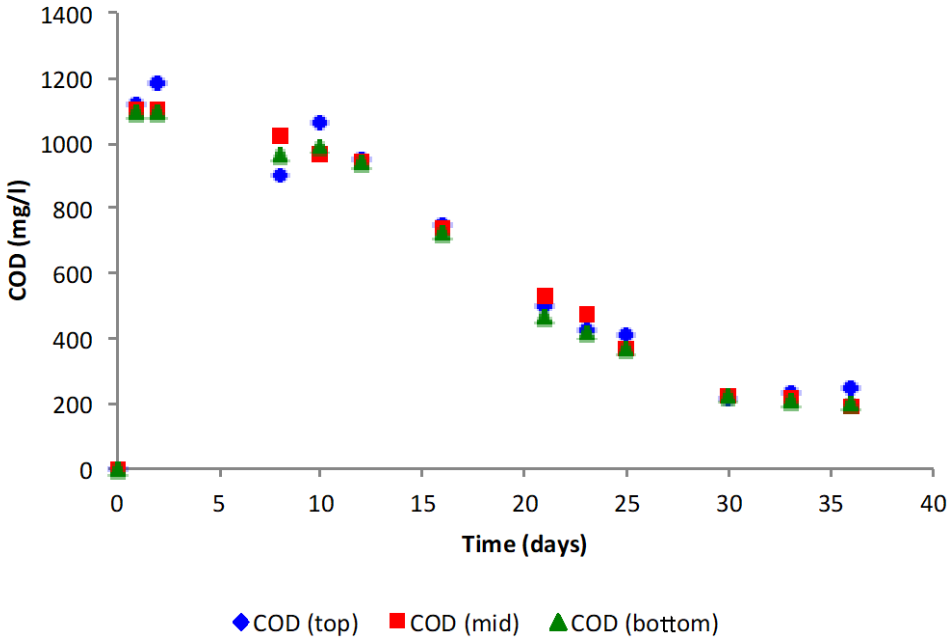

Figure 39: Soluble COD data for the 8 l channel reactor

Soluble COD was measured on the centrifuged samples and this showed concentrations in excess of 1 g/l over the first three days (Figure 39), after which the COD began to decline steadily, finally stabilising between 250 and 300 mg/l. At this stage there did not appear to be significant sulphate reduction which suggests that the residual COD that was being measured was most likely not in a form that could be utilised by sulphate reducing bacteria.

The second potential challenge from a practical application perspective became apparent when the reactor was unpacked. There had been very little change in the structural integrity of the grass, so there was a large mass of saturated biomass that would need to be handled. This would initially require drying to reduce the mass. In addition, a black precipitate that was composed of precipitated metal sulphides, mostly iron, covered the grass. Depending on the nature of the wine water and metal load it is likely that the accumulation of metal precipitates could result in a solid material that would need to be classified as a hazardous waste, further exacerbating the difficulty and cost of disposal.

4.3 COAL SOLUBILISATION

The experiments conducted during this project and the parallel K5/2392 investigation have raised serious concerns regarding the viability of using grass, manure, wood chips or the typical mix of lignocellulosic substrates previously used in passive or semi-passive systems, both from a kinetic perspective, but also it terms of the actual amount of biomass required, the frequency of replenishment and the issue of disposal of partially degraded material.

Direct measurements of the COD of different materials revealed that cut grass had an approximate value of 0.47 g COD/g, which increased to between 1.3 and 1.4 g/g after drying. Manure and microcrystalline cellulose yielded results of 1.03 and 1.11 g/g respectively. However, not all the COD is readily available and experiments with cut pasture grass indicated that approximately 3.7 g of partially dry (2 day old) grass would be required to provide sufficient organic carbon to reduce 1 g of sulphate.

Extending the information presented above to even the pilot scale system, where the intention is to treat 1 kl/d at a sulphate concentration of approximately 3 g/l, would therefore require 11.1 g of grass per l of mine water, or 11 kg/d. Based on the laboratory data, the 2 000 l pilot reactor could hold approximately 44.5 kg of grass, meaning the contents of the entire reactor would need to be replaced every four days. In reality, the kinetics of carbon liberation are slower, so complete sulphate reduction would not be achieved with a two-day HRT, hence the need for multiple reactors in series. Nonetheless, the turnover of substrate will still need to be frequent.

In addition, the majority of the substrate remained intact, even after 75 days in the channel reactor (Figure 40). Therefore, when the substrate has to be replaced there will be a significant mass of residual substrate that will require disposal. The laboratory experiments showed that some sulphate reduction and significant iron removal occurred within the pre-treatment reactor, so the residual substrate was coated in iron sulphide in places. This could make disposal of the residual biomass more challenging.

The same challenge would be encountered with any lignocellulosic or cellulosic biomass.

Figure 40: Photograph showing physical structure of grass after 75 days in laboratory pre-treatment reactor

Coal mining operations produce vast quantities of low grade and ultrafine coal discards, which represent a waste stream and are, in most cases, stored on dumps on site. Coal is carbon-based so represents a potential source of organic carbon to sustain the bio-treatment process, if the complex structure can be depolymerised to soluble molecules. Coal fines from the pilot site were tested to determine the COD, with a mean value of 1.56 g/g recorded. This is higher than the other carbon sources investigated in this study.

Coal has a significantly higher density, meaning a much smaller volume of material would be required to provide an equivalent amount of COD. This is illustrated in Figure 41.

Figure 41: Comparison between 1 g of pasture grass and 1 g of coal fines

The biodegradation of discard coal, particularly by fungal species, has been the subject of a significant amount of research. Certain strains of fungi have been shown to release a range of soluble organic compounds, including single ring aromatics, polyaromatic hydrocarbons (PAHs), aromatic nitrogen compounds and aliphatics. Researchers (Haider *et al*., 2013) have shown that these products could

be used as a substrate for methane production. Methanogenic organisms convert acetate to methane, indicating that the more complex organics can be metabolised to simple VFAs, which are the preferred substrates of many SRB species. Therefore, we initiated a preliminary investigation into the possibility of using fungi to degrade discard coal to produce a substrate for the SRB an SOB.

Fungi with the potential to degrade aromatic compounds were collected from hardwood trees or from enrichments prepared from the coal discard samples. Cultures were initially established by growing on potato dextrose agar plates (Figure 42), from which sub-cultures were prepared in an attempt to isolate individual strains. These were subsequently plated onto plates prepared using bacteriological agar and coal fines (Figure 43).

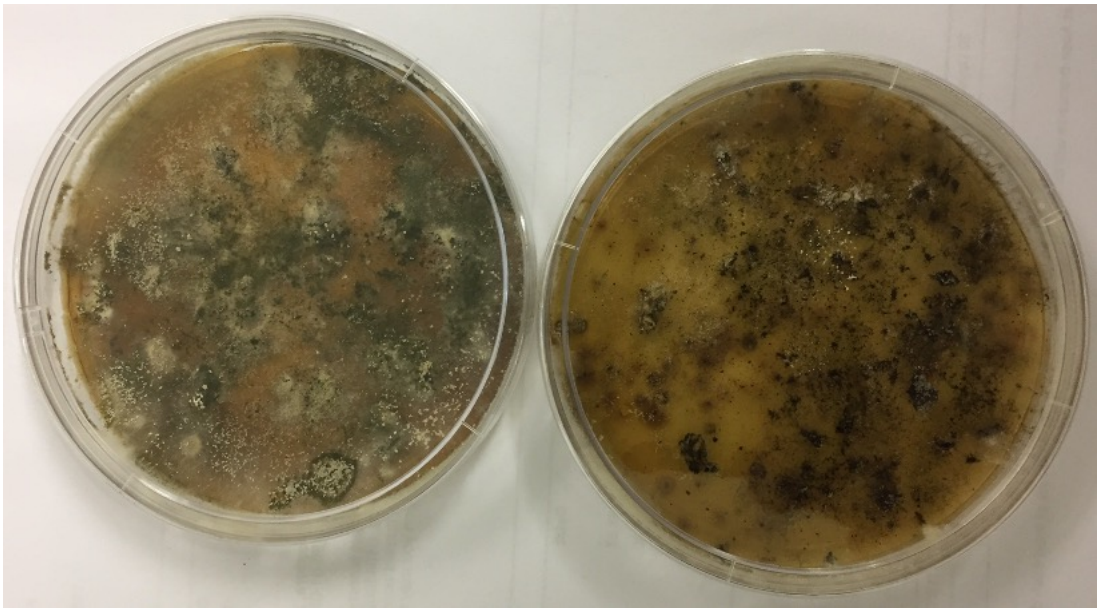

Figure 42: Photograph showing fungal cultures enriched from rotting wood on potato dextrose agar

