INVESTIGATION OF SULPHIDE OXIDATION KINETICS AND IMPACT OF REACTOR DESIGN DURING PASSIVE TREATMENT OF MINE WATER

Report to the

Water Research Commission

by

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EXECUTIVE SUMMARY

Water is increasingly being recognised as an important strategic resource and the sustainability of water resources is becoming the focus of more attention. South Africa has been classified as a water-stressed country. A 1999 report by the Department of Water Affairs and Forestry (DWAF) predicted that the demand for potable water would exceed the supply by 2020. The provision of services to previously disadvantaged communities and the potential for large scale contamination of water resources mean that this may occur sooner than expected.

The threat of acid rock drainage (ARD) to South Africa's water resources, particularly in the gold and coal mining regions was identified over 20 years ago, but the response to the threat has in general been inadequate. Acid rock drainage originates from the oxidation of sulphide minerals that are exposed to oxygen and water. This typically occurs as a consequence of mining or mining-related activities. The process may occur through chemical weathering, but is substantially accelerated by the action of autotrophic iron and sulphur oxidising microbes. Acid rock drainage is a long term issue, with discharges from contaminated sites predicted to persist for decades to centuries. The management of ARD discharges needs to be considered accordingly.

A number of treatment technologies have been developed for the remediation of ARD. The most widely used methods are active, chemical processes that involve oxidation of metal ions to their least soluble state, neutralisation of the acidity, precipitation of metal oxides or hydroxides and solid liquid separation by sedimentation. The end product from such systems is a metal sludge and a water stream that is near neutral pH, with very low concentrations of most soluble metals. However, the sulphate load in the partially treated water is still unacceptably high, typically in the region of 2 000 to 3 000 mg/L. The sulphate concentration is governed by the solubility of gypsum ($CaSO_4$), which forms as a consequence of pH neutralisation with lime or limestone. A number of physical and chemical technologies, such as reverse osmosis and ion exchange, are available to reduce the sulphate load and have been successfully implemented in places. These technologies are costly, particularly in terms of operating costs and are not economically viable as long terms options, unless substantial value recovery from by-products can be integrated into the system.

Biological systems offer a potentially effective alternative to active physical and chemical processes, with lower operating costs and enhanced sustainability. These systems are based on the action of sulphate reducing bacteria (SRB), which reduce sulphate to bisulphide (HS^-) with the generation of bicarbonate alkalinity as a by-product. The primary operating cost associated with these systems is the provision of the electron donor/carbon source. Most commercial systems use ethanol, methanol or volatile fatty acids (VFAs). Recent research has focussed on the utilisation of complex or waste carbon sources, such as sewage sludge and lignocellulosic material. These need to be converted by associated microorganisms to produce the VFAs required by the SRB, which reduces process kinetics. They are more amenable to passive systems, where the retention times within the system are substantially higher.

The integrated managed passive (IMPI) process was developed by Pulles Howard and De Lange in association with Rhodes University. It is a semi-passive process, requiring minimal maintenance. The process incorporates a series of degrading packed bed reactors (DPBRs) to reduce the sulphate and sulphide oxidation reactors to convert the sulphide to elemental sulphur. The DPBRs are packed with discrete layers of manure, straw and wood chips that degrade over time to sustain the sulphate reduction. The sulphide oxidation is facilitated by a floating biofilm that incorporates sulphide oxidising microorganisms. The biofilm acts as a barrier to oxygen mass transfer and creates a redox and pH environment conducive to the partial oxidation of sulphide to elemental sulphur, the desired product. This technology has been implemented at a demonstration scale at BHP Billiton's Middelburg coal mine. The operation of the demonstration plant has been compromised by a number of design and technical issues. The work described in this report was commissioned to provide fundamental information that could be used to enhance process efficiency.

Two units of the IMPI process were simulated at the Department of Chemical Engineering at the University of Cape Town. Two DPBR columns were transported from the Golder Associates Research Laboratories (GARL) in Midrand and set up as saturated upflow systems. Three linear flow channel reactors (LFCRs) were designed and purpose built using similar dimensions to the pilot reactors at GARL. The LFCRs were designed to support a significantly enhanced sampling regime, with 15 sample ports, spread across three

levels, in the front wall. The reactors could be sealed and the headspace gas flushed at a controlled rate, allowing the quantification of sulphide lost to the air.

Several analytical techniques were developed and optimised to facilitate the measurement of a range of sulphur species, including polysulphides, and allow a complete sulphur mass balance to be closed.

Two abiotic control experiments were run, where the sulphide containing solution was pumped into the LFCRs in the absence of microorganisms. In the first case the pH was not controlled and ranged between pH 11.5 and pH 11.9. The solution remained clear and no sulphur was observed to form. The overall sulphide conversion was 24.7%, with the majority forming thiosulphate and polysulphides. Less than 5% of the converted sulphide reported as sulphate. The second abiotic control was run with the pH of the sulphide feed controlled at pH 7. During the operation of the reactor the pH fluctuated between pH 7.1 and pH 7.8 as a result of chemical reactions. The solution had a distinctive yellow-green colour and colloidal sulphur particles were visible for a short period. A total of 33.8% of the sulphide was converted, with no sulphate detected.

A series of experimental runs was performed in which feed from the DPBR columns was used. This effluent contained organisms capable of oxidising sulphide and forming the floating biofilm. The results from these studies illustrated the relationship between organic carbon flux, biofilm formation and efficient partial sulphide oxidation to elemental sulphur. During the first experimental run a discrete biofilm did not form, despite the presence of microorganisms in the liquid phase. The reactor achieved a sulphide conversion of approximately 70%, but the vast majority was fully oxidised back to sulphate. Colloidal sulphur particles were observed below the air-liquid interface. Less than 5% of the converted sulphide exited the reactor as colloidal sulphur, implying that it was an intermediate and the majority was further oxidised to sulphate. High performance liquid chromatography (HPLC) analysis of the DPBR effluent showed that almost no organic carbon (<5 mg/L acetate and no sugars) was leaving the columns. Extracellular polymeric substances (EPS) form an important structural component of biofilms, but are not excreted by cells under carbon limiting conditions. This finding explains the lack of biofilm formation and highlights the need for sufficient organic carbon flux through the system. The high sulphate concentration in the DPBR effluent also suggested that the columns were not reducing sulphate particularly efficiently.

The second reactor run resulted in the formation of a biofilm, but this did not extent across the entire surface of the reactor. The sulphide oxidation efficiency increased to over 90%, with the majority being converted to sulphur. Less than 10% of the consumed sulphide was fully oxidised to sulphate. However, a significant portion (30%) of the sulphur remained as colloidal particles suspended in the liquid and was lost in the effluent. While a discrete biofilm was observed it did not progress through the stages observed in previous studies and was uniformly “brittle” and “flaky”. Scanning electron microscopy, with electron dispersive x-ray analysis (SEM-EDX), showed the presence of discrete sulphur globules, embedded in a matrix consisting of some organic matter, but substantial chemical precipitates. These data again alluded to a limitation in organic carbon, which was confirmed by HPLC analysis of the DPBR overflow.

In order to address the issue of organic carbon subsequent experimental runs were performed with organic supplementation, in the form of 20 g of acetate added to the LFCR on start-up. In this case a complete biofilm was formed and stable operation was achieved after three days. The reactor performed well for the duration of the experiment, with a sulphide conversion efficiency of 82%. Of the converted sulphide 93% was partially oxidised to elemental sulphur, with 98.7% of the sulphur reporting to the biofilm. Although the biofilm did not pass through a distinct “sticky” phase and remained relatively brittle, the structural integrity was significantly improved.

One of the primary aims of this study was the determination of parameters describing the sulphide oxidation kinetics. A detailed hydrodynamic study, performed as part of the WRC solicited project (K5/1834) showed significant inhomogeneity within the reactor, meaning that plug flow could not be assumed, as had been the case for previous work. The complexity of the fluid flow meant that simple kinetic models could not be used. As a first approximation, in order to determine the rate order of the reaction, each of the 15 sampling ports was modelled as a batch reactor. This allowed the determination of rate constants for sulphide disappearance in the bulk fluid, from which the rates could be estimated. For the abiotic controls these were estimated at 0.005 mmoles/day and 0.31 mmoles/day for the uncontrolled and pH controlled systems

respectively. For the biological system where a complete biofilm was formed this increased to 1.61 mmoles/day.

The LFCR data highlighted the importance of organic carbon flux through the integrated system. Analysis of the effluent from the DPBR reactors showed that very little organic carbon present. This was despite the supplementation of the DPBR feed with 1.5 g/L molasses. The molasses feed concentration was increased to 2.5 g/L in an attempt to increase the organic carbon supply to the LFCRs. This had a positive effect for the first week, with the sulphate reduction efficiency of the DPBRs increasing. However, after a week the pH in the DPBRs dropped significantly and sulphate reduction activity was dramatically reduced. The feed was stopped and the columns re-inoculated with an active SRB culture, after which the molasses supplementation was reduced back to 1.5 g/L.

A series of batch tests (1 L) was performed to determine the sulphate reduction efficiency when molasses was the sole carbon source. The reactors were loaded with 2 g/L sulphate and 1.5 g/L molasses and inoculated with increasing volumes (20, 50 and 100 mL) of DPBR effluent. A positive control was run under similar conditions, but inoculated with 10 mL of active SRB sludge. The three reactors inoculated with the DPBR effluent rapidly (24 hours) converted the molasses to VFAs, resulting in a pH decrease to below pH 5. This inhibited sulphate reduction and no sulphide was produced for the duration of the experiment, despite sulphide supplementation (to 50 mg/L) after 24 hours to increase pH and reduce the redox potential. In contrast, the reactor inoculated with SRB sludge maintained the pH around pH 7 and produced sulphide at a linear rate for the first two days. This was followed by a 12 hour period of limited activity, followed by another 48 hours where sulphide was produced at a constant, but slightly lower rate. The data suggest that there are two carbon sources in the molasses that are sequentially metabolised. The sulphide concentration in the reactor reached a maximum of 250 mg/L (36% sulphate reduction) after 111 hours. This value is similar to the highest sulphide concentration detected in the DPBR effluent when the feed was supplemented with 1.5 g/L molasses. This suggests that the molasses, which is added to “kickstart” the utilisation of the lignocellulosic material, is responsible for supporting the majority of the sulphate reduction in the DPBRs. The rapid conversion of molasses to VFAs in the batch tests inoculated with DPBR overflow further suggests that the molasses supplementation has selected for a population that preferentially metabolises molasses.

In conclusion, the work presented in this report has demonstrated that efficient sulphide oxidation is possible in the LFCRs, provided the organic carbon and sulphide concentrations in the feed solution are sufficient and stable. Sufficient organic carbon is required to sustain a stable biofilm, which is necessary to prevent the sulphide from being fully oxidised back to sulphate. The oxygen mass transfer into the system is independent of the sulphide concentration in the influent, so large fluctuations in the feed sulphide concentration (as observed from the DPBR columns) can result in the sulphide to oxygen stoichiometry becoming favourable for oxidation beyond sulphur, to thiosulphate or sulphate. Therefore, while the data indicate that the process could be effective there is currently insufficient data on the stability of the DPBR effluent, in terms of sulphide and organic carbon concentrations, to conclude that performance will be consistent.

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1 GENERAL INTRODUCTION

Acid rock drainage (ARD) is currently a significant problem within the mining and minerals processing sector of South Africa. The biological treatment of mine waters has received increased attention due to its potential as a sustainable and economically attractive alternative to chemical treatment. Moreover, the development of a long-term sustainable technology would be a highly preferable alternative to the current treatment methods. This is due to the fact that many mines have been abandoned and therefore a cheap alternative to process the wastewater is required.

The Integrated managed passive (IMPI) system was developed by Pulles Howard and De Lange, in association with Rhodes University. The process relies on a combination of biological sulphate reduction, utilising complex organic carbon sources, and sulphide oxidation to remediate partially treated mine water. As most ARD is derived from pyrite (FeS_2), the resulting sulphate load is typically higher than the metal load, so even if metal sulphide precipitation is engineered into the process a sulphide mediation step will be required. Studies conducted by the Environmental Biotechnology Research Unit (EBRU) at Rhodes University and Golder Associates Africa (GAA) suggested that biological sulphide oxidation was a potential technology that may be utilised in the passive treatment system to achieve this. This led to the development of the linear flow channel reactor (LFCR) and the application of the IMPI technology at demonstration scale at the Middelburg Mine Site. However, the demonstration plant has not yet operated efficiently at its full potential. A lack of fundamental kinetic and mass balance information has contributed to this.

Therefore, a key aim of this project is to conduct a study on the LFCR in order to generate the necessary kinetic and mass balance information, which is imperative for the characterisation and efficient operation of the demonstration plant.

This report provides background information on the problem of ARD, detailing the mechanisms of formation and impacts on human health and the receiving environment. The conventional treatment options are briefly discussed to place the IMPI technology in context, after which the biological sulphur cycle is reviewed in some detail. Particular focus is placed how the biofilm is likely to affect sulphide oxidation kinetics. The specific aims of the project, explaining how these align with K5/1834, are presented after which the methodology and experimental results are presented and discussed. The key findings of the research are summarised and the implications for operation of the demonstration plant highlighted.

1.1 Acid rock drainage

Acid rock drainage (ARD) and similar effluents continue to be a significant problem within the industrial sector, not only in South Africa but other parts of the world. As the global population and the demand for commodities continue to expand, the rapid increase in industrial activity is resulting in a greater generation of wastewaters. These wastewaters currently pose a threat to the surrounding ecosystems and habitats. These wastewaters are typically generated by the following industries: pulp, paper, chemical, metallurgical and mining (Oyekola, 2008).

The effluents from the above-mentioned industries are generally rich in sulphates, sulphides and dissolved metals. The mining and minerals processing industries is the largest contributor to ARD, posing the greatest risk to the environment and receiving water bodies. As a result, the mitigation and treatment of ARD warrants considerable attention and management (Naicker et al., 2003).

1.1.1 Generation of acid rock drainage

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 coals (Johnson and Hallberg, 2005). The sulphide minerals may be exposed as tailings or waste rock, ore stockpiles or in operating and abandoned mine workings. ARD generation can occur abiotically, through chemical weathering, but the presence of specific iron and sulphur oxidising microorganisms can increase the kinetics of the process up to a thousand-fold. The reactions involved are detailed below (Reactions 1-4) (Akcil and Koldas, 2006).

$$\mathrm{FeS_2} + \frac{7}{2}\,\mathrm{O_2} + \mathrm{H_2O} \rightarrow \mathrm{Fe^{2+}} + 2\mathrm{SO_4^{2-}} + 2\mathrm{H^+} \quad (1)$$

$$Fe^{2+} + \frac{1}{4}O_2 + H^+ \rightarrow Fe^{3+} + \frac{1}{2}H_2O \quad (2)$$

$$Fe^{3+} + 3H_2O \rightarrow Fe(OH)_3 + 3H^+ \quad (3)$$

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{3+} + 2SO_4^{2-} + 16H^+ \quad (4)$$

Pyrite (FeS_2) is the most abundant sulphide mineral and is the primary mineral responsible for acid rock drainage generation (Oyekola, 2008). 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 (reaction 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 reaction 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 reaction 4, thus producing more ferrous iron to drive reaction 2. In the presence of sufficient dissolved oxygen, a continuous cycle of reactions 2 and 3 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 (Figure 1), are typically found in these environments and contribute to ARD formation. This group, which includes *Acidithiobacillus thiooxidans* and *Acidithiobacillus 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 ARD. 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.

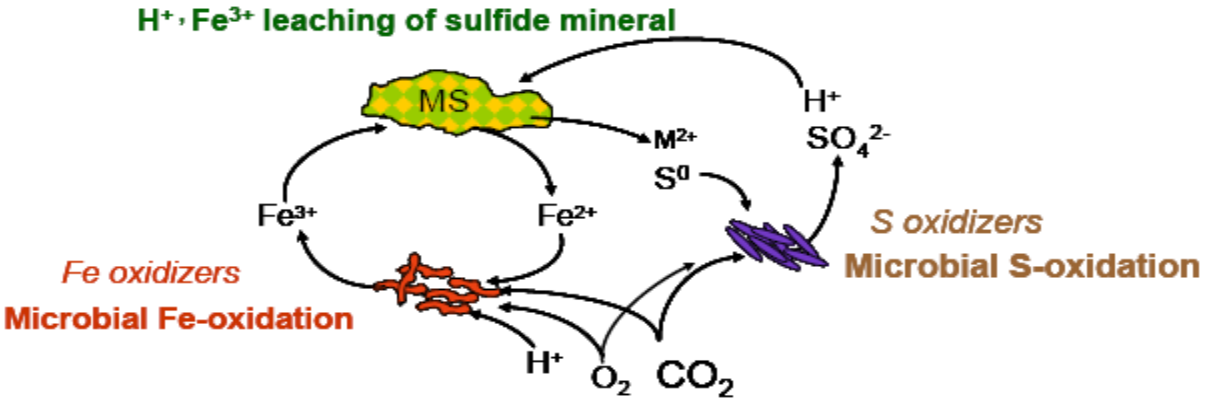

Figure 1: Schematic representation of the reactions catalysed by iron and sulphur-oxidising microorganisms (Ojumu, 2008)

The closure, particularly of deep-level mines poses a serious threat in terms of uncontrolled ARD 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.

1.1.2 Effects on human health

Acid rock drainage may contain a number of heavy metals (copper, lead, nickel, mercury, chromium and zinc) that are acutely toxic to humans and livestock. These elements can be readily removed by neutralisation followed by precipitation. The issue of salinity, particularly sulphate ions, is more difficult to address and may be more likely to affect human health where partial treatment is in place. The acceptable water quality standards set by the American EPA stipulates that the maximum amount of allowable dissolved sulphates is 250 mg/L or less. The water legislations allow for a sulphate content of potable water in the range of 0-200 mg/L (CSIR-Environmental-Services and Holmes, 1996).

Table 1: Effect of elevated sulphate concentrations in drinking water on human health

Sulphate Range (mg/L)	Effects
0 - 200	No health or aesthetic effects are experienced
200 - 400	Tendency to develop diarrhoea in sensitive and some non-adapted individuals. Slight taste noticeable
400 - 600	Diarrhoea in most non-adapted individuals. Definite salty or bitter taste
600 - 1000	Diarrhoea in most individuals. User-adaptation does not occur. Pronounced salty or bitter taste.
> 1000	Diarrhoea in all individuals. User-adaptation does not occur. Very strong salty and bitter taste.

