DEVELOPMENT OF AN IMMOBILISED FIXED FILM SYSTEM FOR SULPHIDE OXIDATION IN PASSIVE MINEWATER TREATMENT

Report to the
Water Research Commission

by

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The publication of this report emanates from a project entitled *Development of an immobilised fixed film system for sulphide oxidation in passive minewater treatment* (WRC project K8/763).

EXECUTIVE SUMMARY

The work reported in this study was undertaken to evaluate, at the pre-feasibility level, the proposition, based on observations by researchers at the Rhodes University Environmental Biotechnology Research Unit over several years, that the immobilised sulphide oxidising fixed film system may be considered for the treatment of sulphide containing wastewaters. The passive treatment application was targeted in these studies where sulphide removal presents a severe technological bottleneck in the development of these treatment systems.

The current Consultancy Project (K8/763) was undertaken to consolidate and confirm work undertaken at EBRU on sulphide oxidising fixed film systems and to investigate the intellectual property position on which further investment in the process may be considered.

The following aims were identified for the study:

- To undertake a preliminary investigation at the pre-feasibility level of the immobilized fixed biofilm system for sulphide oxidation;
- To undertake a preliminary literature review and patent search;
- To investigate optimum process configurations of the tubular reactor;
- To construct and operate a prototype bench-scale plant;
- To make recommendations on the future development of the immobilized fixed biofilm system for sulphide oxidation.

Laboratory-scale studies are reported and the following conclusions may be drawn:

- The feasibility of the sulphide oxidising tubular fixed film system for sulphide removal from wastewaters has been demonstrated;
- This has been demonstrated at the pre-feasibility scale and provides sufficient evidence on which to base larger-scale engineering scale-up studies;
- The results acquired appear to warrant investment in further development of the process;
- Patent and literature searches confirm the originality of the work and that intellectual property residing with the WRC (US Patent, Rose and Rein, 2007) provides a

satisfactory base for the protection of the rights of any further investment in development of the process;

- The intellectual property rights would be protected until approximately 16 May 2023 if the registration is kept current;
- Passive treatment of AMD is certain to grow in importance as the gold and coal mining industries in South Africa reach maturity and large scale mine closure ensues. Biological treatment offers one of the few environmentally sustainable technology options and this is crucially dependent on effective technology for the removal of sulphur species from the treated water;
- It is thus strongly recommended that the patent be maintained and that further work be undertaken to establish an engineering scale-up and implementation of the process at industrial scale.

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LIST OF ABBREVIATIONS

AMD	Acid mine drainage
COD	Chemical oxygen demand
DACST	Department of Arts, Culture, Science and Technology
EBRU	Environmental Biotechnology Research Unit
HPLC	High Performance Liquid Chromatography
HRT	Hydraulic retention time
IC	Ion chromatography
RASBR	Recycling Sludge Bed Reactor
SEM	Scanning electron micrograph
SOB	Sulphate oxidizing bacteria
SRB	Sulphate reducing bacteria
STP	Standard temperature and pressure
TEM	Transmission Electron Microscopy

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1. OBJECTIVES

The following aims and methodology were identified for this study:

1.1 Aims

- To undertake a preliminary investigation at the pre-feasibility level of the immobilized fixed biofilm system for sulphide oxidation;
- To undertake a preliminary literature review and patent search;
- To investigate optimum process configurations of the tubular reactor;
- To construct and operate a prototype bench-scale plant;
- To make recommendations on the future development of the immobilized fixed biofilm system for sulphide oxidation.

1.2 Methodology

1. Undertake a preliminary literature review and patent search on tubular fixed film reactors applied in the treatment of sulphide wastewaters.
2. Based on the above outcomes (if any), and using the fundamental insights acquired in the floating sulphur biofilm study, to attempt to formulate a theoretical basis for approaching the experimental studies.
3. To undertake an empirical approach to process design, informed, at best, by available theoretical insights, and to construct a number of bench-scale process configurations in which the fixed biofilm concept can be evaluated. Existing materials, pumps, sulphide generators etc will be used.
4. Having established at least one prototype process and reactor design, to operate the plant and derive preliminary operational data.
5. Standard analytical techniques already in use at EBRU will be applied including sulphate, sulphide, thiosulphate, sulphur, total organic carbon.