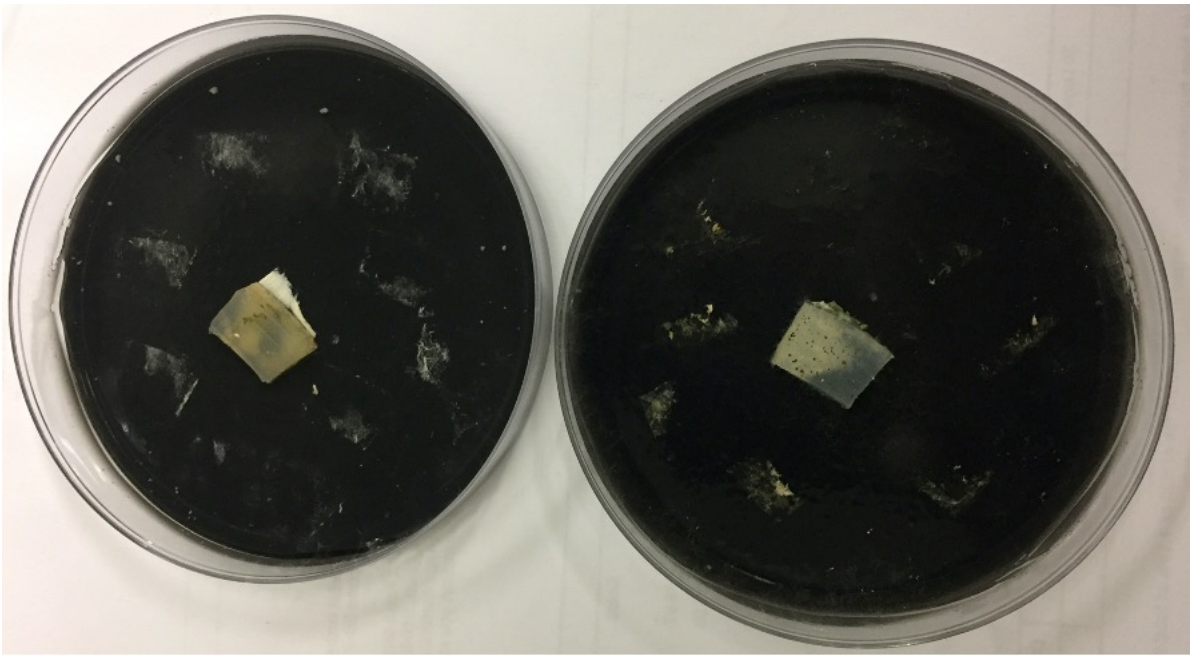

Figure 43: Photograph showing growth of fungal isolates on bacteriological agar enriched with coal fines

Isolates that appeared to grow on the coal plates were picked and used to inoculate flasks containing coal fines in a minimal fungal medium (0.1% ammonium sulphate, 0.1% glucose). The flasks were

maintained at ambient temperature and monitored to determine pH, soluble COD and iron in solution. Periodic samples were taken and prepared for subsequent HPLC analysis.

Evidence of mycelial growth was visible after 48 hours in the flasks inoculated with isolates from the agar plates (Figure 44). The pH of the solution in the flasks dropped rapidly after inoculation to around pH 3.4, after which the pH decreased gradually, but slowly for the duration of the experiment, reaching between pH 3.05 and 3.15 after 40 days. The pH in the control flask also dropped rapidly over the first 24 hours, but the subsequent decrease was slower. While the control flask was not actively inoculated, evidence of mycelial growth was visible after four days, suggesting the presence of spores on the coal fines collected from the pilot site.

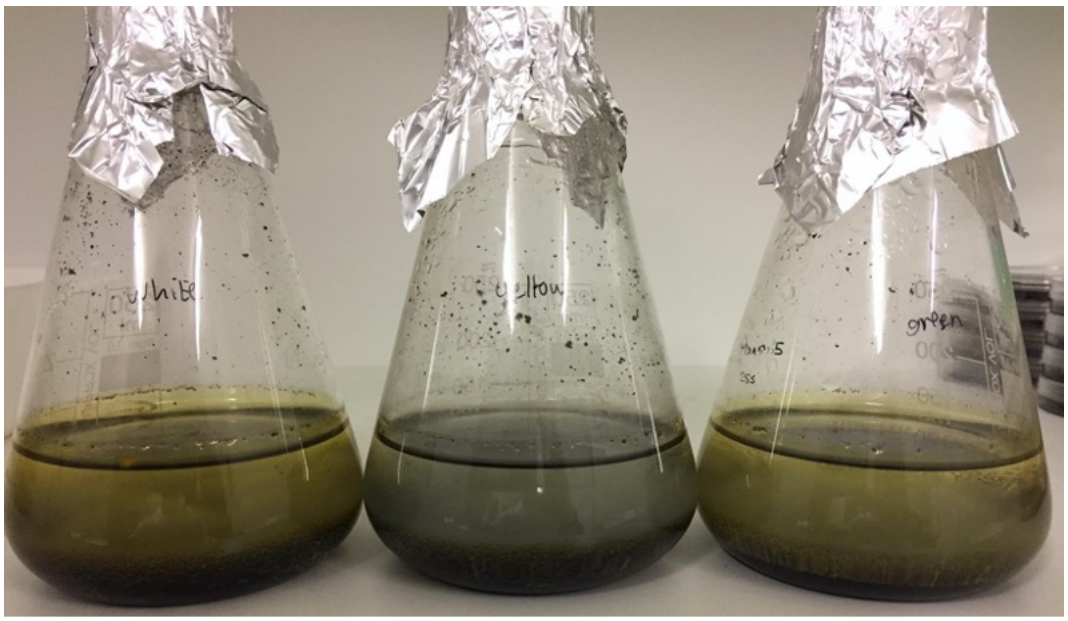

Figure 44: Photograph showing liquid cultures of selected fungal strains in basal media loaded with coal fines

The rapid decrease in pH over the first 24 hours was most likely not biologically mediated. The coal contains traces of sulphide minerals, so it was likely that these were partially oxidised and released some acid when placed in the media. The subsequent, slower decrease in pH could be due to the fermentation of the glucose in the media to VFAs, the liberation of VFAs from coal degradation or the oxidation and hydrolysis of iron liberated from the coal. Most likely a combination of all three contributed.

The soluble COD data (Figure 45) showed an increase over the first seven days in the inoculated flasks, followed by a steady decrease until almost all COD had been consumed by day 40. The increase suggests that some coal solubilisation did take place, after which the rate of carbon consumption exceeded the rate of degradation. Substantial fungal growth was observed in all flasks, clearly indicating biomass accumulation.