From Table 1 it is evident that a concentration of sulphate exceeding 200 mg/L can have adverse effects on the human body. In South Africa the discharge of raw or partially treated ARD into receiving waters of the Vaal River catchment area have led to significant salinisation, primarily as a result of sulphate. Elevated sulphate concentrations have been recorded in surface and groundwater sources and conservative estimates suggest the salinity of the Vaal River has been increasing at a rate of at least 15 mg/L.annum (Roos and Pieterse, 1995; Tutu et al., 2008). Therefore, a sustainable ARD treatment technology needs to address the issue of salinity in order to provide a complete solution.

1.1.3 Effects on the environment

Acid rock drainage has a profound effect on the aquatic ecosystems of receiving environments. In the case of low volume discharges the pH affect is generally low, although acidification of receiving waters can occur where discharge volumes are high. At low (sub-lethal) concentrations heavy metals are accumulated by many aquatic organisms becoming increasingly concentrated higher up the food chain. At higher concentrations, acutely toxic elements (Cu, Pb, Zn, Cd, Ni and Hg) have a devastating effect on aquatic organisms. Their effect is diverse and includes the denaturation of proteins (Meisenberg and Simmons, 2006), damage to nucleic acids (Gupta and Sarin, 2009), the inactivation of enzymes (Teisseire and Vernet, 2000), the destabilisation of membrane structures (Brady and Duncan, 1994; Franklin et al., 2000) and the retardation of electron transport chains and photosynthesis (Asthana et al., 1992; Jin et al., 1996).

While heavy metals such as iron, aluminium and manganese are less acutely toxic, they oxidise and form bulky hydroxide precipitates in oxygen-rich receiving waters. These precipitates can still have a devastating effect on aquatic invertebrates, coating their gills and breathing structures and reducing adhesion points to the substrate (Younger, 1995; Jooste and Thirion, 1999).

1.2 Treatment technologies

A variety of technologies have been developed for the treatment of 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 pH of the water (Gazea et al., 1996). ARD treatment technologies can be divided into two broad categories, active and passive treatment systems.

1.2.1 Active treatment technologies

Active treatment typically involves the installation of agitated reactors or similar units, which require constant energy input and typically require the continuous addition of neutralising chemicals. There is a diverse range of active treatment technologies such as chemical precipitation, ion-exchange, membrane technology or biological sulphate reduction. Many of these have been well researched, are efficient and have been implemented at industrial scale. However, the addition of alkaline chemicals and reagents to treat the acidic effluent can become a costly process, given that the drainage will be on many decommissioned mine sites (Gazea et al., 1996). Similarly, the capital and operational costs of technologies such as reverse osmosis and ion-exchange make their utilisation as long term remediation options economically unattractive, unless significant value add can be derived from end products. Biological sulphate reduction (BSR) technologies has the potential to provide a more cost effective alternative to conventional physical or chemical processes, but despite many years of research and demonstration of technical feasibility, large scale application of the technology has been limited. The cost of the electron donor has been a major stumbling block, although more recent research has focused on the utilisation of complex or waste carbon sources for this.

1.2.2 Passive treatment technologies

Natural processes typically ameliorate ARD pollution. As the contaminated water flows through the receiving systems the toxicity is reduced naturally as a result of chemical and biological reactions and dilution with uncontaminated waters. These phenomena formed the basis for the development of passive treatment technologies, which depend on naturally occurring chemical and biological reactions. Ideally these systems require no further addition of chemicals and little or no operational and maintenance inputs. Passive systems depend on processes that are kinetically slower than those involved in active systems and thus require longer residence times 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.

The interest in passive systems was sparked by research in the late 1980's which indicated that natural *Sphagnum* wetlands improved the quality of mine drainage without incurring any obvious ecological damage (Wieder and Lang, 1992). A number of experimental wetlands were constructed to mimic the *Sphagnum* moss wetlands. However, *Sphagnum* moss was not readily available, proved difficult to transplant and had the tendency to accumulate heavy metals to toxic levels within a few months (Spratt and Wieder, 1988). Despite the initial setbacks research continued and a design evolved that proved tolerant to years of exposure to contaminated mine drainage and was effective at lowering the concentration of dissolved metals. The systems typically consisted of a series of small wetlands (< 1 ha), vegetated with cattails (*Typha latifolia*) (Wieder, 1989).

During later development the importance on anaerobic processes in the metal removal was recognised. It was found that in such situations a complex ecosystem was not required and treatment cells could operate effectively without plants. Recent evidence (White et al., 2011) suggests that plant derived organics actually reduce the efficiency of wetlands, primarily due to the complexation of metals by phenolic compounds.

Pretreatment systems were also developed, where the acidic waters were contacted with limestone in an anoxic environment prior to entering the settling pond or wetland system (Gazea et al., 1996).

The Integrated managed passive (IMPI) system was developed by Pulles Howard and De Lange, in association with Rhodes University and represents a slight departure from a typical passive system in that limited maintenance has been designed into the system. The process is designed around a sequence of degrading packed bed reactors (DPBRs) followed by sulphide oxidation units. The DPBRs are packed with

specifically selected organic carbon sources (wood chips, manure, straw) placed in discrete layers. The system is designed to release volatile fatty acids, which sustain the sulphate reducing organisms, over a sustained period (Coetser et al., 2004).

The effluent stream from the DPBR is rich in sulphide and requires additional treatment in order for the stream to meet discharge requirements. This effluent passes into a sulphide oxidation reactor, where the desire is to achieve partial oxidation of the sulphide to elemental sulphur, without further oxidation back to sulphate.

1.3 The biological sulphur cycle

Sulphur is a key component of all living organisms and forms part of a wide range of organic molecules, from amino acids and antibiotics to complex lipids and carbohydrates. The biological roles of inorganic sulphur compounds are limited, with molecules serving as either sources for sulphur assimilation or as electron donors or acceptors in dissimilatory electron transport (Bruser et al., 2000).

The major compounds and reaction pathways associated with the biological sulphur cycle are presented in Figure 2. In most natural environments the reaction pathways depicted in the diagram are closely associated, with relatively little transfer of sulphur into or out of the system.

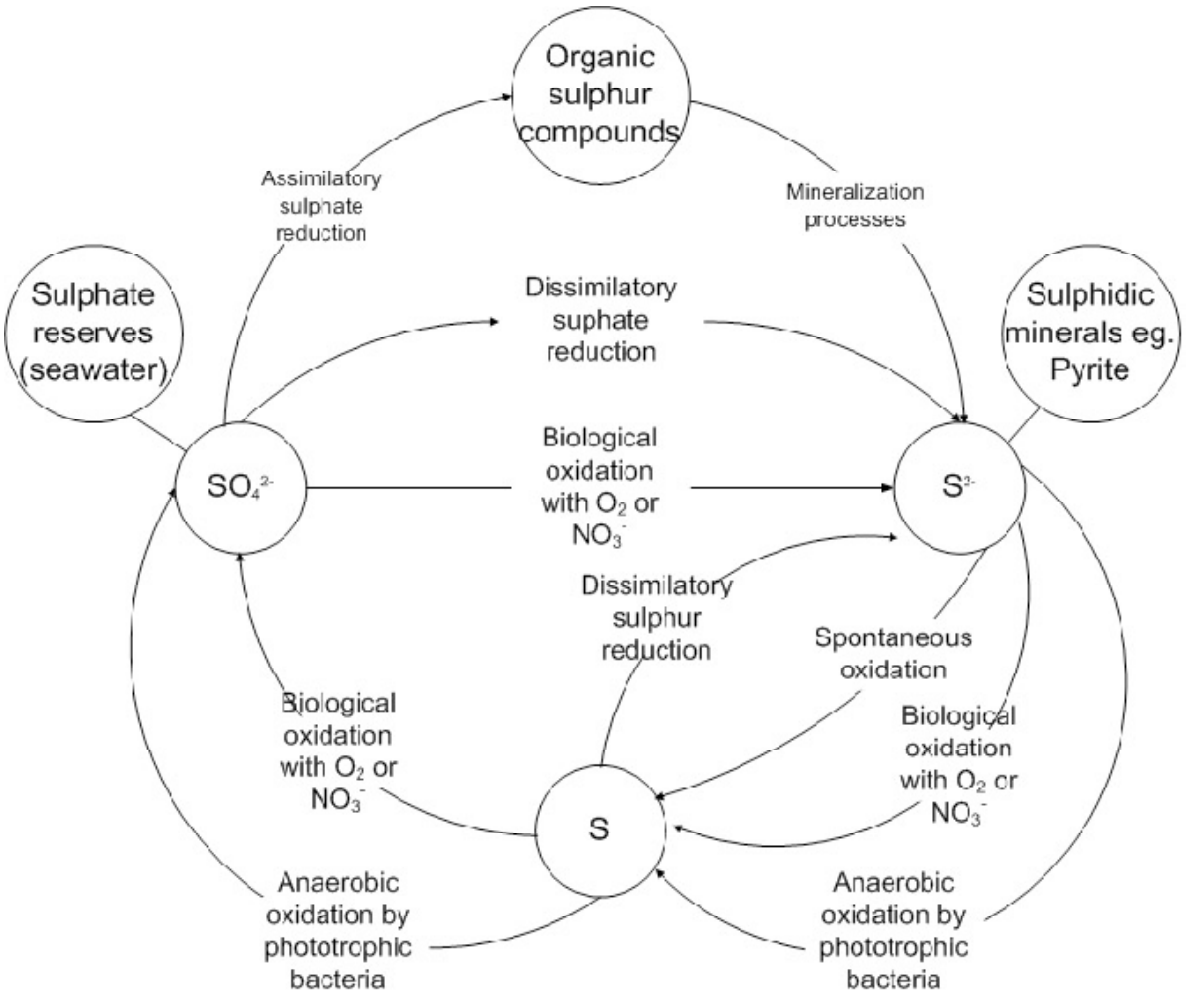

Figure 2: Schematic representation of the sulphur cycle (Robertson and Kuenen, 1992)

The most common inorganic sulphur compounds, available for assimilatory and dissimilatory reactions are sulphide, sulphate, elemental sulphur, thiosulphate, bisulphite, polysulphides and polythionates. Sulphide represents the most reduced form and sulphate the most oxidised (Table 2). The turnover of sulphur compounds in assimilatory pathways is relatively low as the reactions are intimately linked to microbial growth. By contrast the turnover is high in dissimilatory pathways, where the final product is released into the environment.

Table 2: Inorganic sulphur compounds of biological relevance (Bruser et al., 2000)

Compound	Chemical formula	Sulphur oxidation state
Sulphide	HS^-	-2
Polysulphides	$^-S(S)_nS^-$	-1 (terminal S) / 0 (inner S)
Thiosulphate	$S_2O_3^{2-}$	-1 (sulphane S) / +5 (sulphone S)
Polythionates	$^-O_3S(S)_nSO_3^-$	0 (inner S) / + 5 (sulphone S)
Elemental sulphur	S_n rings	0
Bisulfite	HSO_3^-	+4
Sulphate	SO_4^{2-}	+6

The abiotic and biologically mediated reactions involving inorganic sulphur compounds are interconnected and the abiotic formation of substrates can have a profound impact on the species composition in natural environments.

1.3.1 Chemical sulphide oxidation

In the following section the chemistry of sulphide oxidation and the mechanisms and conditions affecting chemical and biological sulphide oxidation are discussed with particular reference to how each process interacts with the other.

Wilmot and co-workers (1988) showed that chemical oxidation played just as significant a role as biological oxidation in the overall total oxidation of sulphides. Therefore, the following section will highlight the importance of chemical oxidation and how this affects biological sulphide oxidation.

Aqueous sulphide can theoretically occur as three chemical species HS^-, S^{2-} and H_2S. The sulphide generated within a biological sulphate reducing system will occur predominantly in an aqueous or dissociated state. The following equations represent the equilibrium between the three species of sulphide and these are strongly dependent on pH (pH=pK*i*; i = 1,2) (Mamashela, 2002; Kuhn et al., 1983).

$$H_2S \rightleftharpoons H^+ + HS^-,\ \mathrm{pKa} = 7.04\ (T = 18^oC) \quad (5)$$

$$HS^- \rightleftharpoons H^+ + S^{2-},\ \mathrm{pKa} = 11.86\ (T = 18^oC) \quad (6)$$

The value of the second dissociation constant has been the subject of much debate, with some calculations implying a value greater than 14. In practical terms the S^{2-} species can essentially be ignored.

The mechanisms involved in chemical sulphide oxidation have been shown to be dependent on the pH, temperature, sulphide and oxygen concentrations and the presence of neutral salts (Kuhn et al., 1983).

Ratio of sulphide to oxygen

In order to achieve the partial oxidation of sulphide to produce elemental sulphur, the desired product, a stoichiometric ratio of sulphide to oxygen of 2:1 is required. (Kuhn et al., 1983).

$$2HS^- + O_2 \rightleftharpoons \frac{1}{4} S_8 + 2OH^- \quad (7)$$

The reaction shown in Equation 8 illustrates the possibility of thiosulphate formation if the sulphide to oxygen ratio falls below 2:1 (Janssen et al., 1995).

$$2HS^- + 2O_2 \rightarrow H_2O + S_2O_3^{2-} \quad (8)$$

A further shift in the reaction stoichiometry, where more oxygen is available, promotes the formation of the sulphate ion.

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \quad (9)$$

Influence of pH

In acidic solutions (pH < 6) H_2S is the dominant sulphide species. However, as the pH increases toward pH 8 the speciation tends toward the bisulphide ion (as per Equation 5), which is far more soluble and allows more sulphide to remain in solution. The H_2S species does not readily oxidise, so the rate of chemical transformation is slow. As the pH increases the oxidation rate increases due to the increase in the HS^- concentration (Mamashela, 2002; Chen and Morris, 1972).

The pH also plays a role in the disproportionation of S^0 particles, leading to the formation of HS^- and $S_2O_3^-$ under alkaline conditions (Equation 10). In addition, at pH values > 9 more thiosulphate and sulphide are formed due to the low chemical stability of sulphur in the S^0 oxidation state. Hydrolysis of the elemental sulphur to sulphide or thiosulphate occurs faster than the hydrolysis of inorganic or biologically produced sulphur (Kleinjan et al., 2005; Van den Bosch et al., 2008).

$$4S^0 + 4OH^- \rightarrow S_2O_3^{2-} + 2HS^- + H_2O \quad (10)$$

The pH also affects the equilibrium between sulphide and polysulphides. This will be discussed in greater detail later in the review.

Influence of temperature

Zhang and Millero (1993) showed that the rate constant increased with increasing temperature from 25-45°C. This is consistent with the Arrhenius equation, which is a function of temperature. Wilmot et al. (1988) also reported an increase in the rate constant with an increase in temperature. The rate constant increased four-fold for a temperature increase from 10-40°C, suggesting a proportional relationship between the rate constant and increasing temperature.

1.3.2 Biological sulphide oxidation

Microbial contribution to the biological sulphur cycle

In nature, sulphur metabolising microorganisms play a vital role in the conversion of the various forms of sulphur. Sulphide can be biologically oxidised by denitrifying organisms, colourless bacteria (in the presence of oxygen) or anaerobically by photosynthetic bacteria (Bowker, 2002). Sulphide, polysulphides, thiosulphate, elemental sulphur, polythionates, bisulphite and sulphate are all inorganic sulphur compounds that can be utilised within a microbial community as an electron acceptor. This may occur via dissimilatory or assimilatory pathways (Bruser et al., 2000). In terms of an ARD remediation process, the preferred product is elemental sulphur, with the removal of all dissolved sulphide species.

The natural sulphur cycle is controlled by heterotrophic bacteria as well as specialised bacteria such as colourless sulphur bacteria, which interconvert the various forms of the element.

Heterotrophic bacteria depend upon organic sources of carbon, whereas autotrophs are able to utilise carbon dioxide from the atmosphere (Bowker, 2002). Most sulphide that accumulates within the environment is formed due to sulphate reduction by sulphate reducing bacteria (SRB). Bacteria such as *Desulfomonas*, *Desulfovibrio* and *Desulfotomaculum* are capable of dissimilatory sulphate reduction using SO_4^{2-}, $S_2O_3^{2-}$, SO_3^{2-}, $S_2O_4^{2-}$ and S^0 as a terminal electron acceptor, instead of oxygen. The heterotrophic SRB are obligately anaerobic organisms and derive energy from simple organic compounds such as pyruvate or lactate; whilst the autrotrophic SRB rely on CO_2 and oxidation of H_2. (Bowker, 2002; Oyekola, 2008; Lens and Kuenen, 2001). Thereafter, sulphide oxidising bacteria (SOB) oxidise the sulphides to elemental sulphur and beyond.

SOB are chemoautotrophic and obtain their energy from the oxidation of sulphur compounds. Sulphide oxidising bacteria can be divided into two main groups, the photosynthetic sulphur bacteria and the colourless sulphur bacteria. Photosynthetic sulphur bacteria use sulphide as the electron donor, CO_2 as the carbon source and the energy is provided by light (Molwantwa, 2007). Colourless sulphur bacteria were among the first group of biogeochemically important bacteria to be studies, in part due to the fact that several species are large and produce macroscopically visible structures such as mats (Gray and Head, 1999). These organisms generally oxidise sulphide to sulphate, generating more metabolically useful energy as opposed to partial oxidation to S^0 (Lens and Kuenen, 2001). In order to obtain sulphur as a product, sulphide oxidation must occur under stringent conditions, such as high sulphide loads and within a narrow redox potential and pH window (Figure 3).