2. BACKGROUND

During the late 1980s work commenced in the Environmental Biotechnology Research Group at Rhodes University on the microbial ecology of tannery ponding systems. These studies focussed in particular on the operation of the biological sulphur cycle and, subsequently, on the application of findings to the treatment of sulphate salinity in acid mine drainage (AMD) wastewater treatment operations (Rose and Cowan, 1992; Rose et al., 1996; Rose et al., 1998; Rose, 2002).

Process development studies on AMD treatment, based on this work and funded by the WRC, resulted in the novel application of the Rhodes BioSURE Process®, in active treatment operations (Rose et al., 2004). Together with Pulles, Howard & de Lange, the WRC and the Department of Arts, Culture, Science and Technology (DACST), the IMPI process was developed for passive treatment of AMD (Pulles and Rose, 2002).

In the course of work on the use of complex biological substrates as carbon and electron donor sources for sulphate reducing bacterial activity, and in a substantial literature on the topic, reviewed among others by Whittington-Jones et al. (2002), the biological principles and engineering applications of the sulphate reduction component of the AMD treatment operations has become fairly well understood. Process development application projects funded by the WRC led to the construction of both pilot and full-scale commercial plants for the treatment of AMD.

However, a technological bottleneck exists in the removal of sulphide, especially, but not only, in passive systems, to ensure that sulphur species present in the minewater are finally removed from the system. While a number of technologies are available for active treatment applications, progress on passive systems has been slight. One exception is the development of the floating sulphur biofilm system developed by the Environmental Biotechnology Research Unit (EBRU) at Rhodes University (Molwantwa et al., 2003; Molwantwa, 2008). This is now the subject of evaluation by industry. However, while offering important advances in this application the process, as currently developed, is a complex operation and presents challenges to engineering modelling and scale-up.

Preliminary work at EBRU has shown that the principles of sulphur biofilm formation could be engineered in an immobilized fixed biofilm system instead of the floating film process configuration (Gilfillan, 2000; Rein, 2002). These studies have provided preliminary data showing that a tubular reactor configuration may be developed and applied effectively in the passive treatment application.

The purpose of this consultancy project was to pull the various observations together and to confirm the work at the pre-feasibility level. Where this would be successful, to then enable detailed follow-up process development and design studies leading possibly to evaluation at pilot plant scale.

3. LITERATURE REVIEW

3.1 Sulphur Cycle

The cycling of sulphur compounds through the biosphere is driven largely, but not only, by the biological sulphur cycle (Figure 1). Where they occur together, the sulphide oxidising and sulphate reducing microorganisms responsible for the cycling of sulphur form a tightly integrated ecological community known as a sulfuretum (Jorgensen, 1982). Tannery wastewater ponds provide an example of such an organisation.

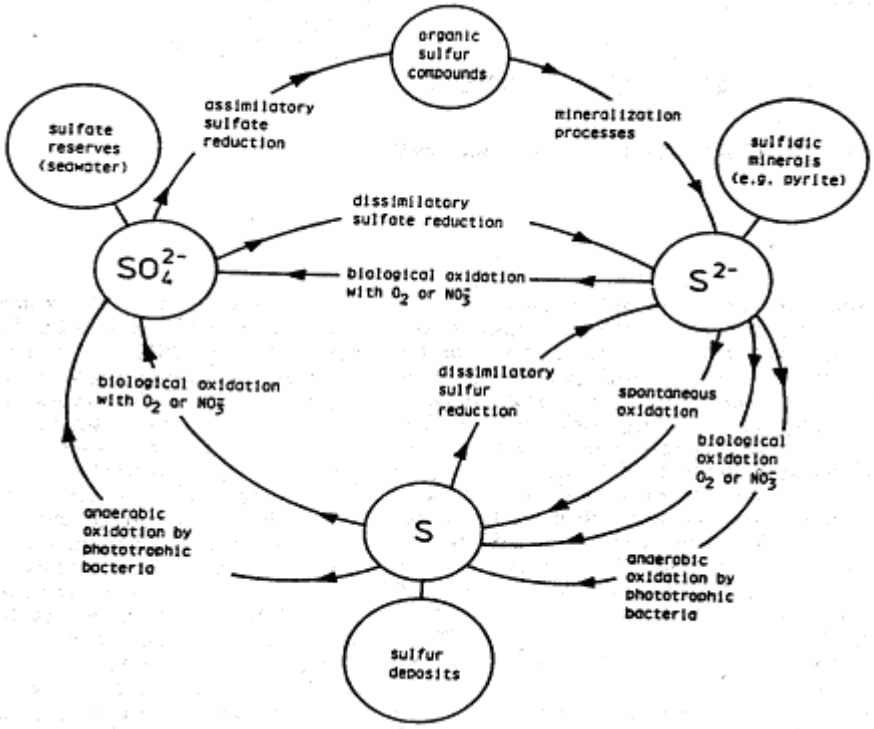


Figure 3.1 The Sulphur Cycle (Kuenen and Robertson, 1992).