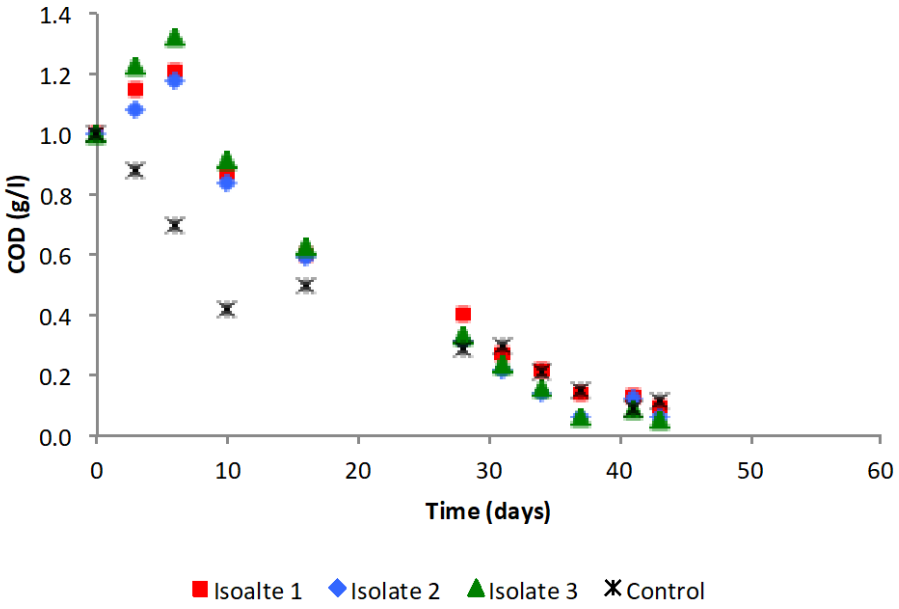

Figure 45: Soluble COD as a function of time for three isolates and uninoculated control

Samples were drawn from the flasks periodically to determine soluble iron (ferrous and ferric) concentrations. There was a steady increase in soluble iron, predominantly as ferrous iron, in the inoculated flasks over the first 14 days, with the iron concentration peaking at around 150 mg/l. This was followed by a decrease in ferrous and increase in ferric concentration, coupled with the development of a yellow/orange colour and ultimately the precipitation of ferric iron. The precipitate appeared to be associated with the fungal mycelia, rather than settling to the base of the flask (Figure 46a). The rate of iron oxidation accelerated as the experiment progressed, suggesting the presence of acidophilic, iron oxidising bacteria. This is not unexpected, as coal spoils are typically colonised by these types of bacteria, accelerating the rate of ARD generation.

There was less evidence of iron oxidation and precipitation in the control flask (Figure 46b), despite the presence of fungi.

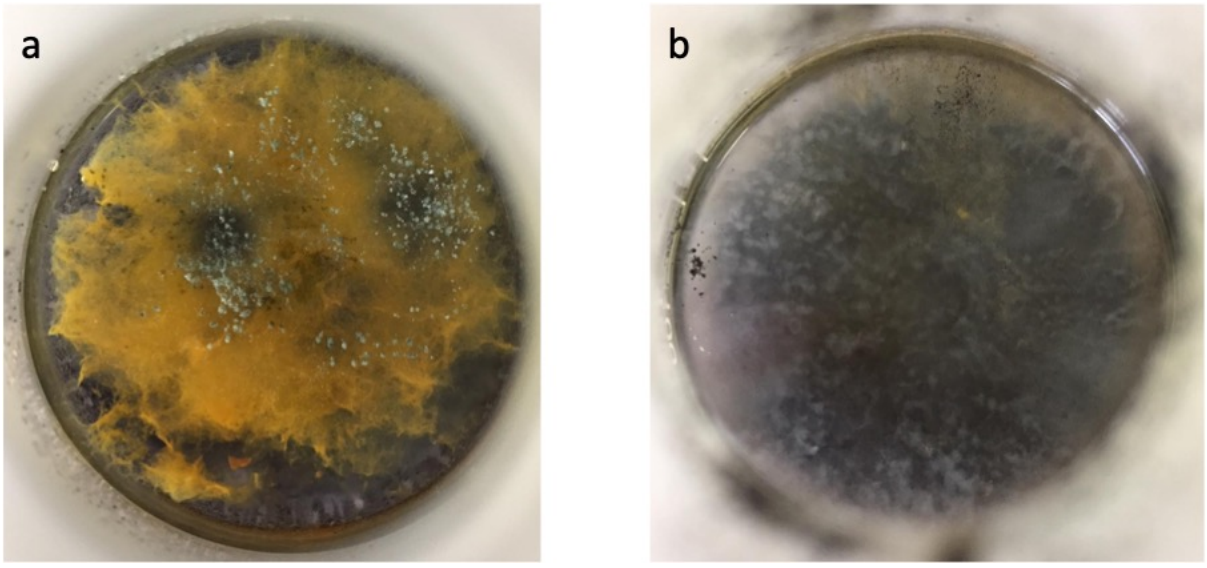

Figure 46: Photograph taken from above illustrating the fungal mycelia and precipitation of iron in the (a) experimental and (b) control flasks

4.4 SULPHUR OXIDATION RESEARCH

An important component of the research into the viability of the integrated process is an assessment of the potential to use the bio-sulphur product as a component of agricultural fertiliser, specifically to achieve the slow release of sulphate. As a precursor to the plant growth study a set of laboratory experiments were initiated to see whether the sulphur oxidising organisms present in the biofilm were able to revive themselves after the biofilm had been dried and if they would continue to oxidise the sulphur present to release soluble sulphate and acidity. Due to the early termination of the pilot-scale system it was not possible to harvest sufficient bio-sulphur to conduct the plant trials in 2019. An alternative source of bio-sulphur was secured in early 2020, from a bio-desulphurisation reactor at a commercial anaerobic digester facility. The intention was to use this sulphur for the plant growth trials, but this was ultimately not possible due to lockdown restrictions in response to the Covid pandemic.

The experiments were conducted in 500 ml shake flasks, maintained at 25˚C in an orbital shake and agitated at 120 rpm. A range of media of increasing salinity was selected to assess the diversity of the community with respect its ability to adapt to increasingly saline conditions. Media composition ranged from municipal tap water to standard artificial seawater (ASW), with a 50/50 and 80/20 mix included. This represented a range of EC values from 2.6 mS/cm to 45 mS/cm. The ASW culture was of interest to a parallel WRC project evaluating the biological treatment of tannery effluent, which has a similar composition, so was included in the study. A final set of flasks was set up using water supplemented with sodium acetate to an acetate concentration of 1 g/l. The flasks were seeded with dried floating sulphur biofilm, harvested of the hybrid LFCR reactors being operated at UCT as part of the K5/2392 project, at an initial loading of 5 g/l.

The flasks were sampled periodically to measure pH, EC, soluble sulphate and for light microscopy to assess microbial activity. The pH data are presented in Figure 47 and show a steady decrease in the pH across all the cultures.

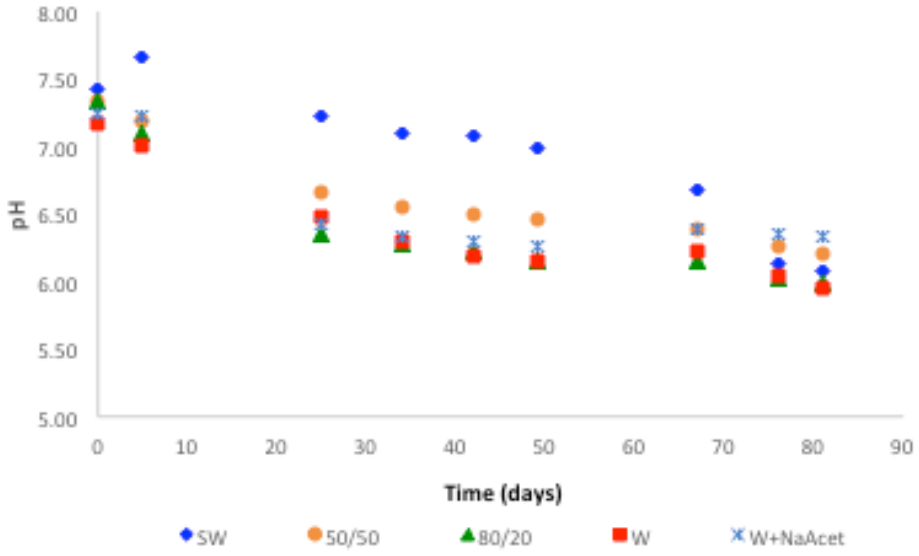

Figure 47: Change in pH over time for sulphur oxidising cultures. Data represent flasks containing artificial seawater (SW), dilutions of artificial seawater (50/50 and 80/20), municipal water (W) and water supplemented with acetate (W+NaAcet)

This is consistent with the oxidation of elemental sulphur to sulphuric acid. The rate of change in the artificial seawater and 50/50 diluted seawater has been slower for the first 50 days, but has increased substantially after that. The ASW does have a higher buffering capacity so would resist pH change

more than the solutions with lower salinity, but the fact that the rate of pH change has increased suggests some adaptation of the community to the higher salinity.