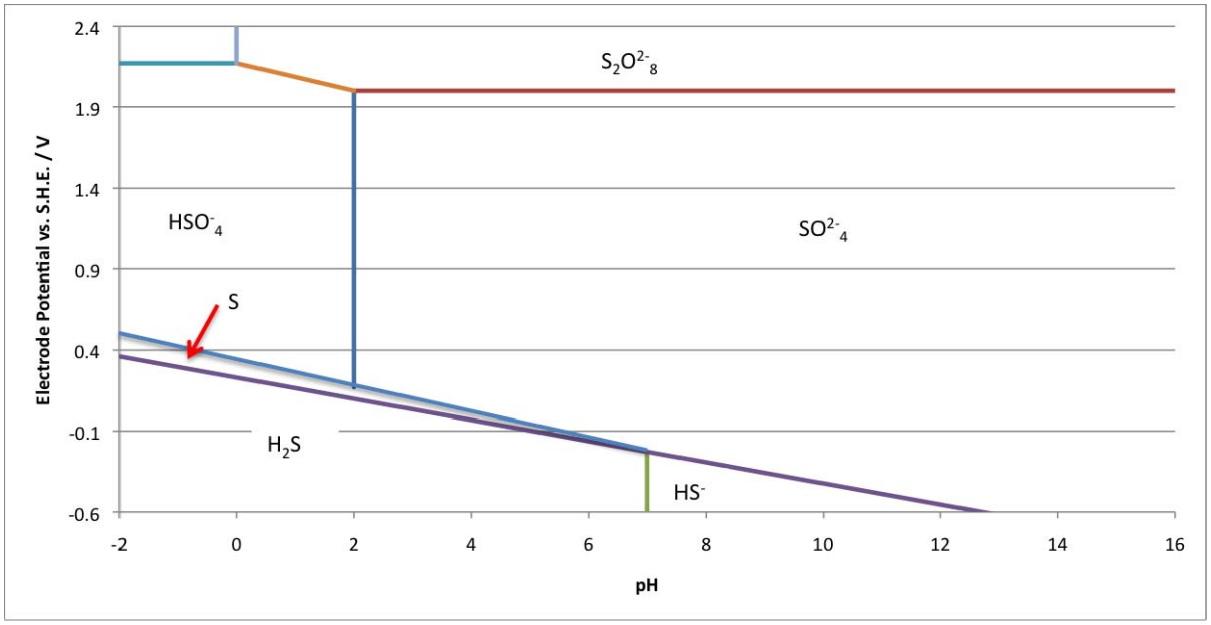

Figure 3: Potential-pH diagram for a sulphur/water system at 298.15K

Ratio of sulphide to oxygen

Similar to the chemical oxidation scenario, the ratio of sulphide to oxygen needs to be carefully controlled in order to achieve partial oxidation to sulphur (Equation 11). If additional oxygen is available the reaction will proceed to the more thermodynamically stable sulphate, either through the further oxidation of the sulphur intermediate (Equation 12) or the complete oxidation of sulphide (Equation 13).

$$2HS^- + O_2 \rightarrow 2S^0 + 2OH^- \quad (11)$$

$$2S^0 + 3O_2 \rightarrow 2SO_4^{2-} + 2H^+ \quad (12)$$

$$2HS^- + 4O_2 \rightarrow 2SO_4^{2-} + 2H^+ \quad (13)$$

Buisman and co-workers (1990) state that in order to minimise sulphate production the sulphide load must be exceedingly higher than that of the oxygen concentration. Therefore, in practical terms the ratio of sulphide to oxygen should exceed 2:1 to ensure the production of sulphur (Equation 11). Janssen and co-workers (1995) demonstrated that when the oxygen supply was increased fourfold sulphide was oxidised directly to sulphate (Equation 13). From a bioenergetic perspective the complete oxidation to sulphate is favoured; since the bacteria derive maximum energy from this reaction. Sulphate production liberates 8 electrons whilst sulphur formation only liberates 2, hence limiting the energy available for cell proliferation (Mamashela, 2002). Furthermore oxygen concentration influences the amount of sulphate formed at low sulphide concentrations (Janssen et al., 1998; Mamashela, 2002).

The production of thiosulphate, via chemical sulphide oxidation is possible, especially during the start-up phase of a sulphide oxidation reactor, when biological activity is low (Van den Bosch et al., 2008). Once sulphide oxidation activity has been established thiosulphate formation is still possible, but only at decreased oxygen concentrations and exceedingly high sulphide loading rates (10 S^{2-}_{tot} : 1.1 O_2)(Buisman et al., 1990; Janssen et al., 1995; Van den Bosch et al., 2008). This can be minimised if the ratio of sulphide to oxygen is maintained between 2:1 and 2.33:1.

Therefore, from a process perspective some degree of control is required to ensure the majority of the influent sulphide is oxidised to sulphur. If the influent sulphide concentration drops, without any change in oxygen provision, complete oxidation to sulphate will be favoured. In addition, the sulphide loading rate is a vital parameter with Stefess (1993), Buisman et al. (1990) and Janssen et al. (1998) all in agreement that this parameter plays a key role in determining the sulphur production and sulphide oxidation rate. In most systems that lack fine control of oxygen addition and redox potential (passive processes) a high sulphide loading rate will be required to prevent complete oxidation. However, at too high a sulphide loading the microorganisms are inhibited, which would lead to a reduction in metabolic activity and thus affect sulphide

oxidation. Research by Buisman and co-workers (1991) determined that cell growth was not inhibited by volumetric loading rates of 200 mg/L.h or sulphide concentrations of 300 mg/L.

The factors described above indicate that optimum sulphur production occurs under a relatively narrow set of operating parameters. In a passive process, or one where management is limited (IMPI), fluctuations in feed sulphide concentration to the sulphide oxidation unit can occur as a result of environmental temperature variation, fluctuations in the sulphate loading to the SRB reactor or a variety of other external factors. It is important that the sensitivity of the sulphide oxidation reactor to such perturbations is quantified.

Influence of pH

The effect of pH on the biological sulphide oxidation is similar to that described earlier for the chemical system. However, pH does play a role in microbial growth. The optimal pH to maximise microbial growth within the biofilm is between pH 7 and pH 8.5.

Influence of temperature

The growth rate of the microorganisms involved increases with an increase in temperature, up to an optimum. The optimum temperature is generally closer to maximum temperature that can be tolerated than the minimum. The optimum and limiting temperatures are normally an indication of the active temperature range of the organism's enzyme systems (Hogg, 2005). The optimal temperature range for most organisms involved in sulphide oxidation is between 20°C and 40°C.

Effect of chemical sulphide oxidation on biological sulphide oxidation

As stated previously, in order to favour partial oxidation to elemental sulphur a high sulphide loading rate and high sulphide to oxygen ratio are necessary. However, these conditions can lead to the formation of thiosulphate and polysulphides via chemical sulphide oxidation.

The groups of Wilmot (1988) and Buisman (1990) showed that chemical oxidation is just as important a component as biological oxidation in the overall oxidation of sulphides.

Gonzalez-Sanchez and Revah (2007) showed that the formation of sulphide intermediates by chemical sulphide oxidation had a positive effect on biological sulphide oxidation, up to the point where the sulphide concentration became limiting. Beyond this point the biomass activity was compromised and sulphur formation was reduced. The formation of polysulphides and sulphide intermediates may also increase biological activity by decreasing the sulphide toxicity where the organisms are exposed to high sulphide concentrations.

Polysulphide formation and its impact on sulphide oxidation

Polysulphides can be formed as a result of chemical sulphide oxidation. Polysulphide species have been observed as intermediates under conditions where elemental sulphur is formed through chemical oxidation (at pH 7). Chen and Morris (1972) found that polysulphide ion oxidation occurred more rapidly than sulphide oxidation and therefore polysulphide ions were found to act as a catalyst for sulphide oxidation. It was confirmed that the polysulphide oxidation followed the reaction stoichiometry in the pH range 7-9. Elemental sulphur was only observed if the average sulphur content (x) exceeded 2 (as per Equation 14).

$$2S_x^{2-} + O_2 + H_2O \rightleftharpoons S_2O_3^{2-} + 2S^{2-} + 2S_{x-1}^{2-} + 2H^+ \quad (14)$$

Polysulphide solutions are generally yellow-orange in colour, whereas thiosulphate solutions are colourless. Therefore, discolouration of the solution and formation of a precipitate (S^0) would indicate reaction below (Equation 15) was taking place (Bruser et al., 2000). This auto-oxidation is known to occur when the solution is exposed to the atmosphere.

$$S_x^{2-} + \frac{3}{2} O_2 \rightarrow S_2O_3^{2-} + (x-2)S^0 \quad (15)$$

At mildly alkaline conditions and in equilibrium with excess inorganic sulphur, the average polysulphide chain length (x) varies from 4.39 - 5.5 (Van den Bosch, 2008). Kamyshny et al. (2004) showed that the specific chain length is not affected by pH or total polysulphide concentration.

The pH of the solution in the reactor plays a critical role in the overall distribution of sulphide species. At pH < 9.17 the majority of the aqueous sulphide is present as HS^-. If the pH is increased above pH 9.17 the majority of the sulphide occurs as polysulphides (S_x^{2-}). Therefore, an equilibrium exists in the region of pH = 9.17, as described by the following expression (Van den Bosch, 2008):

$$K_x = \frac{[S_x^{2-}][H^+]}{[HS^-]} \; x \; \frac{\gamma_{S_x^{2-}} \gamma_{H^+}}{\gamma_{HS^-}} \tag{16}$$

Van den Bosch and co-workers (2008) discovered that over a lower pH range (pH 8.5-9) about 18-40% of the total sulphide was present as polysulphides, while at the higher pH range (pH 9.6-10.1) the polysulphide content increased to between 73% and 89%.

Polysulphide formation may also occur under natron-alkaline conditions (Equation 17). Essentially these are formed by the reaction between sulphide and biosulphur particles. Polysulphide anions comprise (x-1) zero-valent sulphur atoms (S^0) and one sulphide atom in the divalent state (S^{2-}) (x = chain length) (Van den Bosch, 2008).

$$HS^- + (x-1)S^0 \rightleftharpoons S_x^{2-} + H^+ \tag{17}$$

Redox potential

Control of the total sulphide concentration can be achieved via the measured oxidation reduction potential (ORP). The ORP of an aqueous solution is determined by its tendency to either accept or donate electrons and is a result of the proportion of the oxidised and reduced forms of all dissolved compounds. Therefore the tendency for a molecule to donate/accept electrons is expressed by its E*h* value, which is expressed relative to the standard hydrogen electrode (SHE) (Van den Bosch, 2008). Figure 3 shows that only the -2, 0 and +6 oxidation states are stable in water. From the diagram it is evident that elemental sulphur formation occurs within a very small pH range (Kelsall and Thompson, 1993). However, this range is dependent on the sulphide and polysulphide concentration as well as the sulphide to oxygen ratio.

ORP depends largely on the polysulphide concentration, which is controlled by the total sulphide concentration and pH. However, this relationship is only valid for systems where biologically produced sulphur particles are in excess. As a result the ORP increases as pH decreases, provided the total sulphide concentration remains constant.

Therefore the ORP can be related to pH and total sulphide concentration via the following equations (Van den Bosch, 2008):

$$S_x^{2-} + \; xH_2O + (2x-2)e^- \rightleftharpoons xHS^- + \; xOH^- \tag{18}$$

Application of this general equilibrium equation (18) to the Nernst equation (19), the electrode potential can be represented as (equation 20):

$$E_h = E_h^0 - \frac{R.T}{n.F} \; x \; ln \frac{\Pi_j [red]^{n_j}}{\Pi_i [ox]^{n_i}} \tag{19}$$

$$E = \; E^0 - \frac{R.T}{n.F} x \; ln \frac{[HS^-]^x [OH^-]^x}{[S_x^{2-}]^1} \tag{20}$$

Combining the above equation with the equilibrium constant (16), the following equation (22) relates ORP to pH and S^{2-}_{tot}:

$$[S_{tot}^{2-}] = [HS^-] + [S_x^{2-}] \tag{21}$$

$$ORP = \; E^0 - \frac{R.T}{(2x-2)F} \; . ln\left(\frac{10^{-14}}{K_x}\right) - \; (x-1) \frac{R.T}{(2x-2)F} \; . \ln \left(\frac{[S_{tot}^{2-}].K_x}{K_x + 10^{-pH}}\right) \tag{22}$$

Therefore, if there is stringent control of the solution redox, it is possible to oxidise sulphide to elemental sulphur by creating an oxygen-limiting environment (Janssen et al., 1998). This approach is followed in the Paques active sulphide oxidation reactor.

Sulphate formation is minimised at the high sulphide loading rate (50 mg/L.h) and sulphide to oxygen ratio (2.63 : 1). Hence, the optimal redox potential range is -142 to -128 mV (Janssen et al., 1998).

1.4 Biofilms

Since the 1980's biofilms have become the focus of interest in the field of water and wastewater treatment as well as biotechnology. A biofilm is defined as a matrix-enclosed bacterial population adherent to each other and/or to surfaces or interfaces. Bacteria will initiate biofilm development within an aquatic system if there are sufficient nutrients available for cell replication as well as exopolysaccharide (EPS) production (Costerton et al., 1995; Bowker, 2002; Lazarova and Manem, 1995). Thereafter the bacteria adhere to the surface, undergo phenotypic change (that alters many of its structural molecules) and depress exopolysaccharide synthesis (Costerton et al., 1995). This process leads to the development of a micro-colony, the basic unit of biofilm growth. It is these micro-colonies that interact to form a biofilm. Microbial growth and life within a biofilm is very different to that of planktonic life forms. This is due to the fact that biofilm communities develop internal heterogeneity and specific structure/function relationships within the biofilm structure (Zhang and Bishop, 1994; Bowker, 2002).

1.4.1 Biofilm structure

Biofilms form intriguing microbial systems within the aquatic environment with characteristic internal architecture. The organisms produce EPS to facilitate attachment to the substratum, but also the formation and maintenance of the biofilm structure. In addition, the EPS helps to confer a level of resistance to environmental stresses to the component organisms. Research has shown that biofilms are not flat uniform structures, but rather are highly complex heterogenic films; consisting of voids, channels, pores, cell clusters and layers. The biofilm may be made up of thick flat biomasses, organised mushrooms or thin filamentous streamers. It has been hypothesised and observed that the biofilm structure is not a random formation of bacterial cells, but rather an arrangement determined to maximise the influx of nutrients. The biofilm structure is influenced by a diverse range of physical, chemical and environmental factors which affect the adaptation and growth of the organisms (Costerton et al., 1995; Bowker, 2002). Studies show that different populations are restricted to well-defined depth intervals within the biofilm. Therefore the structured distribution of various microbial populations within the biofilm is dependent on its role within the general microbial community. The general principle within a microbial community is that the end products of metabolism by one population is utilised as a resource by another (Bowker, 2002). Biofilms therefore may be considered as microecosystems whereby different microbial strains and species efficiently work together for the general well being of the bacterial community. As highlighted above, biofilms consist of voids and pores which facilitate the transport of nutrients and water. Therefore, the internal structure of the biofilm plays a key role in the mass transport within the film and is discussed further in Section 5.3.

1.4.2 Floating biofilms

Floating biofilms are similar in nature to biofilms that are attached to a solid substrate in that a substrate or interface is required for the development of a biofilm. In this situation the air-liquid interface of an open body of water is able to act as the substrate for the development of a biofilm, provided there are sufficient nutrients. Floating biofilms have been observed on the surface of highly sulphidic tannery wastewater ponds (Molwantwa, 2007). The biofilm is able to form on the surface due to the surface tension of water. A floating sulphur biofilm (FSB) is defined as a system where chemical and biological sulphide oxidation occurs, with the sulphur product remaining within the biofilm. Therefore, if there is a desire to recover sulphur it is necessary to harvest the biofilm as the sulphur forms and integral component.

1.4.3 Mass transport and mass transfer limitations

The mass transport, to and within the biofilm, of sulphide as well as oxygen will play an important role in the biological oxidation of sulphide to sulphur. In biofilms at steady-state, a continuity equation (23) has been used to develop a comprehensive model and provide an accurate description of the nutrient concentration profiles within the biofilm. Equation 23 equates biofilm activity with internal mass transport, assuming constant effective diffusivity and constant biofilm density (Beyenal and Lewandowski, 2002).

$$D_f \frac{d^2C}{dz^2} = \frac{\mu_{max} C X_f}{Y_{\frac{X}{S}}(K_S + C)} \quad (23)$$

The rate of nutrient and sulphide transport to the biofilm is quantified by linking the convective external mass transfer rate to the diffusive mass transport rate across the biofilm (Beyenal and Lewandowski, 2002). Assuming there is no, or negligible substrate consumption, in the bulk solution, the flux of dissolved sulphides must be conserved. Hence the rate external transfer rate ($k_1(Cb - Cs)$) and internal mass transfer rate ($Df(dC/dz)\,|z=Lf$ must be equal at the biofilm surface. In such a case the flux of sulphides into the biofilm at the surface is:

$$N_s = k_1(C_b - C_s) = D_f \frac{dC}{dz}|_{z=L_f} \quad (24)$$

Mass transport within a biofilm is greatly affected by the hydrodynamics of the system. According to De Beer et al. (1996) the mass transfer boundary layer lies parallel to the substratum at low velocities, however at higher velocities (>0.04 m/s) the boundary layer closely followed the irregular biofilm surface. Moreover, the voids within the biofilm enhanced the mass transfer rate and product exchange with the bulk liquid by facilitating convection when the velocity was high ensuring the boundary layer was close to the biofilm surface. It was also demonstrated how an increase in the velocity decreased the mass transfer boundary layer, both above the voids and cell clusters. The oxygen concentration was much greater within the void at a higher velocity (de Beer et al., 1996).

The biofilm consists of cell clusters and voids which form a network of channels and allows the lateral movement of fluid within the biofilm. The oxygen distribution is strongly associated with the biofilm structure. De Beer and co-workers (1994) demonstrated that oxygen was supplied to the cell cluster through the cell cluster-liquid interface. The largest gradient existed at both the cell cluster - pore interface and the cell cluster - bulk liquid interface. It was also determined that the flux from the void to the cell cluster and from the bulk liquid to the cell cluster, was of the same order of magnitude, provided the mass transfer boundary layer was thin. However, the conduits below the cell clusters facilitated oxygen transport in the horizontal direction.

The flow velocity at which the biofilm is cultivated, also plays an important factor in the internal structure of the biofilm. Beyenal and Lewandowski (2002) determined that the flow velocity affected the nutrient transport rate as well as the mechanical pliability of the biofilm structure. When the velocity was too high the biofilm increased its mechanical strength to resist shearing and as a result the density increased, ultimately leading to a less porous structure, where the internal mass transfer rate was significantly reduced.