The role of the sulphide oxidising component of this microbial system in the formation of sulphate saline wastewaters and AMD has been well described and extensively reviewed (Johnson and Hallberg, 2003). Both active (Rose, 2002; Neba, 2006) and passive AMD treatment systems (Pulles *et al*., 1995; Younger *et al*., 1997; Younger, 1998; Zipper and Jage, 2001; Molwantwa *et al*., 2003; Younger, 2004; Coetser *et al*., 2005) have been developed using the biological sulphate reducing component of the sulphur cycle for the removal of sulphate salinity, heavy metal contamination and the neutralization of the acidic stream.

Since sulphate reduction is the central operation of these various biological AMD treatment processes (Johnson and Hallberg, 2003), the sulphide produced in this way needs to be removed from the system in order to prevent its re-oxidation to sulphate and thus defeating the aim of the treatment operation. The objective would thus be the linearization of sulphur flow (total removal and not re-cycling from one form to another) and therefore effective bio desalinisation in a combined treatment operation (Rose, 2002; Molwantwa *et al*., 2007).

3.2 Sulphide Consuming Processes

Sulphide may be removed from the environment by one of four processes (Figure 3.1):

1) Reaction with metal ions to form insoluble metal sulphide complexes, an example of which is pyrite formation. This represents a large pool of inert sulphur if maintained under anaerobic conditions. The kinetics of pyrite formation are slow with predictions based on the fastest mechanism of pyrite formation known, indicating that only $9x10^{-13}$ mol FeS_2 $.L^{-1}$ of sediment.day^{-1} may be formed (Rickard, 1997);

2) Reaction with other sulphur compounds e.g. elemental sulphur to produce polysulphides or other compounds containing sulphur of mixed oxidation state. These compounds can be regarded as intermediates of aqueous sulphide oxidation (Chen and Morris, 1972; Millero, 1986; Steudel, 1996) and oxidation of metal sulphides (Smart *et al.,* 2000). These intermediates are important in the biological cycling of sulphur compounds between oxic and anoxic compartments in the environment (Van den Ende, 1997);

3) Oxidation on reaction with molecular oxygen, the ultimate product of which is sulphate (Chen and Morris, 1972);

4) Biological oxidation by bacteria. Sulphide may be oxidised with either oxygen or nitrate as the electron acceptor by bacteria belonging to the group of colourless sulphur bacteria (Jorgensen, 1982) or under anaerobic conditions by photosynthetic sulphur oxidising bacteria (Van Niel, 1931)

The major portion of sulphide is biologically oxidised at anoxic/oxic interfaces (Stefess, 1993). Sulphur biofilms have been noted to develop on natural sulphur springs (Figure 3.2), tannery waste stabilisation ponds (Figure 3.3) and on the surface of biological sulphate

reducing laboratory reactors (Rose et al., 1998; Gilfillan, 2000; Rein, 2002; Molwantwa, 2008). Studies on these biofilms undertaken at EBRU are shown in Figures 3.4 and 3.5.

Figure 3.2 Photograph of a floating sulphur biofilm present on the surface of a hot spring in Namibia

Figure 3.3 Photograph of floating sulphur biofilm development on the surface of tannery waste ponds in Wellington, South Africa.