The soluble sulphate data (Figure 48) does follow the same general trend as the pH data, although there is some inconsistency. The barium chloride method of sulphate analysis is not the most precise, so replicate samples are analysed. Variation between replicates can be as high as 10-15%.

The initial data points show the differences in sulphate concentration as a function of the media composition, with the ASW and its dilutions having considerably more sulphate than the water-based media, although the sulphate in the latter is not insignificant. The sulphate in the water and acetate-based media was introduced with the dried biofilm. The freshly harvested biofilm contains a significant amount of entrained, sulphate containing, liquid (up to 80% of the wet mass). During drying the sulphate will crystallise out, but will dissolve again when the biofilm powder is submerged.

Despite the variability in the data a clear trend of increasing sulphate concentration is apparent, with the most significant increase occurring in the lower salinity media. The sulphate concentration in the water-based medium has increased from around 4000 mg/l to almost 12 000 mg/l over the duration of the experiment. This is an encouraging result in terms of the potential for the bio-sulphur as a fertiliser component.

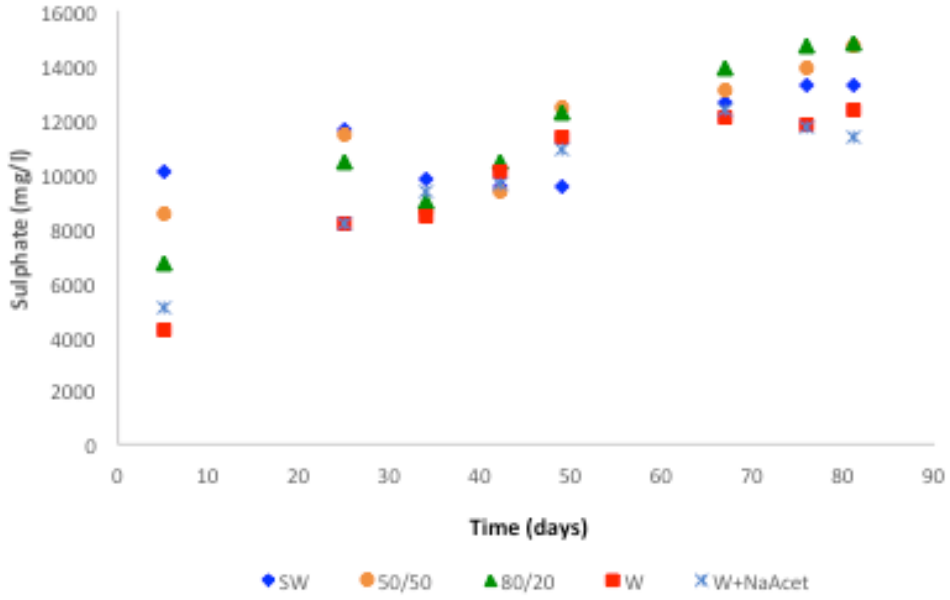

Figure 48: Change in sulphate concentration over time for sulphur oxidising cultures. Data represent flasks containing artificial seawater (SW), dilutions of artificial seawater (50/50 and 80/20), municipal water (W) and water supplemented with acetate (W+NaAcet)

Samples were taken periodically from the flasks and examined using light microscopy to assess culture viability and obtain qualitative information on cell concentration and morphological diversity. Some examples are presented in the figures below.

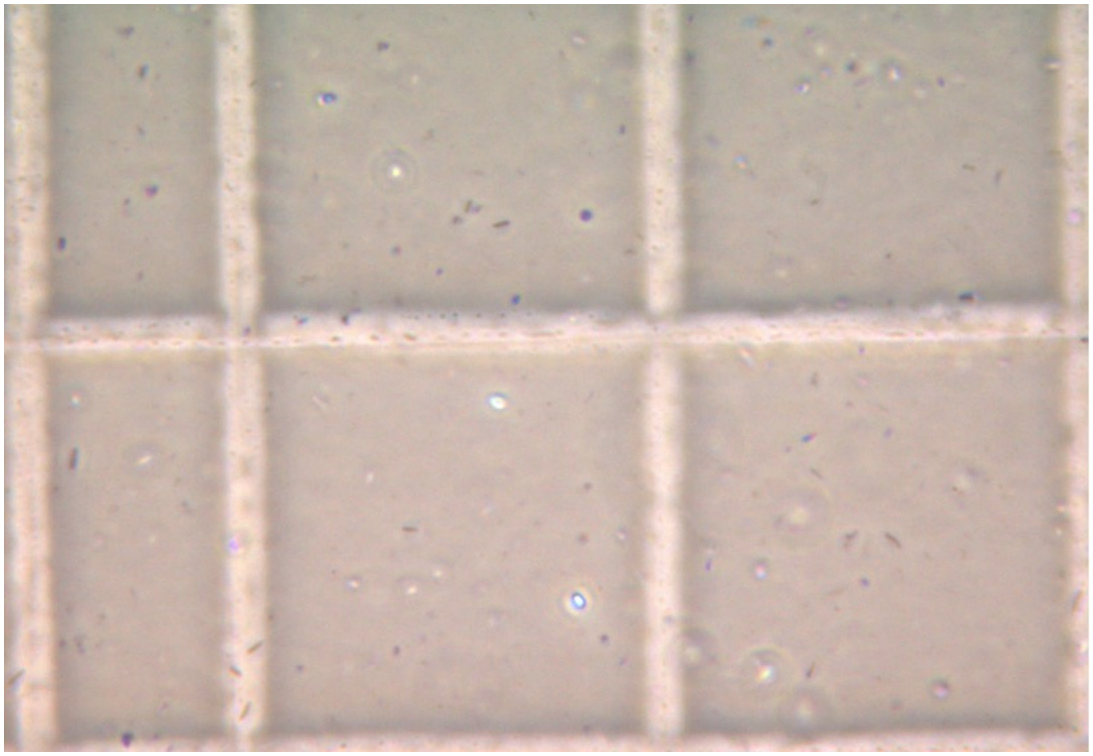

Figure 49: Light microscope image showing cell number and morphological diversity in autotrophic sulphur oxidising culture (water) after 70 days (100 X magnification)

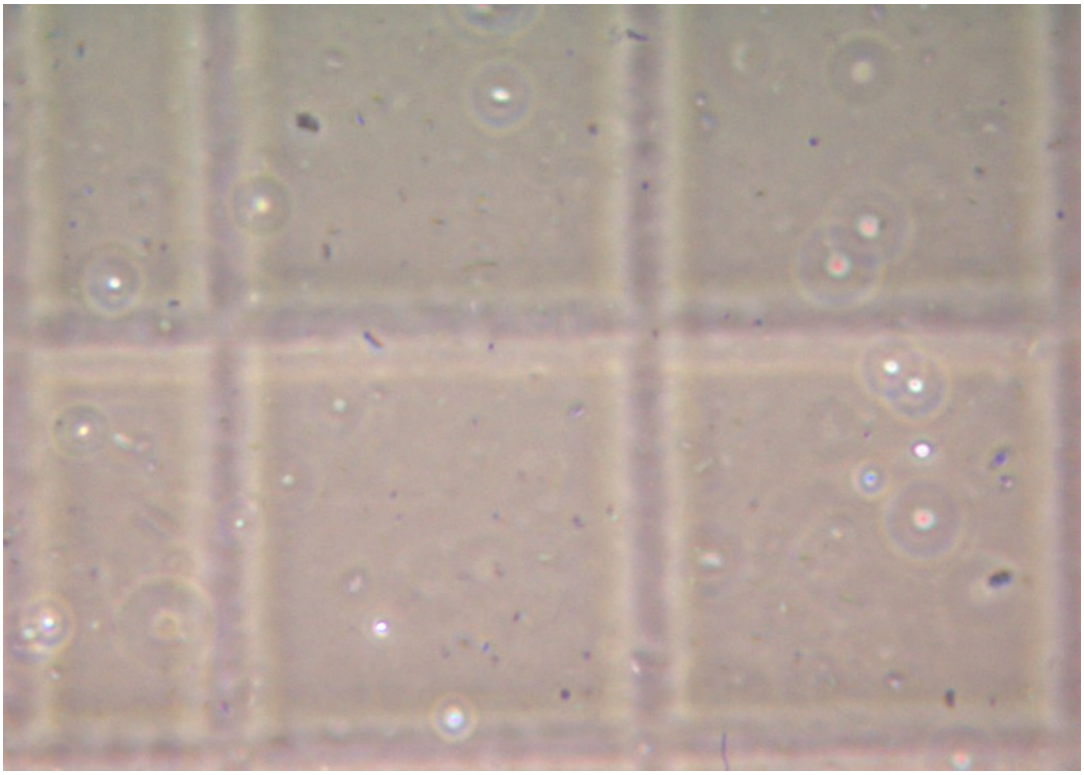

Figure 50: Light microscope image showing cell number and morphological diversity in sulphur oxidising culture supplemented with acetate after 70 days (100 X magnification)