A key aim of the current project is to determine the relationship between biofilm structure and mass transport, particularly with respect to oxygen penetration into the film. As the biofilm matures it passes through a series of phases, each characterised by different physical properties. Molwantwa (2007) collected data on pH, redox potential and sulphide gradients through the biofilm, but was not able to relate this information to changes in mass transfer.

2 AIMS AND OBJECTIVES

2.1 Project terms of reference

This project is closely aligned with the WRC solicited project (K5/1834), managed by Golder Associates Africa (GAA), with a number of the experimental streams running in parallel. The overall aims of this project (K8/941), according to the proposal, were laid out as follows:

1. Literature review covering biological treatment of AMD with particular focus on passive systems and biological sulphide oxidation.
2. Theoretical model, based on stoichiometry, of oxygen required to achieve efficient sulphide oxidation for a range of sulphide concentrations and hydraulic residence times (HRTs).
3. Design of a linear flow channel reactor suitable for kinetic and hydraulic studies, which allows quantification of aqueous and gaseous sulphide. Overall reactor dimensions and aspect ratio will be consistent with LFCR's at Golder Associates Research Laboratories (GARL) being operated as part of the K5/1834 project in order to maximise the integration of the two data sets.
4. Optimisation of analytical techniques for all sulphide species, including elemental sulphur and soluble polysulphides.
5. Determine kinetic parameters for oxidation of sulphide in LFCR. Investigate the effect of sulphide concentration, pH, HRT and harvesting frequency.
6. Qualitative characterisation of the microbial population at different stages during biofilm formation and development.

Through the early stages of research aimed at achieving the specific goals of this project a number of previously held assumptions were proven not to hold and deficiencies in the relatively limited sampling regime possible in the pilot scale work were identified. In order to keep the solicited project moving forward it was necessary to report these findings to the Steering Committee and project managers. To avoid duplication of reporting these issues are highlighted in the text and the relevant documents cited.

2.2 Deliverable 3 terms of reference

According to the project proposal, Deliverable 3 would report on the following:

1. Theoretical model of oxygen requirement and associated reactor surface area.
2. Qualitative characterisation of the microbial population.
3. Preliminary kinetic data on sulphide oxidation.

At a meeting of the Joint Passive Treatment project group (23 June 2010), where the theoretical model was described, the predictions of the model were questioned, based on previous pilot scale data. Data on the reactor hydrodynamics, generated as part of K5/1834 (Van Hille and Mooruth, 2010), clearly illustrated that the previous assumption of plug flow in the LFCR did not hold and that resulting stratification and short circuiting had not been accounted for in the pilot scale work. As a result of the discussion, the theoretical model, coupled with the influence of hydrodynamics, were reported to the K5/1834 project team.

During the renegotiation (between GAA and the WRC) of the K5/1834 deliverables and timelines, the qualitative assessment of the microbial population was included as a major deliverable into K5/1834 and as such cannot be presented in this project.

The assessment of the sulphide oxidation kinetics remains part of this project. In addition, this project will report on the extended work on closing the sulphur species mass balance across the reactors, as well as research conducted to assess the relationship between organic carbon loading of the DPBR and availability to in the LFCR and biofilm structure. This information feeds into the question of harvesting strategy and product sulphur purity.

3 MATERIALS AND METHODS

3.1 Linear flow channel reactor studies

3.1.1 Integrated system setup and operation

The experimental setup consists of two DPBR columns, transported from GARL, and three purpose built LFCRs (described in detail in K5/1834/7). The reactor configuration is illustrated in Figures 4 and 5.

The DPBRs were fed from the bottom as fully saturated reactors. This was done to minimise channelling and promote reactor stability. The columns were fed a synthetic, partially treated AMD (Appendix A) at a rate of 4 L/day. The feed was based on a thermodynamic prediction of the composition of partially treated Landau colliery ARD. The synthetic AMD was supplemented with molasses (1.5 g/L) to promote sulphate reduction and mimic the operation of the demonstration plant. The molasses supplementation concentration was decided upon following consultation with the K5/1834 project team and was substantially lower than 2.5% reportedly used in the pilot study at GARL (Golder Associates Africa, 2009), although the accuracy of the report has been questioned. The molasses concentration in the feed was increased to 2.5 g/L (in consultation with the K5/1834 project team) for a period of time to assess the impact on organic carbon flux through the system. The column effluent was sampled every second day to determine pH, redox potential, sulphate, sulphide and more recently VFA concentration.

3.1.2 Linear flow channel reactor tests

The standard operating procedure for the LFCR experiments is described below. The LFCRs were operated with a liquid volume of 25 L. The reactors were closed to the surroundings (gasket sealed lid) leaving a headspace of 12.5 L. The headspace was flushed with air at a rate of 2.08 L/hour, with the exit gas passing through an alkaline scrubber to recover any in H_2S in the gas phase. The lid was removed only when microprobe measurements were taken.

Unless otherwise stated the reactors were fed, via the uppermost inlet port, at a rate of 5 L/day and the effluent exited via the uppermost exit port. This resulted in a residence time of approximately 5 days.

The LFCRs were designed with 15 sampling ports, across three levels, on the front wall of the reactor. Each sample port was fitted with a rubber septum (GC injection septum) and samples were withdrawn using a 100 mm hypodermic needle. Samples (5 mL) were collected from 10 of the 15 sample ports (Figure 6) on a daily basis, along with influent and effluent samples.

The samples were analysed for pH, redox potential and aqueous sulphide concentration. Colloidal sulphur was detected by transferring 200 µL of sample to an eppendorf tube and adding 1.3 mL of chloroform. The tube was agitated and once all the sulphur had dissolved (1 hour) the solution was filtered through a 0.45 µm nylon membrane filter and the filtrate retained for HPLC analysis. A further 4 mL subsample was centrifuged at 13 000 rpm and the supernatant retained for HPLC analysis (sulphate, polysulphide, VFA). Where possible, the biofilm was harvested, dried and the sulphur content determined. A small fraction of the biofilm was used to prepare samples for SEM-EDX analysis (Van Hille and Mooruth, 2011).

The exact operating parameters for the LFCR studies are summarised in Table 3.

Table 3: Summary of operating conditions for LFCR studies

Run	Air flow rate (L/day)	Feed flow rate (L/day)	Hydraulic residence time (Days)	Acetate addition (g)	Abiotic
1	48	5	5	0	
2	48	5	5	0	
3	48	5	5	0	✓
4	48	12.5	2	20	
5	48	12.5	2	0	✓

Figure 4: Reactor configuration showing DPBR columns and LFCR reactor inlets

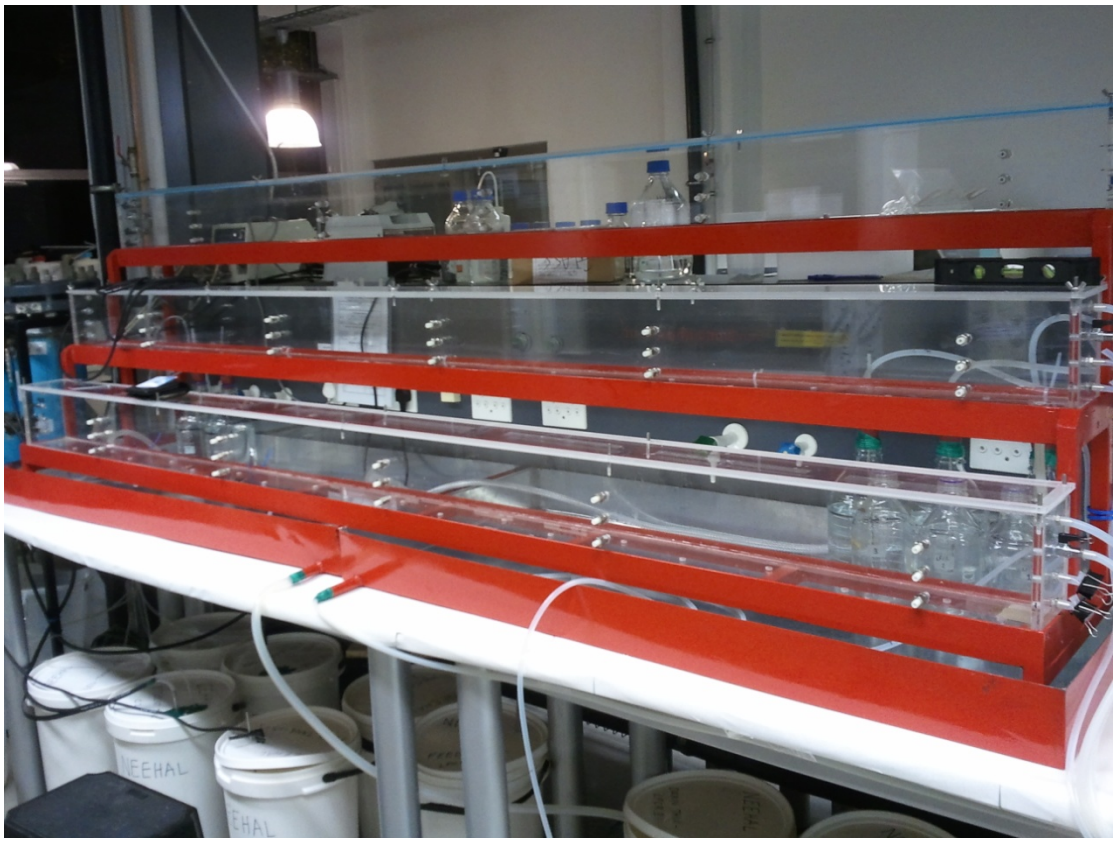

Figure 5: Reactor configuration showing tiered LFCRs, feed and effluent storage and reactor sampling ports

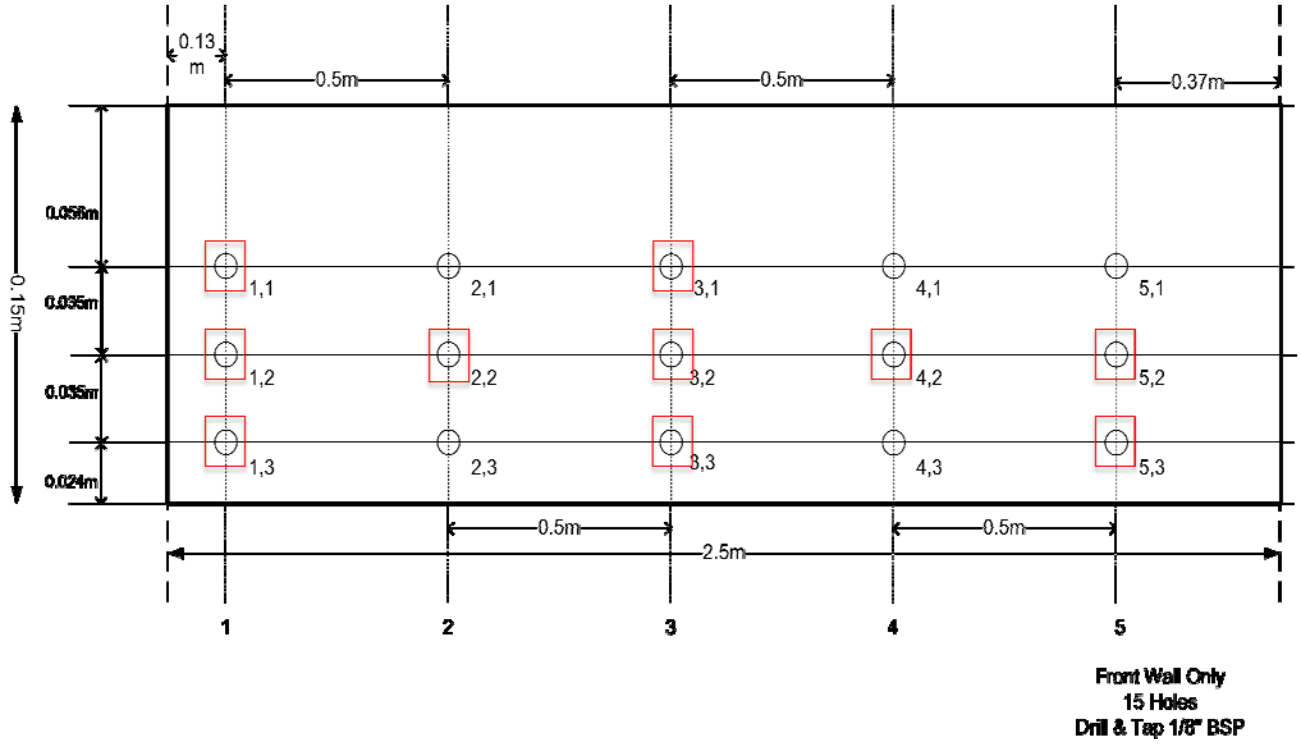

Figure 6: Schematic image of front wall of LFCR showing location of sampling ports and sample designation. Highlighted ports were sampled daily.

3.1.3 Determination of kinetic parameters

The kinetic parameters were determined using a rate law equation typically used for batch reactors (equation 25).

$$\frac{dC_{HS^-}}{dt} = r_{HS^-} = kC_{HS^-}^{\alpha} C_{O_2}^{\beta} \tag{25}$$

Equation 25 shows that the rate of change in sulphide concentration with time is a function of the sulphide and oxygen concentrations, to the power of the rate order (α and β), multiplied by a rate constant. For the purpose of this study an initial assumption was made that each of the 15 sampling points could be modelled as a batch reactor. This was done for the sake of numerical simplicity and with the intention of determining the order of the reaction rate. Once the reaction order was determined the complexity of the model could be increased to include the hydrodynamic flow patterns.

A more complete model of a differential reactor, such as the LFCR, can be generated using a mass balance approach. Here, the influent flow minus the effluent flow plus the rate of generation is equal to the rate of accumulation. Assuming that no sulphide is generated in the LFCR, but only consumed, the equation can be represented as:

$$-r_A = v_{in}C_{in} - v_{out}.C_{out} \tag{26}$$

This can be simplified to:

$$-r_A = v_0 C_{product} \tag{27}$$

However, due to the complex nature of the hydrodynamics within the LFCR and the existence of a dead zone at the back end of the reactor the rate of accumulation cannot accurately be determined without a detailed hydrodynamic model. Furthermore, the existence of multiple products introduces and additional level of complexity. Therefore, the assumption that each sample port represents a batch reactor represents a good first approximation.

The models described above can only be used to model changes in sulphide concentration in the bulk phase. A different approach is required to determine the rate law within the biofilm. In the case of the biofilm, where oxygen mass transfer limitation is a function of biofilm thickness and composition, it can be assumed that the reactions are mass transfer, rather than reaction rate controlled. Therefore, in order to determine the rate law for sulphide conversion in the biofilm the following equation can be used:

$$-r_A = \frac{P_{O_2}}{\frac{1}{k_{Ag.a}} + \frac{1}{\frac{H_A}{K_{Al.a.E}}} + \frac{H_A}{kC_{A_S.f}}} \quad (28)$$

Each term within the denominator of Equation 28 accounts for a resistance within the "film system". The first accounts for gas film resistance, the second liquid-biofilm resistance and the third the bulk liquid volume resistance. The biofilm model development depends data generated using the microprobe analysis of the biofilm and will form a key component of an ongoing PhD investigation.

3.2 Organic carbon flux and impact of molasses supplementation

Visual observation of the development of the biofilm during the operation of the LFCRs, coupled with SEM EDX results and data on process performance suggested the availability of organic carbon in the LFCRs played an important role in the process. The structure of the biofilm differed from that observed in the pilot reactor at GARL, particularly with respect to the absence of the "sticky", organic rich phase. This prompted an investigation into the carbon flux through the integrated system.

3.2.1 Analysis of VFA content in DPBR overflow

Volatile fatty acids in the DPBR effluent were determined by HPLC. The concentration of each VFA was determined using a Waters Breeze 2 HPLC system equipped with a Bio-Rad Organics Acids ROA column and a UV (210 nm) 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 2000 psi. Sample injection volumes of 100 µL were used. To quantify the VFA concentrations standard solutions (50, 100, 500 and 1000 mg/L for each acid) were prepared. A sample chromatogram for the standards is illustrated in Appendix B.

Based on the results of the initial analysis and in consultation with the project team it was decided to increase the molasses concentration in the feed to 2.5 g/L in an attempt to increase the organic carbon flux through the system.

DPBR column effluent was sampled every second day. A portion of the sample was centrifuged at 13 000 rpm for 10 minutes, to remove particulate matter, and then filtered through a 0.22 µm membrane filter (Millipore) prior to storage for HPLC analysis.

3.2.2 Determination of COD value of molasses

The COD to SO_4^{2-} ratio has traditionally been used as a control measure in biosulphidogenic systems. In order to determine this ratio based solely on the DPBR feed solution, assuming no contribution from the lignocellulosic material, the COD of a 1.5 g/L molasses solution was determined. The suitably diluted sample (3 mL) was added to a COD reaction tube containing 0.3 mL of COD solution A and 2.3 mL of COD solution B and mixed well. The tubes were incubated at 148°C for two hours, allowed to cool and the absorbance measured at 610 nm. COD values of molasses samples were determined against a standard curve (Appendix C) generated using potassium hydrogen phthalate.

3.2.3 Batch sulphate reduction tests

To decouple the contribution of the lignocellulosic material to overall sulphate reduction in the DPBR reactors a series of batch tests were performed. Four 1L batch reactors were prepared by dissolving 2.85 g of sodium sulphate (Na_2SO_4), 0.1 g of magnesium chloride ($MgCl_2.6H_2O$), 0.1 g of potassium dihydrogen phosphate (KH_2PO_4) and 1.5 g molasses in 500 mL tap water. The solution was buffered by adding 0.068 g sodium bicarbonate ($NaHCO_3$), to give a final concentration of 50 mg/L. The initial redox potential was reduced to below -250 mV by adding 2.5 mL of a 10 g/L sodium sulphide (Na_2S) stock solution (final concentration 25 mg/L). The pH effect of the sulphide addition was balanced by adding 0.78 mmoles of sulphuric acid (H_2SO_4), which increased the overall sulphate load to 2 g/L. The volume was made up to the

appropriate volume (1 L less inoculation volume) with tap water and a time 0 sample (5 mL) taken for analysis (pH, redox, sulphide and sulphate). Reactors 1-3 were inoculated with increasing volumes (20, 50 and 100 mL) of DPBR effluent. Reactor 4 was used as a positive control and inoculated with 10 mL of biosulphidogenic sludge granules. The sludge was obtained from a demonstration scale active BSR reactor and the sulphidogenic activity had previously been determined using ethanol as the carbon source. The contents of the reactors were agitated using a magnetic stirrer at 300 rpm. All reactors were sampled (5 mL) 1-2 times per day.

4 RESULTS AND DISCUSSION

The data presented and discussed below for the LFCR studies focuses on the overall reactor performance, the closing of the sulphur species mass balance and the relationship between organic loading, biofilm structure and performance. The distribution of dissolved and suspended particles within the reactors is complex and inhomogeneous due to the nature of the flow within the system. Detailed analyses of these are not presented in this study as reactor hydrodynamics forms a key component of the K5/1834 project.

The ability to close the sulphur species mass balance has been enabled by the development and optimisation of an HPLC technique to analyse polysulphides. This had not previously been reported wastewater studies. The method development has been described in detail as part of K5/1834. An example chromatogram is presented in Appendix D.

4.1 Abiotic control LFCRs

The purpose of the control studies was to allow the decoupling of chemical sulphide oxidation from biological sulphide oxidation.

4.1.1 No pH control

The first control reactor was operated with no pH control. A synthetic sulphide solution (207 mg/L) was used to feed the reactor. The detailed analysis of sulphide distribution (Van Hille and Mooruth, 2011) showed distinct vertical stratification during the early part of the experiment, with the establishment of a homogeneous distribution by day 3. The lack of a gradient profile suggested that limited abiotic sulphide oxidation was occurring. This is confirmed by the preliminary mass balance data (Table 4).

Table 4: Summary of sulphur species over duration of experiment

	Sulphide mmoles	Sulphur mmoles	Sulphate mmoles	Thiosulphate mmoles	Polysulphide mmoles
In	314.86	0	17.09		-
Out	236.88	0	12.48		33.4
Consumed	77.98	-	4.61	-	-
Produced	-	-	-	-	33.4

The thiosulphate data still need to be integrated as HPLC data have only recently become available.

The pH of the system was not controlled and remained stable between pH 11.5 and pH 11.9 for the duration of the experiment, being dictated by the high concentration of aqueous sulphide. The solution remained clear throughout, which is consistent with a sulphide dominated system, and no elemental sulphur particles were observed. The observation was confirmed by HPLC analysis. The overall sulphide conversion was 24.7%, at a rate of 51.1 mg/day. The indication, supported by the absence of a yellow/green colour and HPLC data, is that relatively little of the sulphide was converted to polysulphide, with the majority going to thiosulphate.

The high pH which prevailed during the experiment was not representative of normal operation, so a second experiment was conducted where the pH was controlled around pH 7.

4.1.2 pH controlled system

The second abiotic control was conducted at a higher flow rate, resulting in a hydraulic retention time of two days (Table 3). The reactor was filled with sulphide solution that had been adjusted to pH 7 with HCl. The reactor feed solution was similarly pH adjusted.

The dissolution of crystalline sodium sulphide produces a clear solution. However, if the pH is reduced to between pH 6 and pH 9, in the presence of free dissolved oxygen, polysulphide formation occurs and the solution is characterised by a “strawish-green” colour (Chen and Morris, 1972). This was clearly evident during the preparation of the solutions. Due to the high concentration of aqueous sulphide a portion of the sulphide may be chemically oxidised to elemental sulphur by the reaction depicted in Equation 29. This reaction resulted in the formation of a small amount of sulphur and an increase in pH.

$$2HS^- + O_2 \rightleftharpoons \frac{1}{4} S_8 + 2OH^- \quad (29)$$

The presence of a substantial excess of aqueous sulphide most likely led to the reaction of the elemental sulphur with free sulphide to form more polysulphide, by the reaction represented by Equation 30. The combination of the two reactions led to an increase in pH from pH 7.1 to approximately pH 7.8 and the solution becoming clear, with the characteristic yellow-green colour of polysulphides evident.

$$HS^- + (x-1)S^0 \rightleftharpoons S_x^{2-} + H^+ \quad (30)$$

The fresh feed pumped into the reactor was initially at a lower pH (pH 7) than the bulk solution (± pH 7.8). As a result, the formation of a transient plume of colloidal sulphur was observed. The pattern of sulphur formed was consistent with the hydrodynamic regime determined using tracer studies (Van Hille and Mooruth, 2010) confirming the reaction between the influent and bulk solutions (Figure 7). The relevant reaction is depicted by Equation 31.

$$S_9^{2-} + H^+ \rightleftharpoons S_8^0 + HS^- \quad (31)$$

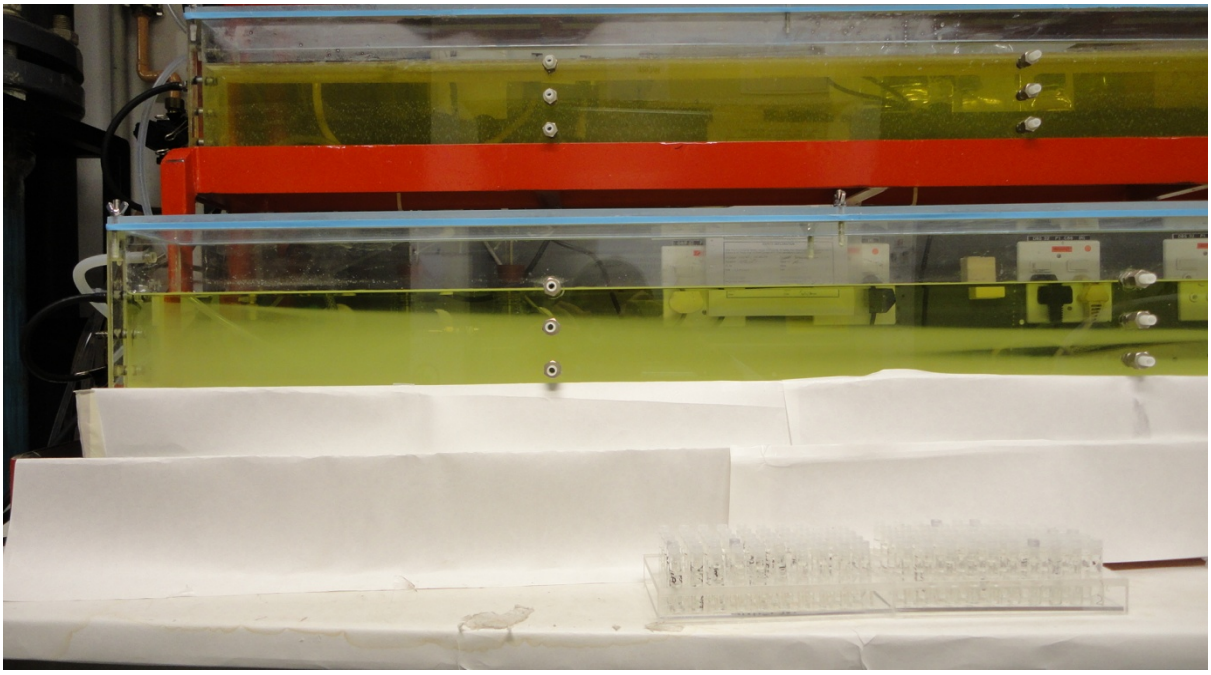

Figure 7: Evidence of colloidal sulphur formation during pH-controlled abiotic control experiment

The plume of colloidal sulphur was short live, most likely due the reaction with free sulphide (Equation 2). The hydrogen ions liberated during this reaction caused the pH to decrease back to around pH 7 and once this had occurred no further colloidal sulphur was observed at the inlet.

The mass balance data is consistent with this explanation. A total of 256.8 mmoles (33.8%) of the influent sulphide was consumed. At steady state no sulphate or colloidal sulphur was detected, implying that the sulphide had been converted to polysulphides. The integration of the HPLC data will confirm or refute this.

4.2 Biological LFCRs

Data from three LFCR reactors are presented in this interim report. Each data set represents a distinctly different level of process efficiency and highlights the complex relationship between sulphide loading, organic carbon flux and biofilm structure. In all cases the amount of sulphide lost to the gas phase was insignificant, based on the analysis of the solution in the sulphide trap.

4.2.1 LFCR 1 – limited biofilm formation

The data from this study are characteristic of a poorly performing system. Effluent from the DPBR was fed into the LFCR without the feed sulphide concentration being manipulated to ensure consistency, resulting in some fluctuation in feed sulphide concentration. The DPBR was not operating effectively, evidenced by the fact that the amount of sulphate entering the LFCR was significantly higher than the sulphide value (Table 5).

Table 5: Summary of sulphur species over the duration of the experiment. Thiosulphate and polysulphide data pending

	Sulphide mmoles	Sulphur mmoles	Sulphate mmoles	Thiosulphate mmoles	Polysulphide mmoles
In	90.65	18.65	545.29		
Out	24.86	14.93	634.09		
Consumed	65.79	3.72	-	-	-
Produced	-	-	88.8	-	-

A continuous biofilm did not develop at any stage during the operation of this reactor. As a result oxygen mass transfer at the interfacial surface was not impeded. This, coupled with the relatively low (<100 mg/L) and inconsistent feed sulphide concentration, resulted in a sulphide to oxygen stoichiometry significantly below two for the duration of the experiment. This resulted in all the sulphide that reacted being fully oxidised to sulphate (Equation 4). In addition, a portion of the elemental sulphur present in the reactor was further oxidised to sulphate. The relative distribution of colloidal sulphur and sulphate in the LFCR (Figures 8 and 9) is consistent with this, showing higher sulphur concentrations near the inlet (length 1) and along the bottom of the reactor (depth 3). The sulphur decreases toward the outlet (length 5) and closer to the surface (depth 1), where sulphate levels increase. Despite the lack of a biofilm, light microscopy observations confirmed the presence of planktonic microorganisms, so the reaction represented by Equation 32 was biologically catalysed.

$$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+ \quad (32)$$

The pH in this system fell to blow pH 7, confirming the generation of acidity by the above reaction.

The mass balance information indicates that just under 20 mmoles of sulphate cannot be accounted for the oxidation of influent sulphide of sulphur. There are two likely explanations for this. The first is that the influent stream from the DPBR contained a substantial amount of polysulphide and this was oxidised to sulphate by the microorganisms. The validity of this will become clear when the polysulphide data are available.

An alternative mechanism could be that the relatively poor sulphate reduction in the DPBR resulted in calcium and sulphate concentrations sufficiently high to induce gypsum ($CaSO_4$) precipitation. A portion of this could re-dissolve in the LFCR due to the greater liquid volume. Thermodynamic modelling of the solution composition using software such as Visual MinteQ or OLI Systems will be employed to validate this. A combination of these two phenomena is also possible.

The absence of a biofilm can most likely be accounted for by a deficiency in organic carbon in the LFCR. Biofilms require extracellular polymeric substances (EPS), secreted by the cells to contribute to the structural integrity. Volatile fatty acids (VFAs) are the most likely source of organic carbon, given that the DPBR is an anaerobic system and its organic carbon is derived from sugars (molasses or depolymerised cellulose). The HPLC analysis (Figure 10) showed a single peak at a retention time of around 16 minutes, corresponding to acetate. Integration of the peak areas showed that the acetate concentration at steady state was approximately 5 mg/L.

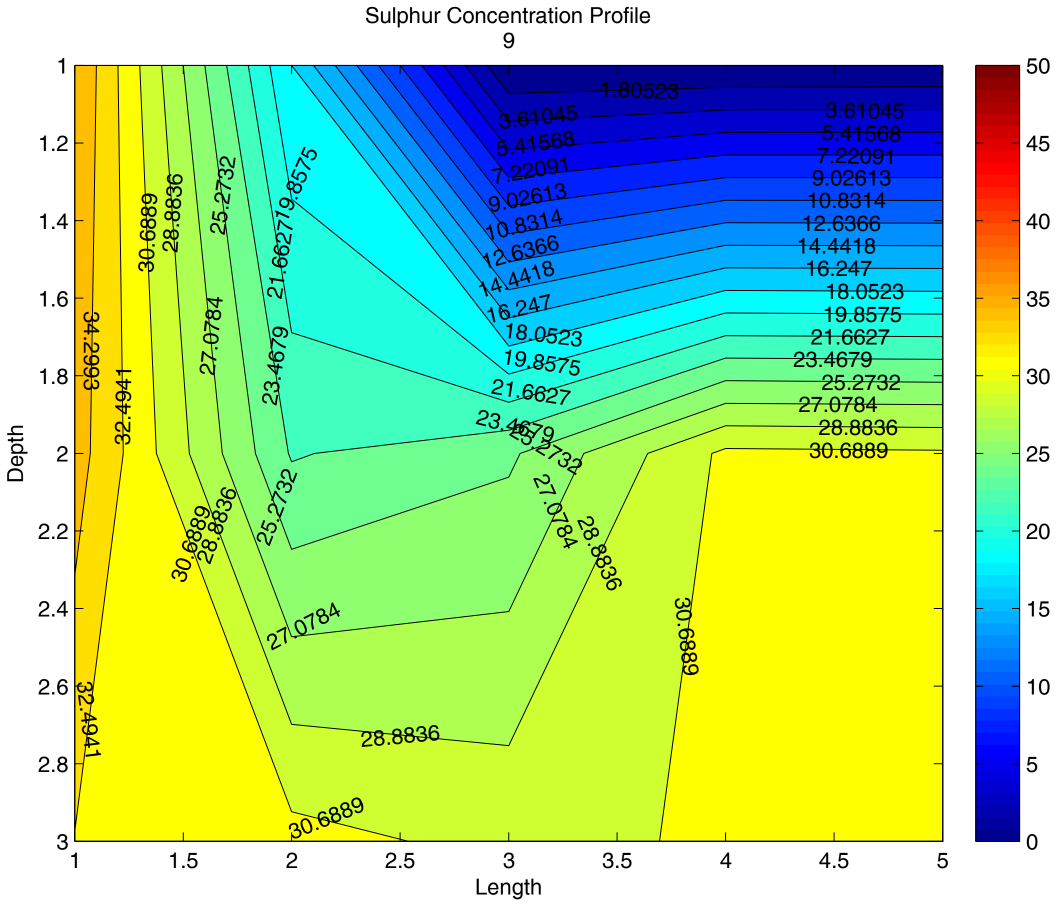

Figure 8: Colloidal sulphur profile during LFCR operation (day 9). Values expressed as mg/L

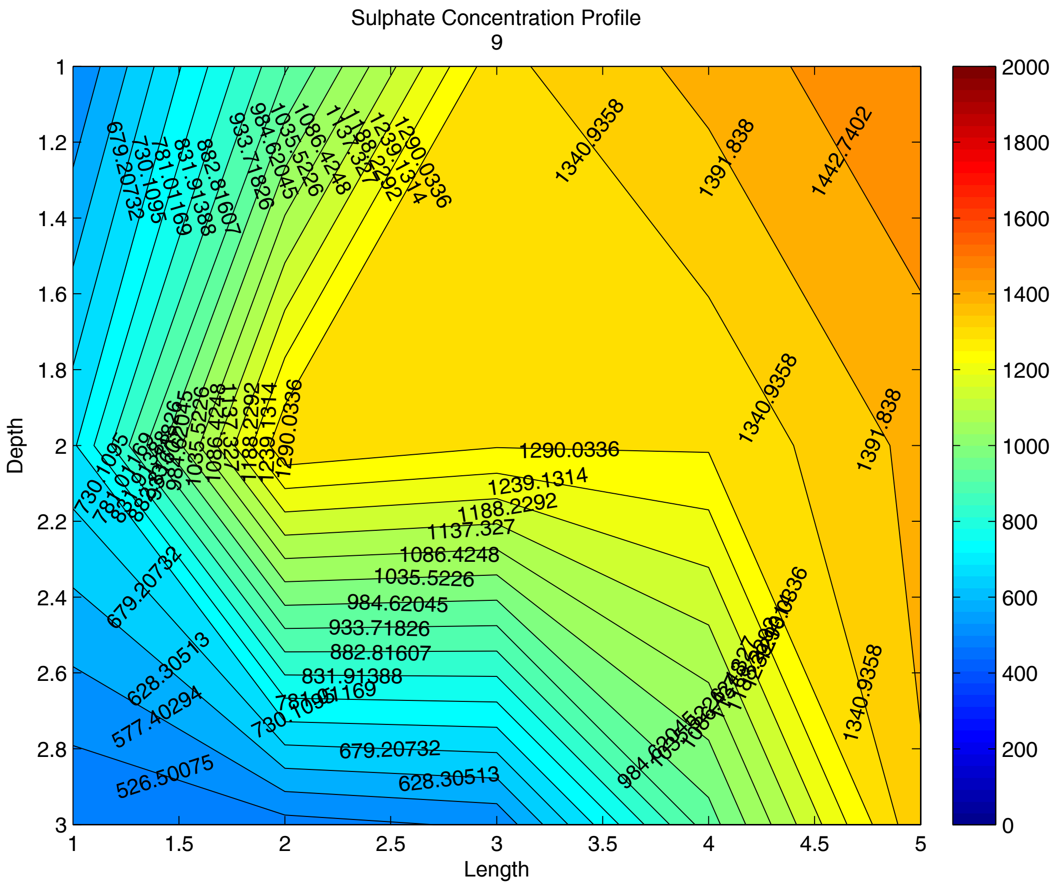

Figure 9: Soluble sulphate profile during LFCR operation (day 9). Values expressed as mg/L

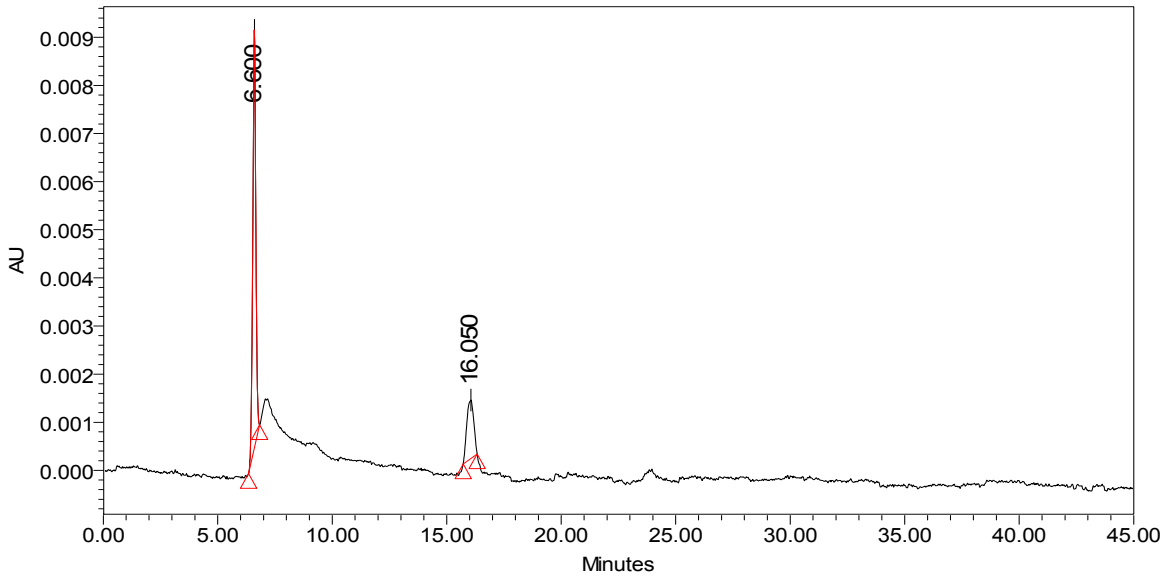

Figure 10: HPLC chromatogram of DPBR column 2 effluent sample illustrating a single VFA peak at 16 minutes, corresponding to acetate

The sample was run through a second HPLC protocol to detect mono or disaccharide sugars, with the data indicating that no sugars were present in the sample.