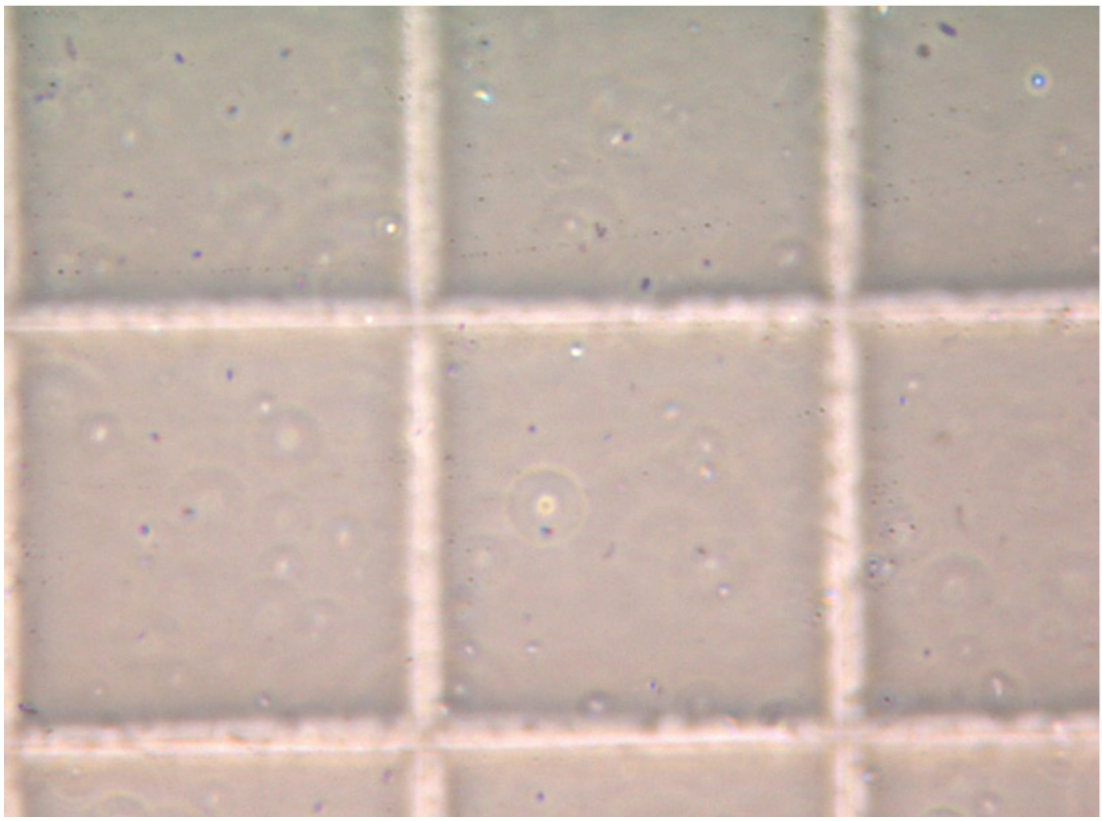

Figure 51: Light microscope image showing cell number and morphological diversity in sulphur oxidising culture on artificial seawater after 70 days (100 X magnification)

It is difficult to capture still images of motile bacteria as they move through the liquid film layer. For future research heat-fixed mounts should be Gramm stained to better represent the morphological diversity.

Based on the light microscopy observations it is possible to draw a number of qualitative conclusions. All flasks showed clear evidence of microbial activity and the cell concentration has clearly increased as the experiment has progressed. There is a relationship between cell concentration and morphological diversity and the salinity of the media, with the less saline media supporting a larger and more diverse community. The addition of acetate did not support a significantly higher cell number, but the morphological diversity did appear greater, specifically with respect to rod-shaped cells.

CHAPTER 5: CRITICAL ASSESSMENT OF SYSTEM PERFORMANCE AND MODELLING OF SUBSTRATE REQUIREMENTS

5.1 INTRODUCTION

The analysis of the laboratory scale experiments suggests that while it is theoretically possible to achieve the levels of pH adjustment and sulphate reduction required to treat the type of mine water at the pilot site, the kinetics of the process are severely constrained by the rate of hydrolysis and acidogenesis. In order to assess the requirements for successful operation of the pilot scale system a modelling exercise was conducted, based on the stoichiometric requirements of COD to reduce sulphate (COD:SO_4^{2-} of 0.7:1) at different feed concentrations and hydraulic residence times. The COD of the potential substrates were calculated from first principles for the simple substrates or measured directly for the complex substrates. The values are presented in Table 4.

Table 4: COD values for different substrates used in the model

Substrate	COD (g/g)	Determined by
Lactate	1.07	Calculation
Acetate	1.07	Calculation
Ethanol	2.09	Calculation
Grass (dry)	0.95	Measurement
Grass (wet)	0.28	Measurement
Molasses	0.85	Measurement

The model evaluates the requirements for removing up to 1500 mg/l of sulphate at a feed rate of up to 2 000 l/d, which equates to a 2 day HRT for the a system consisting of two channels of 2 000 l in series.

The model also assumes that all the soluble COD provided would be consumed by SRB and that there is no competition for substrate from either fermentative bacteria or methanogenic archaea. In the case of fresh grass cuttings, the model is based on the mean rate of liberation of soluble COD measured in the batch reactors over the first 48 hours.

The three-dimensional surface graph showing the COD requirements is presented in Figure 52. The laboratory studies in the 2 l channel reactor, using lactate as the carbon source at a controlled temperature of 30˚C, indicated an optimal HRT of two days to maximise the extent of sulphate reduction and sulphide oxidation. Higher sulphate reduction rates were achieved at lower HRTs, but the residual sulphate concentration increased. Under those ideal conditions, the pilot system consisting of two channels in series would require just over 1 kg of soluble COD or 500 g per day to reduce the sulphate concentration from 2 000 mg/l to below 500 mg/l, which is the target for discharge.

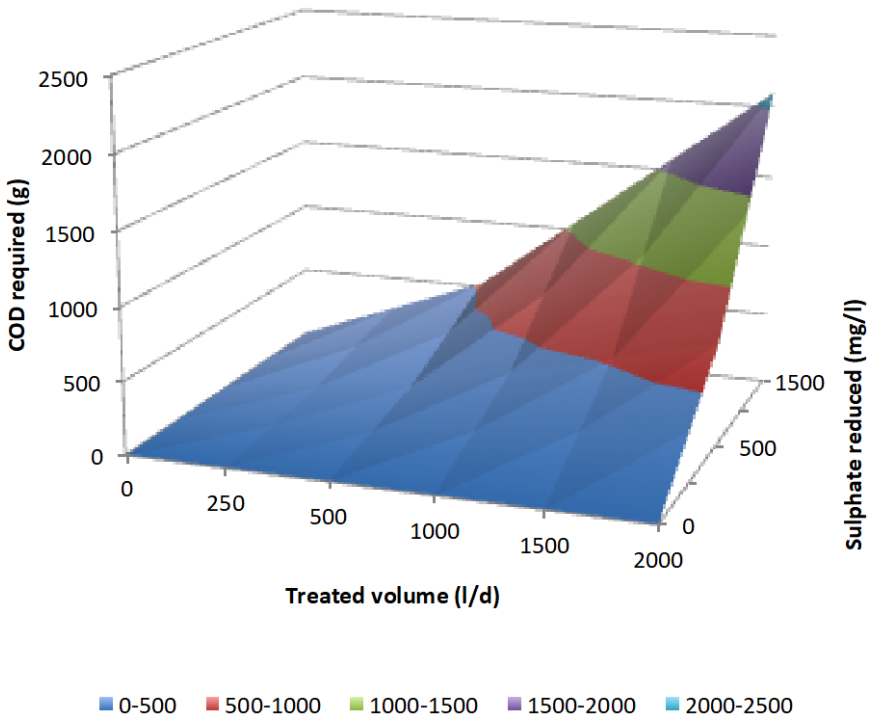

Figure 52: Surface plot showing the amount of soluble COD required to support sulphate reduction for increased loading and decreasing HRT

There are a number of commercial scale, active processes for treating industrial effluents with elevated sulphate concentrations and these often use ethanol or methanol as the electron donor and carbon source. Using the COD of absolute ethanol the volumes required are shown in Figure 53.

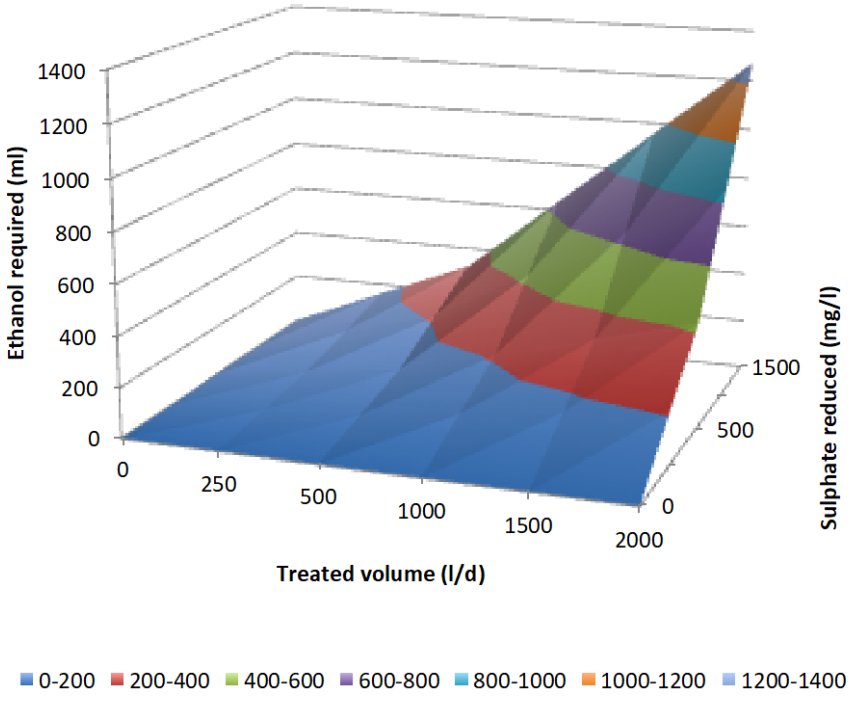

Figure 53: Volume of absolute ethanol required to achieve desire levels of sulphate reduction

To remove 1500 mg/l of sulphate at a feed rate of 2 000 l/d (2 day HRT over system) would require around 600 ml of absolute ethanol per day. Industrial grade ethanol costs in the region of R200 per litre so the substrate cost would be around R120/d or R3600 per month, to treat 2 000 l per day of mine water. This cost escalates rapidly as the volumes increase, which is why active SRB systems tend to be confined to treating effluent from the oil and gas industry.

Molasses is a carbohydrate-rich by-product of the sugar industry and has been used as a substrate for sulphate reduction, including as a supplementary feed in some semi-passive systems. Assuming that all the COD is available for sulphate reduction, which may not be true as carbohydrates will need to be fermented to volatile fatty acids, the system would require around 850 ml per day to treat 2 000 l of mine water. Molasses is substantially cheaper than ethanol, at around R50/l, but would need dilution to reduce the viscosity to make it easier to feed into the reactor.

Molasses has not been used a substrate in any of the laboratory-scale research, so any challenges related to competition by non-SRB for a carbohydrate feedstock have not been assessed.

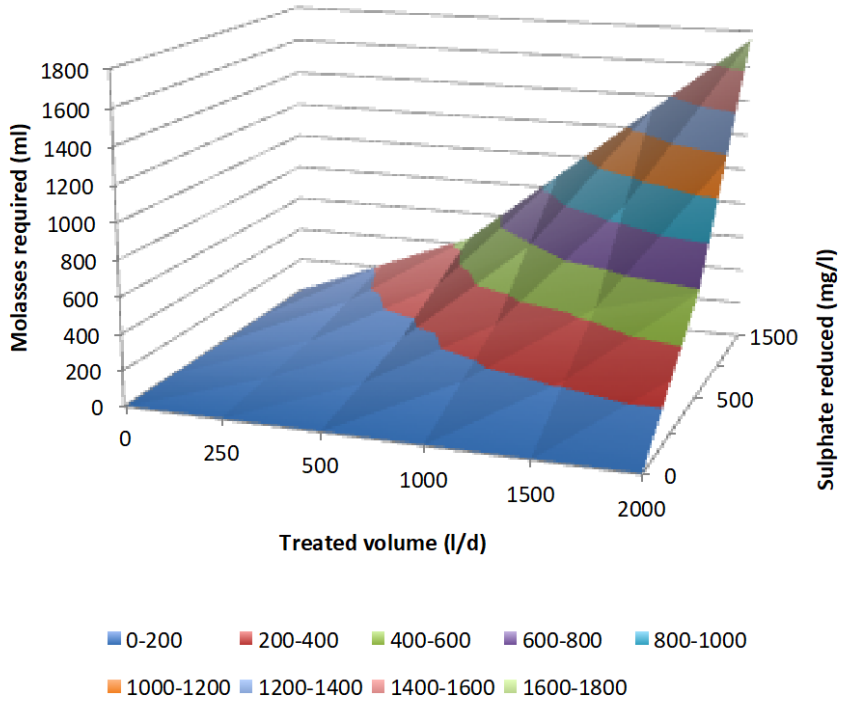

Figure 54: Volume of molasses required to achieve desire levels of sulphate reduction

Application of the model to fresh grass cuttings was not based on the measured COD of the material, but rather the amount of soluble COD liberated during the batch tests. The long term batch studies showed that when using partially dried (2 day old) grass as the substrate the COD:SO_4^{2-} ratio required was between 3.5 and 4.2, rather than 0.7, which showed that even with extended contact times the majority of the COD locked up in the grass is not accessible.

The model for substrate requirement using grass cuttings is presented in Figure 55. This shows that to treat 2 000 l/d of mine water to remove 1500 mg/l of sulphate would require almost 140 kg of grass cuttings. Furthermore, to sustain the release of soluble COD at the required rate the biomass would need to be turned over every 48 hours.

The laboratory tests, conducted in the 8 l reactor, showed that the reactor could hold 185 g of grass cuttings, so assuming a similar packing density the 2 000 l reactor could only hold 46.26 kg of grass. The reactor would therefore need to be significantly bigger.

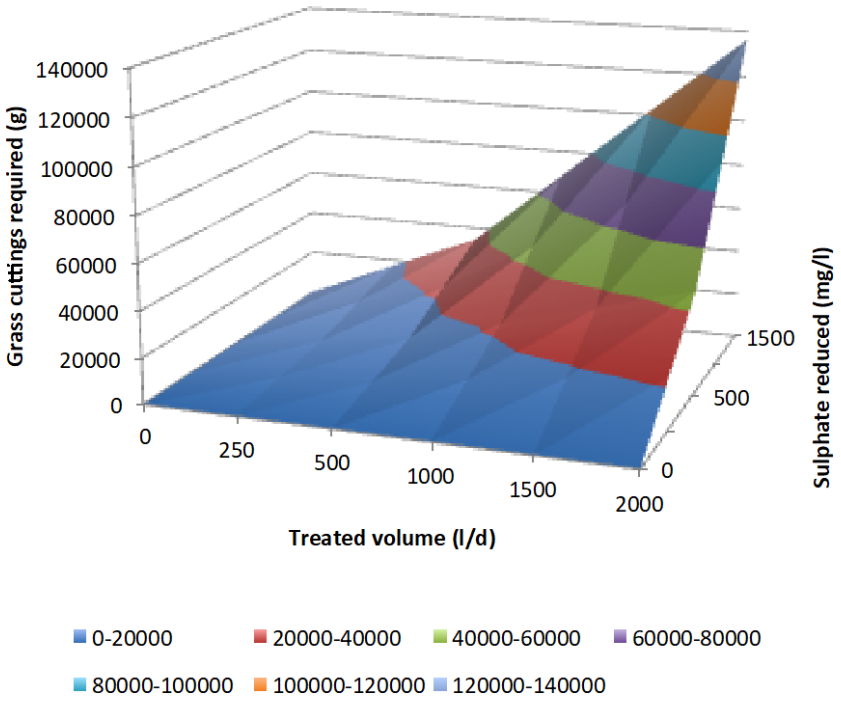

Figure 55: Amount of fresh grass cuttings required to provide sufficient soluble COD to sustain the required sulphate reduction rates

Considering the performance data generated at laboratory scale and the model for substrate required it is clear that grass cuttings are not a viable substrate to sustain the semi-passive system at the desired hydraulic retention times. This is before the logistical challenges of moving the tonnages of grass required and disposing of the potentially hazardous residues are considered. The lack of feasibility, together with the serious damage to two of the reactors meant that test work at the site ceased.