4.2.2 LFCR 2 – partial biofilm formation

This reactor was operated under similar conditions as described in Section 4.2.1. The sulphur balance data (Table 6) illustrates that there was still significant amounts of sulphate in the DPBR effluent, which is indicative of sub-optimal performance. In total 130 mmoles of sulphide was converted (93% conversion), with the majority most likely being transformed to elemental sulphur. Due to the physical structure of the biofilm it was not possible to effectively harvest it upon completion of the experiment, so the value presented in Table 6 was theoretical, based on the quantification of all other sulphur species. A significant portion of the sulphur (± 30%) occurred as colloidal particles and was lost from the reactor in the effluent stream. From a practical perspective this is undesirable as this would likely oxidise in the receiving water body. In contrast to the previous case, very little of the sulphide was fully oxidised to sulphate.

Table 6: Summary of sulphur species over the duration of the experiment. Thiosulphate was not detected in significant amounts

	Sulphide	Sulphur		Sulphate	Polysulphide
		Colloidal	In biofilm		
	mmoles	mmoles	mmoles	mmoles	mmoles
In	139.80	18.84	-	719.70	-
Out	9.76	53.25	-	732.12	11.07
Consumed	130.04	-	-	-	-
Produced	-	34.41	83.21[1]	12.42	11.07

[1] theoretical value

The biofilm formed during this study was thin, brittle and had a marbled appearance. It did not pass through the transition phases described by Molwantwa (2007) or observed in the pilot scale studies at GARL. The SEM-EDX data (Figures 11-13) illustrate the presence of sulphur granules embedded in what appears to be a chemical rather than organic matrix. Microbial cells were not readily visible in the SEM preparations.

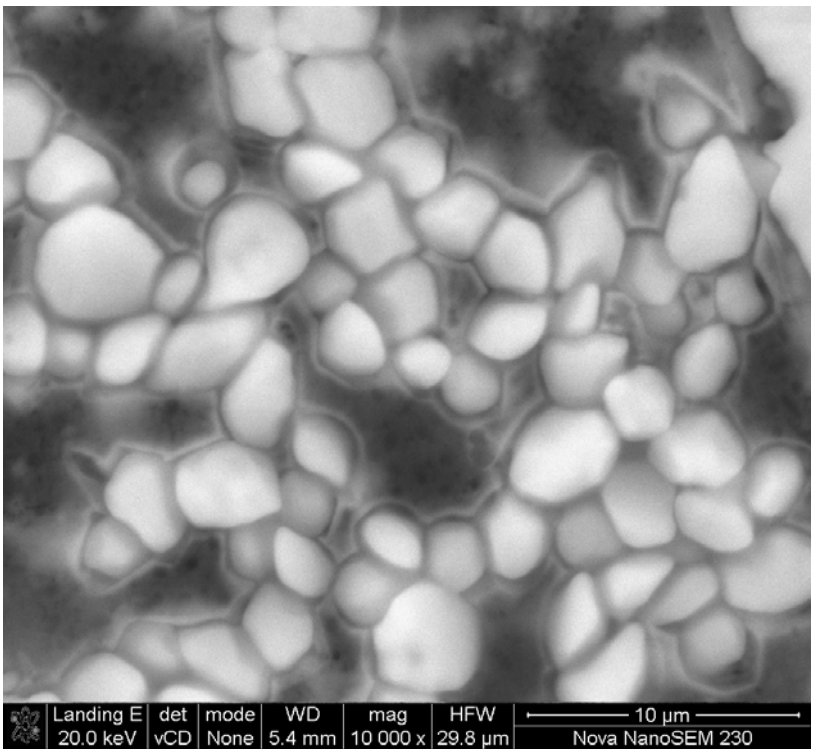

Figure 11: SEM micrograph of sampled biofilm. The bright crystalline structures represent elemental sulphur

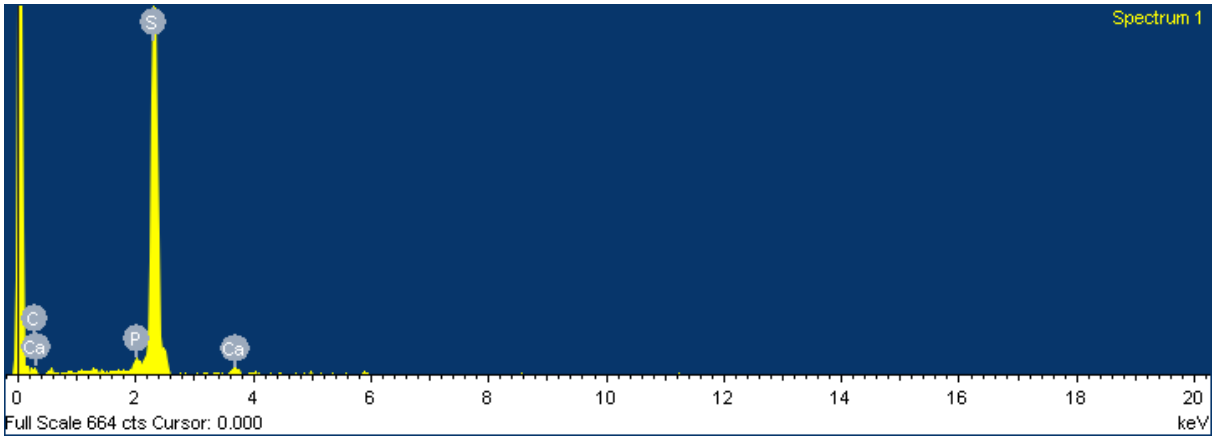

Figure 12: Elemental composition, determined by EDX analysis, of a portion of a sulphur grain. Based on the analysis the sulphur content is greater than 95%

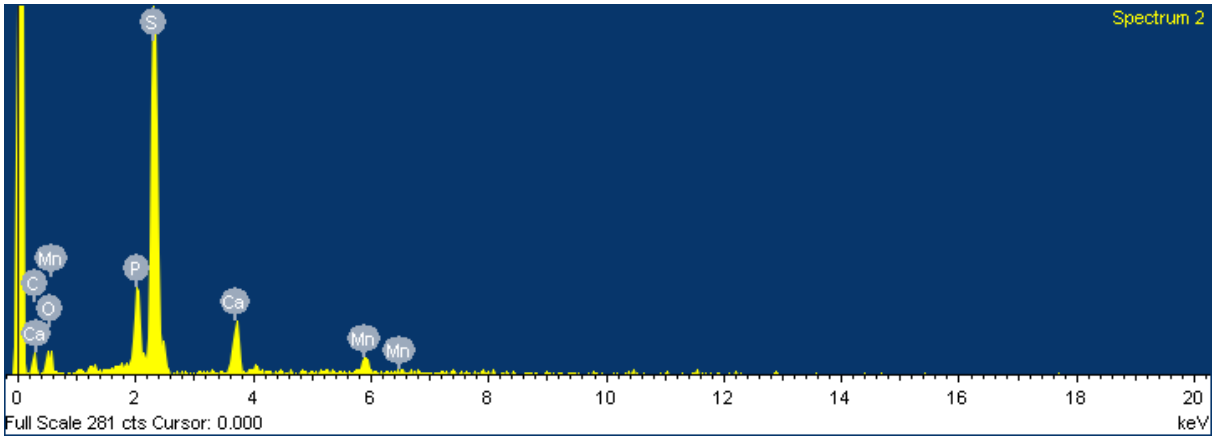

Figure 13: Elemental composition, determined by EDX analysis, of a portion of the non-sulphur matrix. Based on elemental analysis the dominant elements are S (47%), O (32%), P (9%) and Ca (7.5%)

The structural integrity of the biofilm was compromised by the limited organic content. As with the previous case the VFA content of the DPBR effluent was below 10 mg/L, comprising solely of acetate. As a result it was decided to supplement subsequent runs with additional acetate.

4.2.3 LFCR 3 - complete biofilm formation

The previous studies highlighted the importance of biofilm formation in achieving consistent sulphur formation as well as the benefit of a consistent sulphide concentration in the influent. The inconsistent performance of the DPBR columns necessitated some manipulation of the operating parameters to achieve this consistency. The sulphide concentration in the feed was maintained at approximately 200 mg/L with the addition of sodium sulphide (when required). To counteract the low organic carbon content of the DPBR effluent 20 g of acetate (as sodium acetate) was added to the LFCR at initiation. A final change was the increase if feed rate to 12.5 L/day, giving a hydraulic retention time of two days.

The reactor attained stable performance after three days. The sulphur balance across the system for the duration of the experiment (12 days) is summarised in Table 7. A total of 725.52 mmoles of sulphide, 1184.1 mmoles of sulphate and 18.84 mmoles of colloidal sulphur were fed to the reactor. The various analytical techniques employed allowed the sulphur mass balance to be closed to within approximately two millimoles. The overall sulphide conversion was 78.9%, which increased to 82.2% once the stable state had been achieved. Of that, 93% was converted to sulphur with the vast majority (98.7%) reporting to the biofilm and only a small fraction remaining as colloidal sulphur.

Table 7: Summary of sulphur species over the duration of the experiment. Thiosulphate was not detected in significant amounts

	Sulphide	Sulphur		Sulphate	Polysulphide
		Colloidal	In biofilm		
	mmoles	mmoles	mmoles	mmoles	mmoles
In	725.52	18.84	-	1184.1	-
Out	152.79	25.49	-	1217.1	4.0
Consumed	572.73		-	-	-
Produced	-	6.65	527	33.0	4.0

Figure 14 presents an example of the sulphide concentration profile in the LFCR as a function of position in the reactor. The consistency of these profiles was used to assess the stability of the system.

The situation depicted in Figure 14 differed significantly from that in the abiotic controls. The contour plots of the sulphide distribution in the control reactor have been reported previously (Van Hille and Mooruth, 2011) and showed stratification initially, in line with the hydrodynamic flow, but homogeneous distribution once steady state had been achieved. The consistent gradient presented above provides evidence for the microbial action.

A stable biofilm formed within three days during this experiment. While the biofilm was not as thick as those observed in the pilot scale reactor at GARL it was structurally sound. The biofilm did not pass through a "sticky" phase, but it the progressive increase in the sulphur content could be visually observed. At the end of the experiment the biofilm was harvested, dried and a portion dissolved in chloroform to determine the sulphur content. The results indicated a sulphur content of 66%, with the remainder comprising organic material, cells and inorganic precipitates.

4.3 Determination of sulphide oxidation kinetics in bulk phase as a function of time

The rate law was used to determine the rate constant for the two abiotic controls and the most effective of the biofilm experiments. The analysis showed that the reaction rate (α) was first order with respect to sulphide concentration. For the abiotic controls the rate constant (k) was 0.0234 and 0.068 for the uncontrolled (Figure 15) and pH controlled (Figure 16) experiments respectively.

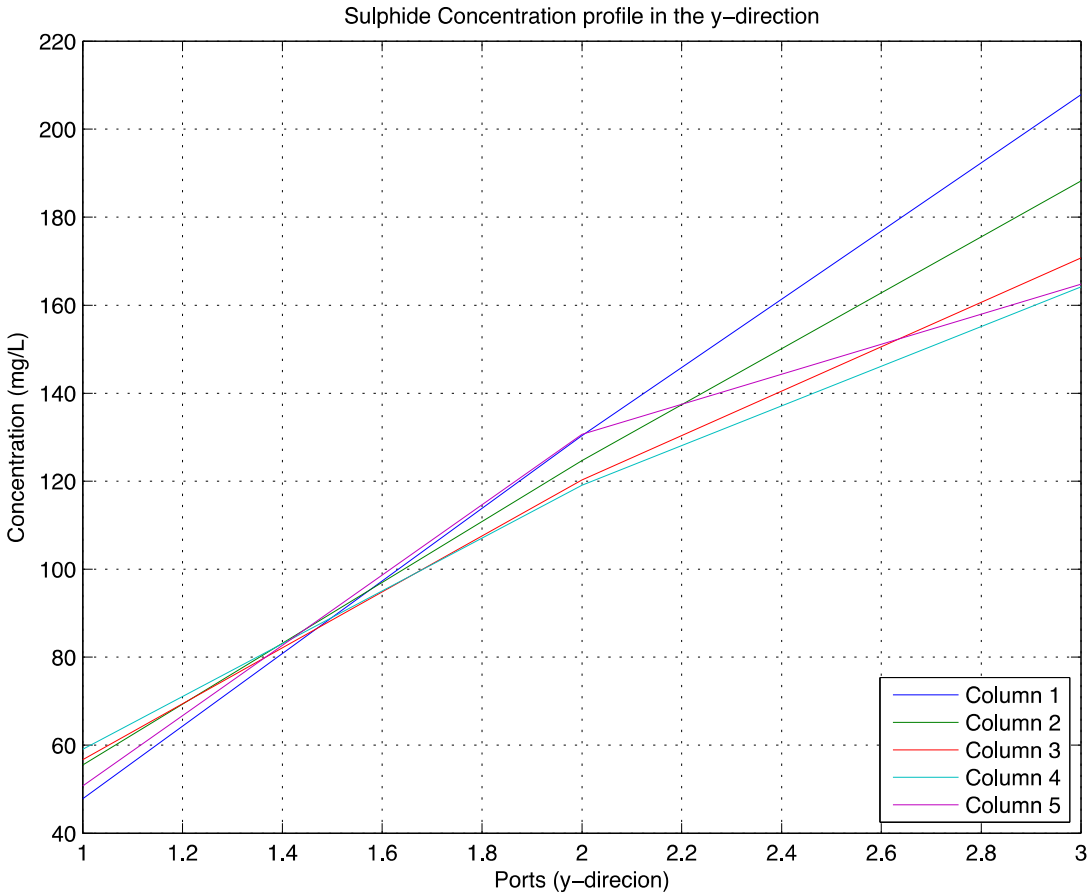

Figure 14: Sulphide concentration as a function of depth below surface (port 1 = uppermost sample port and port 3 = lowest) and horizontal position (column 1 = closest to inlet, column 5 = closest to outlet)

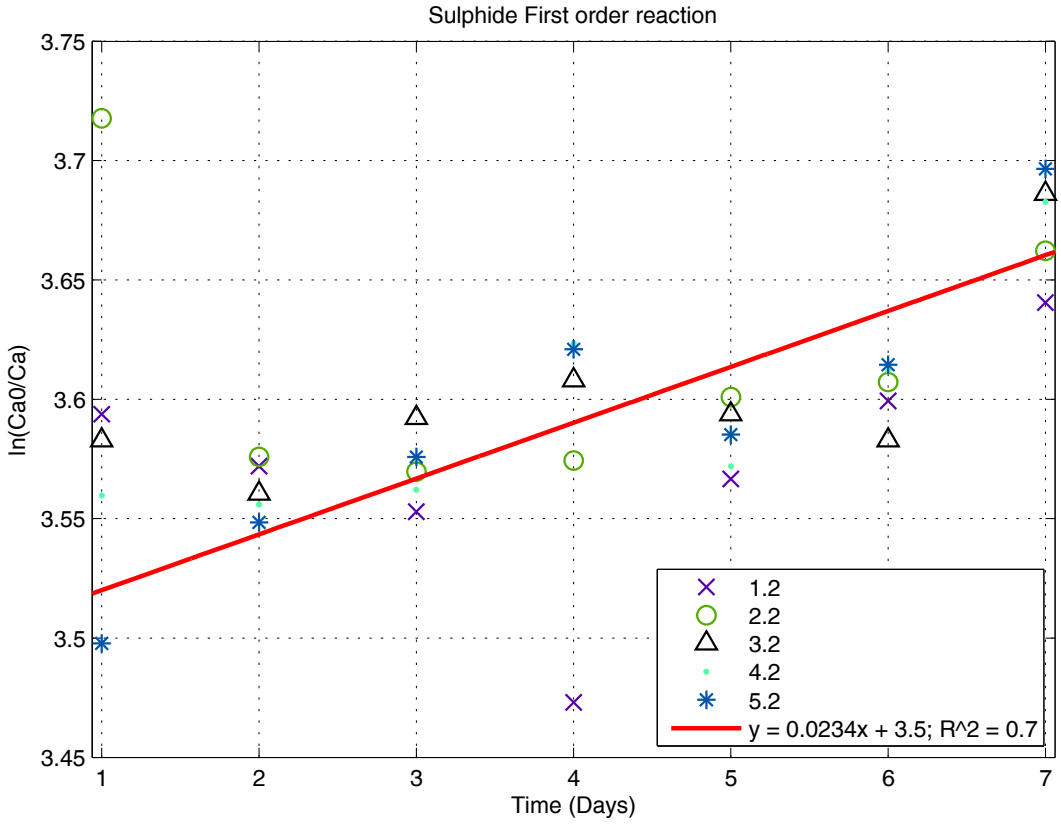

Figure 15: Determination of reaction order (α) for sulphide conversion in the abiotic control reactor with no pH control. The data points represent the mid-level sample port along the length of the reactor

In both cases the fit (R^2) is in the region of 0.7. The scatter in the data is a function of the inhomogenity within the reactor. The rate constant is significantly higher for the reactor run at a lower pH. At high pH the elevated concentration of hydroxide ions suppresses the oxidation of sulphide to elemental sulphur. In addition, any sulphur that may forms at this pH is unstable, reacting rapidly with hydroxide ions to from thiosulphate and bisulphide (Equation 10). This is consistent with the sulphur species data which showed relatively little sulphide conversion, with the majority most likely accounted for by thiosulphate.