CHAPTER 6: CONCLUSIONS AND CHALLENGES THAT NEED TO BE ADDRESSED

6.1 CONCLUSIONS

The tests conducted at the first mine site showed that it was possible to replicate the floating biofilm formation in the pilot-scale hybrid linear flow channel reactor. Biofilm formation was complete and a significant amount of elemental sulphur could be recovered. Sulphate reduction in the reactor was demonstrated if a suitable soluble organic carbon source was provided.

Unfortunately, attempts to transition to the use of a complex organic carbon source as the substrate proved unsuccessful and further analysis indicated that a massive improvement in the rates of hydrolysis and acidogenesis would be required to release soluble organic carbon at the required rate. Upon critical evaluation of the proposed system the following challenges would need to be overcome to make the process viable:

1. A cost effective organic carbon source that can provide at least 0.7 g soluble, bioavailable COD per g of sulphate that needs to be reduced needs to be found. It is very unlikely that a bed packed with cellulosic or lignocellulosic material could achieve this, unless it was many times larger than the sulphate reducing reactors so the HRT in the hydrolysis reactor could be greatly extended.
2. The substrate is available year-round at a location close to the treatment plant to ensure continuity of supply and reduce transport costs.
3. If the process needs to treat acidic or metal laden effluent it either needs to be able to generate sufficient alkalinity to neutralise the acidity in the feed and precipitate metals or the water needs to pass through an effective pre-treatment stage. The hydrolysis reactor could play a role in pre-treatment.
4. If the substrate does not degrade extensively and needs periodic replacement the management of the residual material needs to be viable from a logistic and economic perspective.
5. The channel reactors will need some form of covering to ensure that the floating biofilm if not prematurely disrupted my wind or rain.

6.2 POTENTIAL FOR FUTURE APPLICATION

The work conducted to date has shown that the LFCR is an effective reactor to manage the sulphide generated during biological sulphate reduction, reducing the aqueous concentration to acceptable levels and managing the odour problem, while producing a bio-sulphur product that may have economic value.

Therefore, the reactor could be applied as a dedicated sulphide oxidation unit to treat liquid effluent from other types of SRB reactor. This was the original rationale behind the development of the LFCR and the work conducted in this, and the preceding WRC funded projects has overcome the problems that caused the failure of the original channel reactors.

The hybrid LFCR may still have potential as a single reactor unit that supports both sulphate reduction and sulphide oxidation if a suitable organic carbon source is available. The hybrid reactor has been tested, with some success, at laboratory scale to pre-treat tannery effluent, significantly reducing the sulphide concentration and the total sulphur species load. The treated effluent was shown to be more amenable to anaerobic digestion to produce biogas.

An increasing number of commercial anaerobic digesters are being commissioned in SA, primarily to treat agricultural and agri-processing residues (manure, slaughterhouse waste, crop residues). The digestate from these reactors still contains several grams of soluble COD, which may be in a form that is accessible to SRB. Co-location of a large-scale AD plant with the hybrid LFCR system may allow the digestate to be used as a substrate for mine water treatment.

Therefore, while the research has not been able to meet several of the primary aims of the project it has still generated a number of valuable insights.

REFERENCES

ADAMS, B., GALE, I., YOUNGER, P.L., LERNER, D. and CHILTON, J. (2000). Groundwater. In: ACREMAN M (ed.) *The Hydrology of the UK. A study of change*. Routledge, London, pp 150-179.

AKCIL, A. and KOLDAS, S. (2006). Acid Mine Drainage (AMD): causes, treatment and case studies. Journal of Cleaner Production **14** 1139-1145.

ALI, M. and SREEKRISHNAN, T.R. (2001). Aquatic toxicity from pulp and paper mill effluents: A review. Advances in Environmental Research **5** 175-196.

ALI, T.A. and WAINWRIGHT, M. (1995). Effect of *Phanerochate chrysosporium* on the transformations of urea, sulphur-coated urea and peptone in soil. Bioresource Technology **53** 91-93.

BAILEY, J.S., DENG, Y. and SMITH, R.V. (2001). Changes in soil organic carbon storage under grassland as evidenced by changes in sulphur input-output budgets. Chemosphere **42** 141-151.

BIJMANS, M.F.M., BUISMAN, C.J.N., MEULEPAS, R.J.W. and LENS, P.N.L. (2011). Sulphate reduction for inorganic waste and process water treatment. In: MOO-YOUNG M (ed.) *Comprehensive Biotechnology*. Elsevier Academic Press, pp 435-446.

BLAKE-KALFF, M.M.A., ZHAO, F.J., HAWKESFORD, M.J. and MCGRATH, S.P. (2001). Using plant analysis to predict yield losses caused by sulphur deficiency. Annals of Applied Biology **138** 123-127.

BRIMBLECOMBE, P. (2014). The global sulphur cycle. In: HOLLAND H and TUREKIAN K (eds) *Treatise in Geochemistry 2nd Edition*. Elsevier, pp. 559-591.

COETSER, S.E., HEATH, R., MOLWANTWA, J.B., ROSE, P.D. and PULLES, W. (2005). Implementation of the degrading packed bed reactor technology and verification of the long-term performance of passive treatment plants at Vryheid Coronation Colliery. Research report no. 1348/1/05, Water Research Commission, Pretoria, South Africa.

DAI, J., HUF, D.R. and SCHLOSSBERG, M.J. (2009). Salinity effects on seed germination and vegetative growth of greens-type *Poa annua* relative to other cool-season turf grass species. Crop Science **49** 696-703.

DOMAGAL-GOLDMAN, S.D., MEADOWS, V.S., CLAIRE, M.W. and KASTING, J.F. (2011). Using biogenic sulphur gases as remotely detectable biosignatures on anoxic planets. Astrobiology **11** 419-441.

ERCOLI, L., ARDUINI, I., MARIOTTI, M., LULLI, L. and MSONI, A. (2012). Management of sulphur fertiliser to improve durum wheat production and minimise S leaching. European Journal of Agronomy **38** 74-82.

ERIKSON, J. (2009). Sulfur cycling in temperate agricultural systems. Advances in Agronomy **102** 55-89.

FOX, A., GAHAN, J., IKOYI, I., KWAPINSKI, W., O'SULLIVAN, O., COTTER, P.D. and SCHMALENBERGER, A. (2016). *Miscanthus* biochar promotes growth of spring barley and shifts bacterial community structures including phosphorous and sulphur mobilizing bacteria. Pedobiologia – Journal of Soil Ecology **59** 195-202.

FRAUSTO DA SILVA, J.J.R. and WILLIAMS, R.J.P. (2001). *The Biological Chemistry of the Elements.* Oxford University Press, Oxford.

GAZEA, B., ADAM, K. and KONTOPOULOS, A. (1996). A review of passive systems for the treatment of acid mine drainage. Minerals Engineering **9** 23-42.

GREBEN, H., BALOYI, J., SIGAMA, J. and VENTER, S.N. (2009). Bioremediation of sulphate rich mine effluents using grass cuttings and rumen fluid microorganisms. Journal of Geochemical Exploration **100** 163-168.

GÜNTHER, P. and NAIDU, T. (2008). Mine water reclamation − Towards zero disposal. *Proceedings of the WISA Biennial Conference*, Sun City, South Africa.

HAIDER, R., GHAURI, M.A., SAN FILIPO, J.R., JONES, E.J., OREM, W.H., TATU, C.A., AKHTAR, K. and AKHTAR, N. (2013). Fungal degradation of coal as a pretreatment for methane production. Fuel **104** 717-725.

HAMMACK, R.W., DVORAK, D.H. and EDENBORN, H.M. (1993). The use of biogenic hydrogen sulphide to selectively recover copper and zinc from severely contaminated mine drainage. In: TORMA AE, WEY JE and LAKSHMANAN VL (eds) *Biohydrometallurgical Technologies*, The Minerals, Metal & Materials Society, pp 631-639.

HAVLIN, J.L., BEATON, J.D., TISDALE, S.L. and NELSON, W.L. (1999). *Soil Fertility and Fertilizers.* Prentice-Hall, New Jersey.

HEDIN, R.S., NAIRN, R.W. and 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.

HOVE, M., VAN HILLE, R.P. and LEWIS, A.E. (2009). The effect of different types of seeds on the oxidation and precipitation of iron. Hydrometallurgy **97** 180-184.

JANSSEN, A.J.H., SLEYSTER, R., VAN DER KAA, C., JOCHEMSEN, J,. BONTSEMA, J. and LETTINGA, G. (1995). Biological sulphide oxidation in a fed-batch reactor. Biotechnology and Bioengineering **47** 327-333.

JOHNSON, D.B. and HALLBERG, K.B. (2003). The microbiology of acidic mine waters. Research in Microbiology **154** 466-473.

JOHNSON, D.B. and HALLBERG, K.B. (2005). Acid mine drainage remediation options: a review. The Science of the Total Environment **338** 3-14.

JOVANOVIC, N.Z., BARNARD, R.O., RETHMAN, N.F.G. and ANNANDALE, J.G. (1998). Crops can be irrigated with lime-treated acid mine drainage. Water SA **24** 113-122.

LOEWENTHAL, R.E., MORGAN, B.E. and LAHAV, O. (2001). Iron and heavy metals in acid mine drainage waters – equilibrium and treatment considerations. Water Sewage & Effluent **21** 15-23.

LORAX INTERNATIONAL (2003). Treatment of Sulphate Mine Effluents. Technical report, International Network for Acid Prevention (INAP).

MCCARTNEY, D.M. and OLESZKIEWICZ, J.A. (1993). Competition between methanogens and sulphate reducers: Effect of COD: sulphate ratio and acclimatization. Water and Environmental Research **65** 655-664.

MOHAMED, A.A., EWEDA, W.E.E., HEGGO, A.M. and HASSA, E.A. (2014). Effect of dual inoculation with arbuscular mycorrhizal fungi and sulphur-oxidising bacteria on onion (*Allium cepa* L.) and maize (*Zea mays* L.) grown in sandy soil under green house conditions. Annals of Agricultural Science **59** 109-118.

MOKONE, T.P., VAN HILLE, R.P. and LEWIS, A.E. (2010). Effect of solution chemistry on particle characteristics during metal sulphide precipitation. Journal of Colloid and Interface Sciences **351** 10-18.

MOGOWO, W.E., SHERIDAN, C. and RUMBOLD, K. (2020). Global co-occurrence of acid mine drainage and organic rich industrial and domestic effluent: Biological sulfate reduction as a co-treatment option. Journal of Water Process Engineering **38** 101650.

MOLWANTWA, J.B. and ROSE, P.D. (2013). Development of a Linear Flow Channel Reactor for sulphur removal in acid mine wastewater treatment operations. Water SA **39** 649-654.

MOLWANTWA, J.B., COETSER, S.E., HEATH, R., PULLES, W., BOWKER, M. and ROSE, P.D. (2010). *Monitoring, evaluation and long-term verification of the passive water treatment plant at Vryheid Coronation Colliery*. Research report no. 1623/1/10, Water Research Commission, South Africa.

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.

MULOPO, J. (2016). Pilot scale assessment of the continuous biological sulphate removal from coal acid mine effluent using grass cuttings as carbon and energy sources. Journal of Water Process Engineering **11** 104-109.

NATHOO, J., JIVANI, R. and LEWIS, A.E. (2009). Freezing Your Brines Off: Eutectic Freeze Crystallization for Brine Treatment. *Proceedings of the International Mine Water Conference 2009*. International Mine Water Association, 424-430.

OYEKOLA, O.O. (2008). *An investigation into the relationship between process kinetics and microbial community dynamics in a lactate-fed sulphidogenic CSTR as a function of residence time and sulphate loading*. Ph.D. thesis, University of Cape Town.

PULLES, W. and HEATH, R. (2009). The evolution of passive mine water treatment technology for sulphate removal. *Proceedings of the International Mine Water Conference 2009,* Pretoria, South Africa. International Mine Water Association 2-15.

RAMLA, B. and SHERIDAN, C. (2015). The potential utilisation of indigenous South African grasses for acid mine drainage remediation. Water SA **41** 247-252.

ROMAN, H. (2004). *The degradation of lignocellulose in a biologically-generated sulphidic environment*. PhD thesis, Rhodes University.

SCOTT, R. (1995). *Flooding of Central and East Rand gold mines: An investigation into controls over the inflow rate, water quality and predicted impacts of flooded mines*. Research report no. 486/1/95, Water Research Commission, Pretoria, South Africa.

THOMAS, S.G., HOCKING, T.J. and BILSBORROW, P.E. (2003). Effect of sulphur fertilisation on the growth and metabolism of sugar beet grown on soils of differing sulphur status. Field Crops Research **83** 223-235.

TRUTER, W., JONKER, R., OLIVIER, J., NELL, B. and JANSEN VAN RENSBURG, M. (2011). Rehabilitation of Mined Land: Irrigation of pastures with gypsiferous mine water. Final Report, Project 8.3, Coaltech.

VAN BILJON, J.J., FOUCHE, D. and BOTHA, A.P.D. (2004). Threshold values for sulphur in soils of the main maize-producing areas of South Africa. South African Journal of Plant and Soil **21** 152-156.

VAN HILLE, R.P. and MOORUTH, N. (2011.) *Investigation of sulphide oxidation kinetics and impact of reactor design during passive treatment of mine water.* Research report no. KV 268/11, Water Research Commission, Pretoria, South Africa.

VAN HILLE, R.P. and 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.

WHITE, C.L., TABEL, M., DOVE, H., HAMBLIN, J., YOUNG, P., PHILLIPS, N., TAYLOR, R., GULATI, S., ASHES, J. and HIGGINS, T.J.V. (2001). Increased efficiency of wool growth and live weight gain in Merino sheep fed transgenic lupin seed containing sunflower albumin. Journal of the Science of Food and Agriculture **81** 147-154.

YOUNGER, P.L. (1997). The longevity of minewater pollution: a basis for decision making. The Science of the Total Environment **194/195** 457-466.

YOUNGER, P.L. (2000). Predicting temporal changes in total iron concentrations in groundwaters flowing from abandoned deep mines: a first approximation. Journal of Contaminant Hydrology **44** 47-69.

ZAGURY, G.J., NECULITA, C.M. and BUSSIÈRE, B. (2007). Passive treatment of acid mine drainage in bioreactors using sulfate-reducing bacteria: critical review and research needs. Journal of Environmental Quality **36** 1-16.

ZHAO, F.J., FORTUNE, S., BARBOSA, V.L., MCGRATH, S.P., STOBART, R., BILSBORROW, P.E., BOOTH, E.J., BROWN, A. and ROBSON, P. (2006). Effects of sulphur on yield and malting quality of barley. Journal of Cereal Science **43** 369-377.

ZHAO, F.J., LEHMANN, J., SOLOMAN, D., FOX, M.A. and MCGRATH, S.P. (2006). Sulphur speciation and turnover in soils: evidence from sulphur K-edge XANES spectroscopy and isotope dilution studies. Soil Biology and Biochemistry **38** 1000-1007.