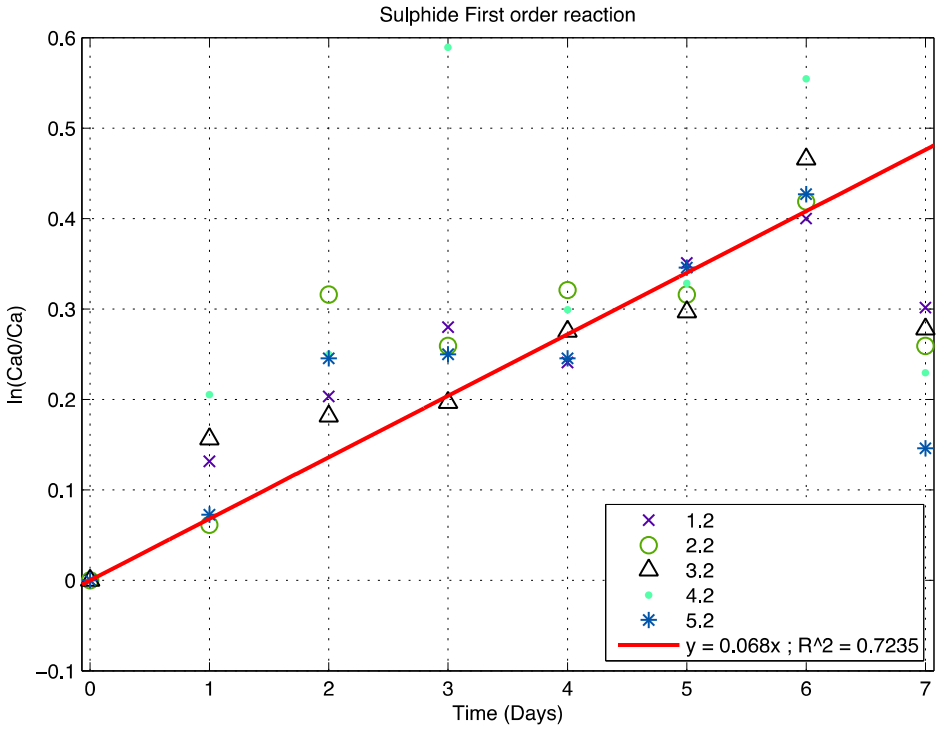

Figure 16: Determination of reaction order (α) for sulphide conversion in the abiotic control reactor with pH control. The data points represent the mid-level sample port along the length of the reactor

The rate constant for sulphide conversion was significantly higher in the pH controlled system. Again this is consistent with the physical observation of the system, where the solution turned a yellow-green colour and colloidal sulphur formation and consumption was visible (Figure 17). The yellow-green colour was not consistent throughout the reactor, being more apparent near the feed point and disappearing near the effluent point. This is consistent with the HPLC data, which showed little polysulphide in the effluent stream. Chen and Morris (1972) indicated that polysulphide species have been observed where elemental sulphur was formed by chemical oxidation at pH 7. This is consistent with the observation depicted in Figure 7. They continue to say that polysulphide oxidation occurred more rapidly at that pH than sulphide oxidation and they therefore occur as intermediates. The most likely reaction product was thiosulphate, which is not coloured.

The data (Figure 17) show that the conversion of sulphide was first order in the biological system, with a rate constant of 1. This is significantly higher than the rate constants for either of the abiotic systems (0.0234 and 0.068) and is consistent with the sulphur species data which show efficient conversion of sulphide to elemental sulphur and the deposition of the sulphur in the biofilm.

Based on the preliminary kinetic analysis, the rate of sulphide conversion in the two abiotic reactors was 0.005 and 0.31 mmoles/day and 1.61 mmoles/day for the system where the biofilm was complete.

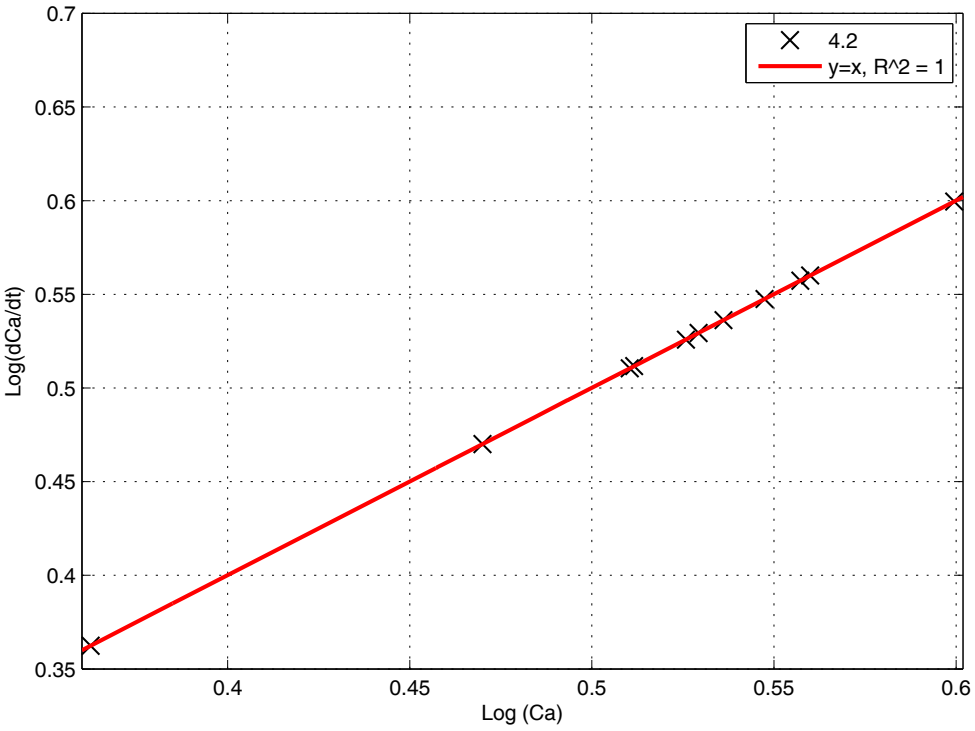

Figure 17: Determination of reaction order (α) for sulphide conversion in the bioreactor where a complete biofilm was observed

4.4 Organic carbon flux and impact of molasses supplementation

The biofilms formed in the LFCR studies described above were structurally different from those observed in the pilot reactor at GARL in that they were thinner, more brittle and did not progress through the stages previously described. Observations of the pilot reactor showed that biofilm development across the various channels followed a consistent pattern. The biofilm started forming near the feed inlet point and progressed further with time. Further from the sulphide source the biofilm was a brownish colour and has a more "sticky" consistency. Closer to the sulphide source the biofilm became creamy white in colour and more brittle, which is consistent with an increasing sulphur content. This suggests that the biofilm was initially primarily organic in nature and the sulphur content increased with time. This progression was not observed in the current study, suggesting that organic carbon may be limiting. This was confirmed by VFA and sugar analysis on the DPBR column effluent, even for DPBR 1 where the effluent sulphide concentration was consistently below 100 mg/L.

4.4.1 Effect of molasses feed concentration on DPBR performance

In consultation with the K5/1834 project team the decision was taken to increase the molasses concentration in the DPBR feed from 1.5 to 2.5 g/L. The change was made on the 1st of March and had a positive impact initially, with the sulphide concentration in the both columns increasing (Figure 18). However, after one week the pH of the effluent decreased significantly (Figure 19). This was accompanied by a rapid decrease in the sulphate reduction efficiency. Between the 8th and 19th of March the sulphide concentration in the effluent from DPBR 1 decreased from 96 to 0 mg/L, while for DPBR 2 the decrease was 306 to 61 mg/L. HPLC analysis of the effluent from the columns at this stage showed that the VFA concentrations had increased significantly. Acetic and iso-valeric acids were the two major constituents. The acetic acid values were similar for the two columns (157 and 173 mg/L), but the iso-valeric acid concentration was significantly higher in DPBR column 2 (661 mg/L) than column 1 (173 mg/L). Example chromatograms are displayed in Appendix B.

On March 20 the feed to the columns was stopped. A sample (250 mL) was drawn from DPBR column 2 and used to inoculate a series of batch reactors. The columns were then supplemented with 1 L of active

SRB inoculum from another experiment, after which feeding was resumed at 1.5 g/L molasses. The sulphide concentration recovered back to pre-perturbation levels.

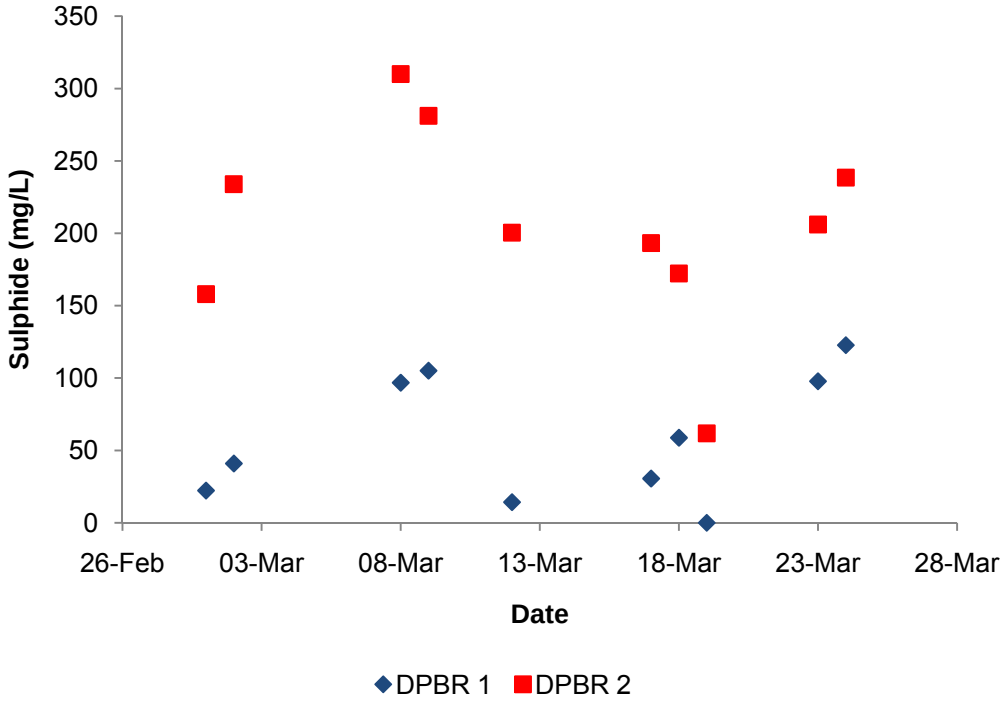

Figure 18: Sulphide concentration in the DPBR column effluent as a function of time. Feed molasses feed concentration was increased from 1.5 to 2.5 g/L on 1 March. On 20 March column feeding was stopped, columns were inoculated with 1 L of active culture and feeding resumed at 1.5 g/L molasses

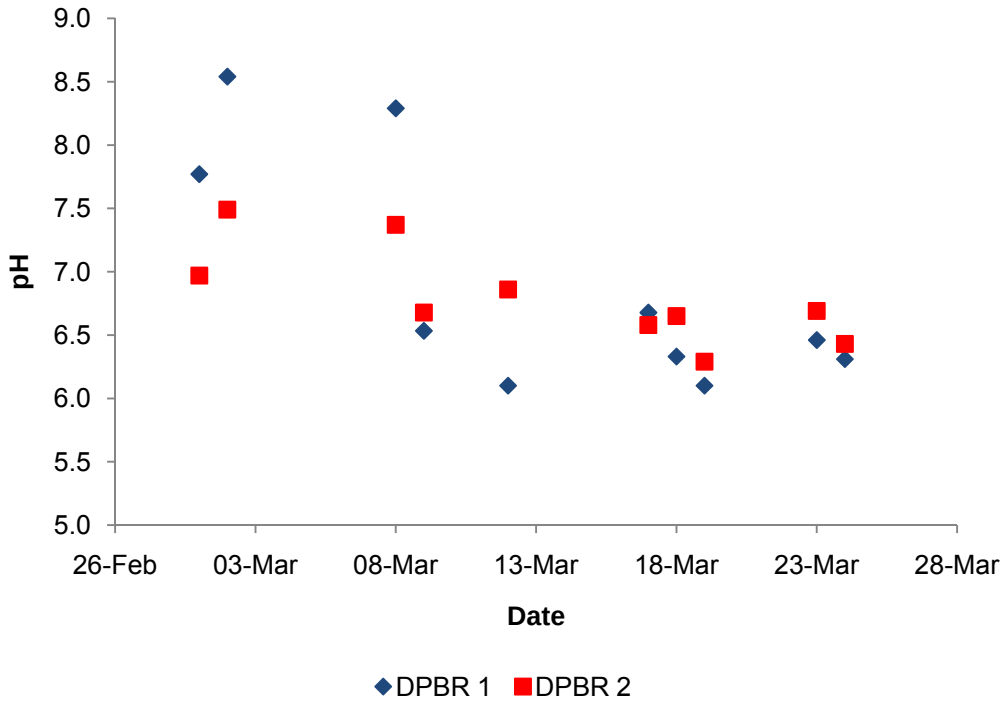

Figure 19: pH in the DPBR column effluent as a function of time. Feed molasses feed concentration was increased from 1.5 to 2.5 g/L on 1 March. On 20 March column feeding was stopped, columns were inoculated with 1 L of active culture and feeding resumed at 1.5 g/L molasses

4.4.2 Batch flask tests

The decision to increase the molasses feed concentration had a dramatic effect on the performance of the DPBR columns. The implication is that the molasses, used to "kick-start" the degradation of the complex carbon was playing a more significant role. The sulphide concentration in the column effluent was typically below 250 mg/L for DPBR 2, the more efficient of the two reactors. The batch tests were designed to investigate the utilisation of molasses as a carbon source under more controlled conditions and determine the amount of sulphate reduction that could be sustained.

The COD of a 1.5 g/L molasses solution was determined to be 1 200 ± 26 mg/L, giving a COD to sulphate ratio for the batch tests of 0.6.

The pH, redox potential and sulphide concentration data are presented below (Figures 20-22). The data for the three reactors inoculated with DPBR effluent show a consistent trend. There was a rapid decrease in pH over the first 24 hours, despite the addition of sulphide and bicarbonate prior to inoculation. The most likely explanation is the rapid conversion of the sugars in the molasses to VFAs. The reactor inoculated with the SRB sludge performed significantly differently, with no decrease in pH observed after 24 hours.

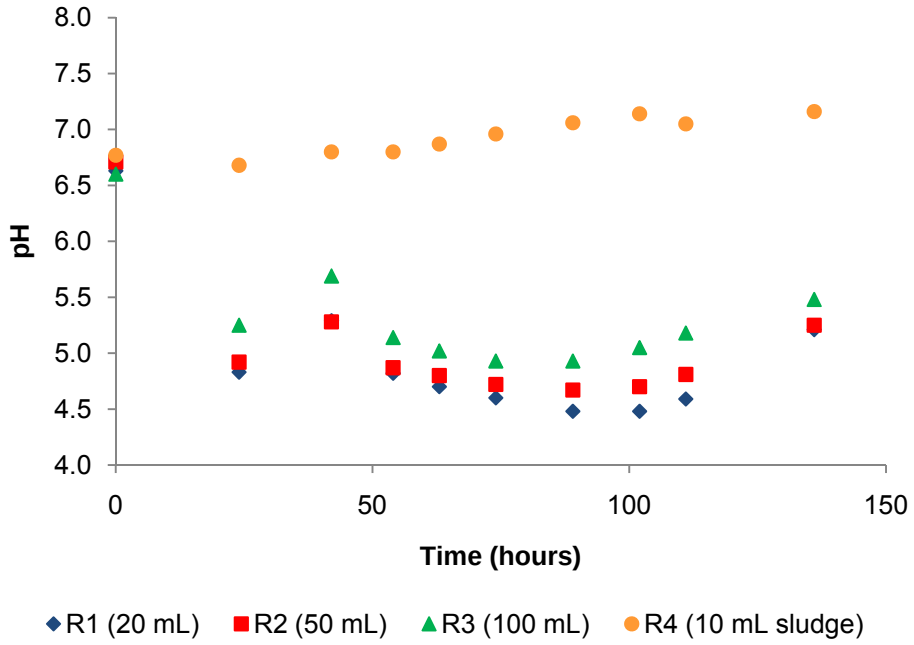

Figure 20: pH profile for the batch sulphate reduction test investigating molasses as a sole carbon source and electron donor. For reactors 1-3 the number in parentheses represents the volume of DPBR effluent used as an inoculum. Reactor 4 was inoculated with 10 mL of active sulphidogenic sludge

The redox potential (Figure 21) and sulphide (Figure 22) data are consistent with the pH data. The rapid decrease in pH inhibited sulphate reduction, with no sulphide formed in reactors 1-3. Consequently, the redox potential of those reactors became less negative. The sludge inoculated reactor did show significant sulphate reduction during the first 24 hours, with the sulphide concentration increasing to over 80 mg/L. This was coupled to a decrease in redox potential to -364 mV.

In an attempt to revive the effluent inoculated reactors sufficient sulphide to increase the aqueous concentration to 50 mg/L was added. Reactor 4 did not receive additional sulphide. The sulphide addition had a short term effect, with an increase in pH and decrease in redox potential observed in the subsequent sample. However, the sulphide data showed that no additional sulphide had been formed and the pH and redox effect were due to the added sulphide. During the same period reactor 4 continue to produce sulphide at a consistent rate, resulting in a slight increase in pH and reduction in redox potential.

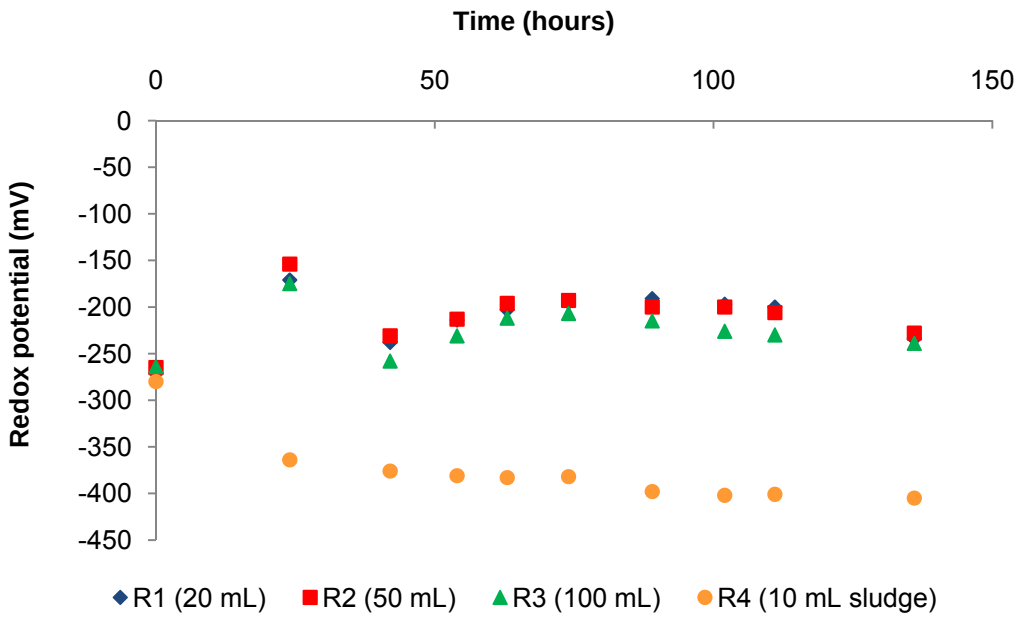

Figure 21: Redox potential profile for the batch sulphate reduction test investigating molasses as a sole carbon source and electron donor. For reactors 1-3 the number in parentheses represents the volume of DPBR effluent used as an inoculum. Reactor 4 was inoculated with 10 mL of active sulphidogenic sludge

Reactor 4 continued to generate sulphide at a linear rate (2.95 mg/L.h) until hour 54. Between hour 54 and 63 no further sulphide was produced, suggesting electron donor exhaustion. However, between 63 and 111 hours sulphate reduction resumed, initially at a linear although slightly reduced rate, before finally ceasing after 111 hours. These data suggest that the molasses either contained, or was metabolised to produce, two potential electron donors which were utilised sequentially.

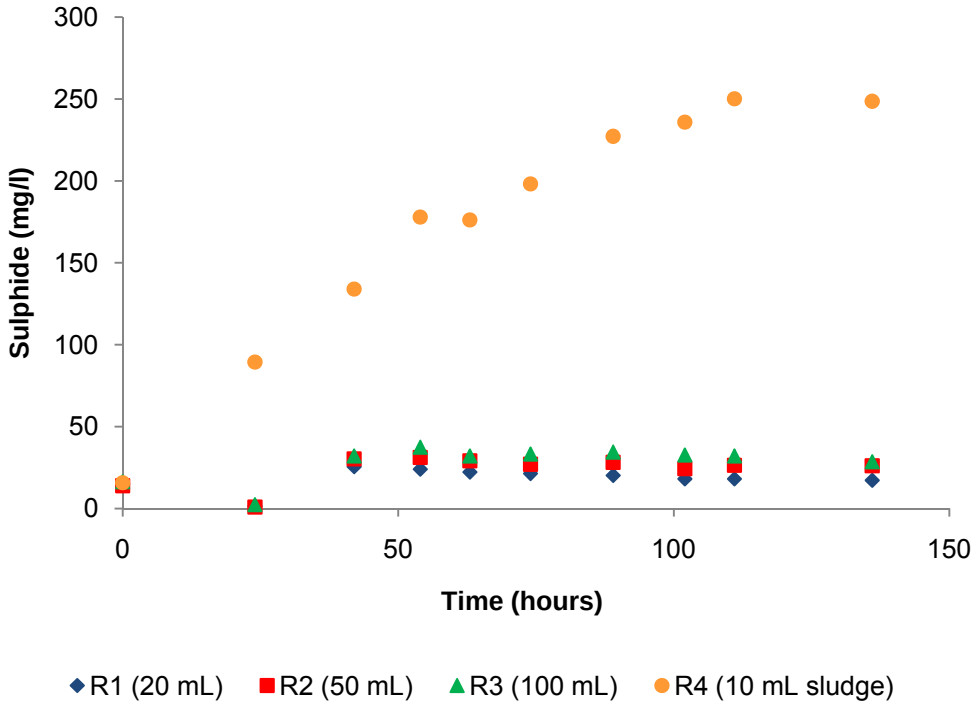

Figure 22: Aqueous sulphide profile for the batch sulphate reduction test investigating molasses as a sole carbon source and electron donor. For reactors 1-3 the number in parentheses represents the volume of DPBR effluent used as an inoculum. Reactor 4 was inoculated with 10 mL of active sulphidogenic sludge

The maximum sulphide concentration attained was just over 250 mg/L, similar to the highest obtained in DPBR 2 when fed with 1.5 g/L molasses, equating to the reduction of 727 mg/L of sulphate. The data suggest that sulphate reduction in the DPBR columns is largely driven by molasses metabolism. The sludge used to inoculate reactor 4 had previously been tested under identical operating conditions with ethanol as the electron donor at a COD to sulphate ratio of 0.7. The linear sulphate reduction rate achieved in those tests was 5.06 mg/L.h. Control tests conducted in the absence of ethanol showed no sulphate reduction, confirming that the sludge did not contain any residual electron donor.

The outcome of this trial suggests that the DPBR columns contain a population that effectively converts molasses to VFAs and this population is either significantly larger or more active that the sulphate reducing population. This would explain the response of the columns to the increase in molasses load and the batch studies.

5 CONCLUSION AND IMPLICATIONS FOR PLANT OPERATION

The data presented in this report represents the most complete characterisation of the LFCR system to date. The extensive sampling protocol and suite of analyses showed the inhomogeneity within the reactor, primarily driven by the hydrodynamics. Despite this, an almost complete sulphur species mass balance across the system was possible. The results from the experimental runs detailed in this report and ongoing experiments highlight a number of important issues for large scale process operation.

- The ratio of oxygen to sulphide in the region of the biofilm is critical to ensure the desired product formation. The oxygen partial pressure remains constant implying that significant fluctuations in the sulphide concentration entering the system could have a substantial effect on system performance, particularly during the early stages of biofilm development. Once a continuous biofilm is in place the oxygen mass transfer is regulated by diffusion across the boundary.
- Heterotrophic sulphur oxidising microorganisms play an important role in biofilm formation so the provision of sufficient organic carbon in critical to ensure effective biofilm formation. Insufficient organic material compromises the structural integrity of the biofilm and results in process inefficiency.
- The long term performance of the DPBR should be monitored with respect to the organic carbon released. The columns used in this study had been operated at GARL for over a year prior to being moved to UCT. The effect on performance of changing the molasses feed concentration and the data from the batch tests imply that the microbial activity (acidogenesis and sulphate reduction) in the DPBR columns is controlled by molasses availability, rather than the hydrolysis and metabolism of the lignocellulosic material.

The integrated reactor system has been operated successfully, achieving over 80% sulphide oxidation, with the majority being converted to sulphur in the biofilm. However, this was achieved under conditions where the sulphide loading into the LFCR was controlled and additional organic carbon was provided. The implication is that efficient operation of the sulphide oxidation units of the IMPI process will be dependent of stable, reliable DPBR performance.

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Appendix A: Composition of synthetic AMD fed to DPBR columns

The composition is based on Landau effluent after neutralisation with lime. Feed pH adjusted to pH 6.1-6.5

Component	Concentration mg/l	Concentration mM
SO_4^{2-}	2 000	20.8
Al^{3+}	100	3.7
Ca^{2+}	230	5.8
Fe^{2+}	35	0.6
Mg^{2+}	210	8.6
Mn^{2+}	96	1.7
Na^{+}	8	0.3

Appendix B: Sample chromatograms from VFA analyses

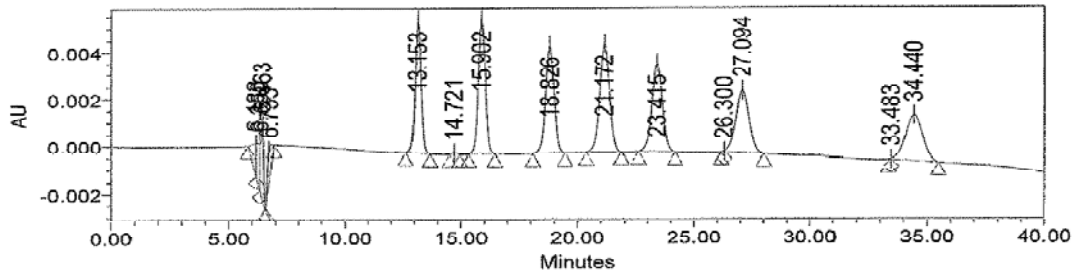

Figure B1: Chromatogram of VFA standards illustrating characteristic retention times

Volatile fatty acid	Retention time (min)
Lactic	13.15
Acetic	15.90
Propionic	18.83
Iso-butyric	21.17
Butyric	23.42
Iso-valeric	26.91
Valeric	34.45

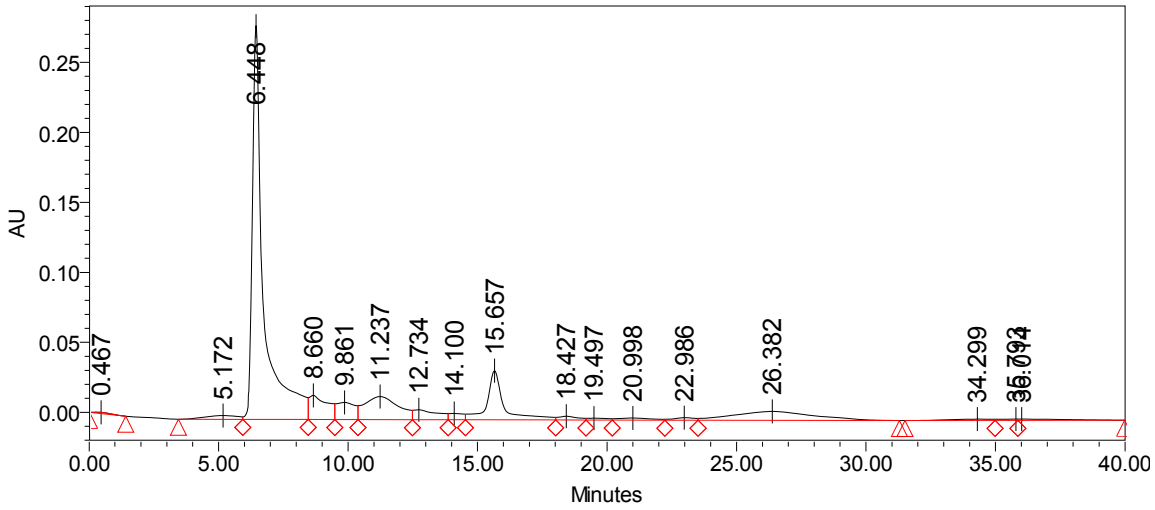

Figure B2: Chromatogram showing VFA distribution in column 1 effluent after molasses feed was increased to 2.5 g/L (19 March)

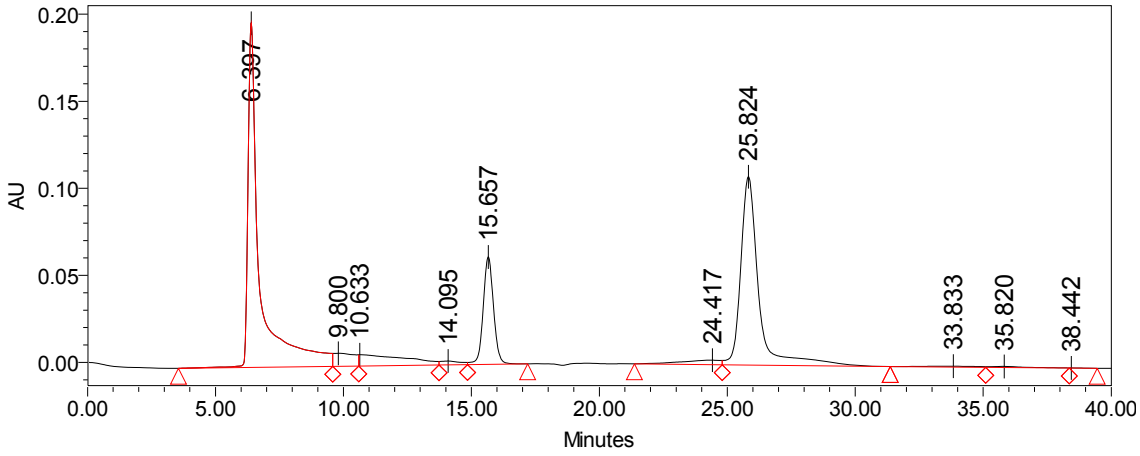

Figure B3: Chromatogram showing VFA distribution in column 2 effluent after molasses feed was increased to 2.5 g/L (19 March)

Appendix C: Standard curve for COD analysis

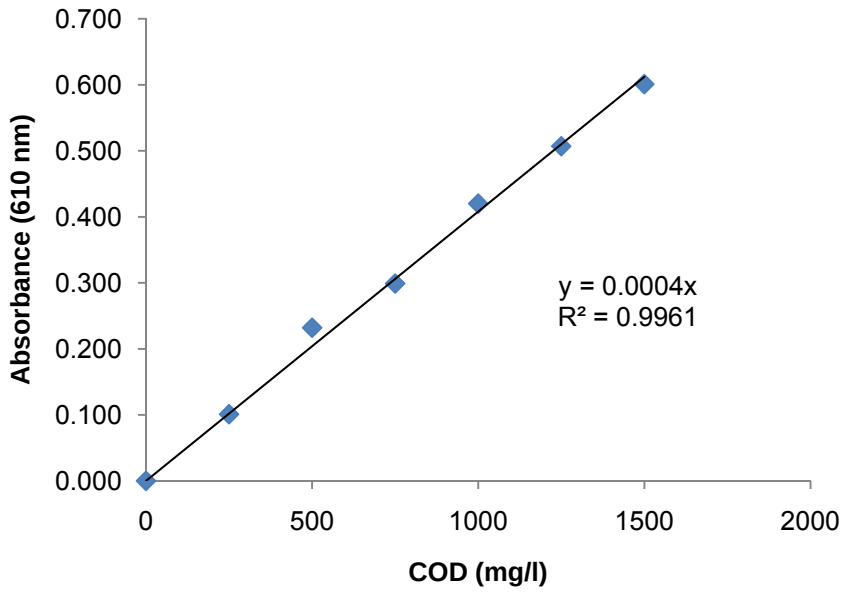

Figure C1: Standard curve for COD analysis, using potassium hydrogen phthalate as the standard material.

Appendix D: Polysulphide standards

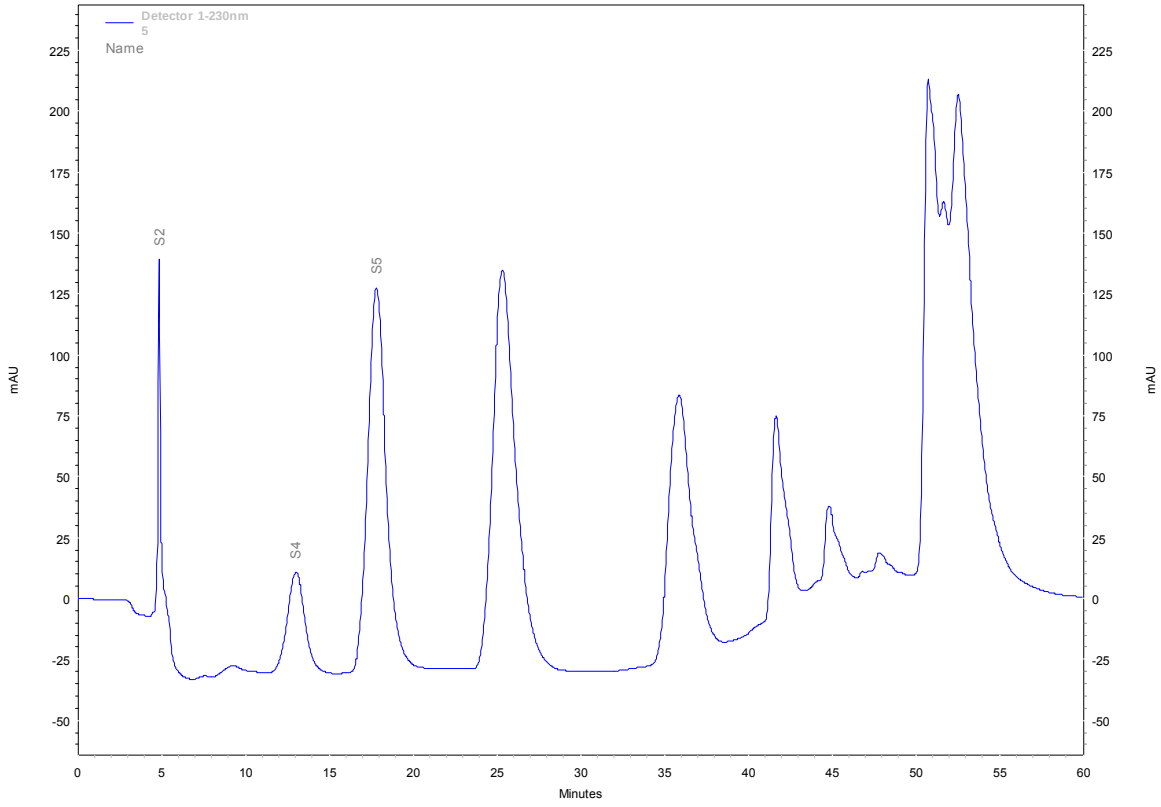

Figure D1: Chromatogram showing distribution of peaks for polysulphide standards