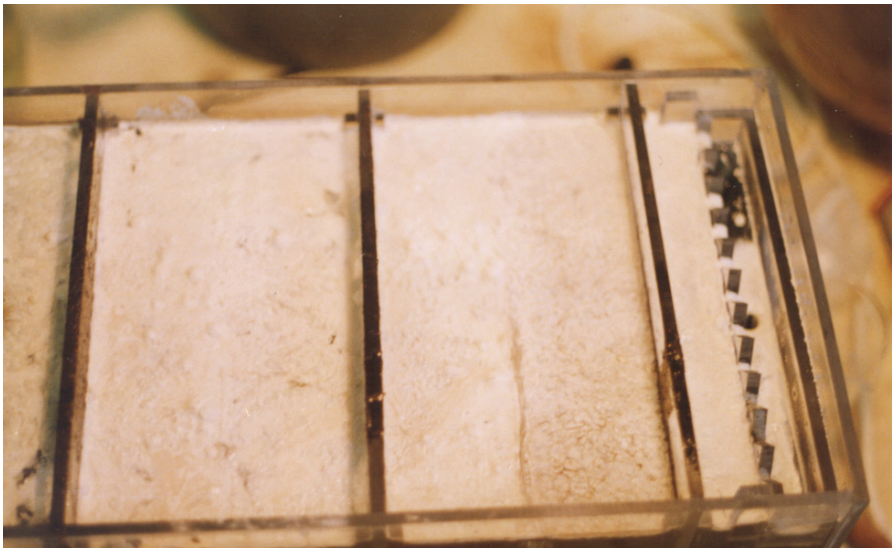


Figure 3.4 Photograph of a well-developed sulphur biofilm on the surface of a Recycling Sludge Bed Reactor (RSBR) used to investigate biofilm formation under laboratory conditions. Rhodes University, South Africa.

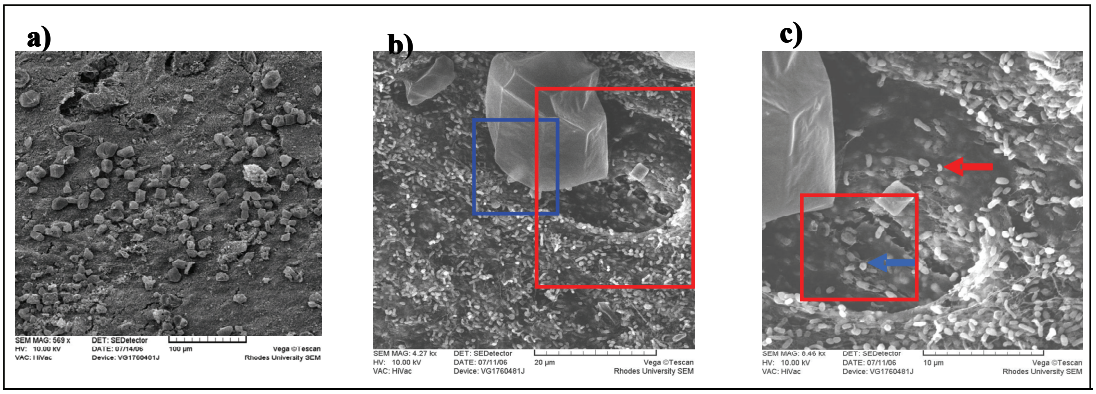


Figure 3.5 Electron micrograph of crystalline sulphur formed within the floating biofilms that develop on the surface of sulphate reducing reactors (Molwantwa, 2008).

3.3 Chemistry of Sulphide Oxidation

Hydrogen sulphide (H_2S) is a weak acid which dissociates into HS^- (pK_{a1}= 7.04) and S^{2-} (pK_{a2}). The pK_{a2} has variously been reported to be in the range $12<pK_{a2}<19$ (Loewenthal et al, 2001) and for practical purposes is disregarded. The term sulphide is commonly used for any of the reduced species that may be present. The two most important biologically relevant oxidation reactions which sulphide may undergo are (Kuenen, 1975):

$$2HS^- + O_2 \rightarrow 2S° + 2OH^- \qquad \Delta G°' = -129 \text{ kJ./mol } HS^- \qquad (1)$$

$$2HS^- + 4O_2 \rightarrow 2SO_4^{2-} + 2H^+ \qquad \Delta G°' -772.43 \text{ kJ/mol } HS^- \qquad (2)$$

These are overall equations for oxidation of sulphide. Other possible products of oxidation include thiosulphate ($S_2O_3^{2-}$) and polythionates ($^-S0_3\text{-}S_n\text{-}SO_3^-$) (Steudel, 1996; Steudel, 2000). In addition to this polysulphides (S_n^{2-}, n = 2-5) have been identified as important intermediates of oxidation of sulphide by oxygen according to Steudel (1996); Millero (1986) and Chen and Morris (1972). The following reaction mechanism (Figure 3.6) for the oxidation of sulphide has been proposed by Chen and Morris (1972).

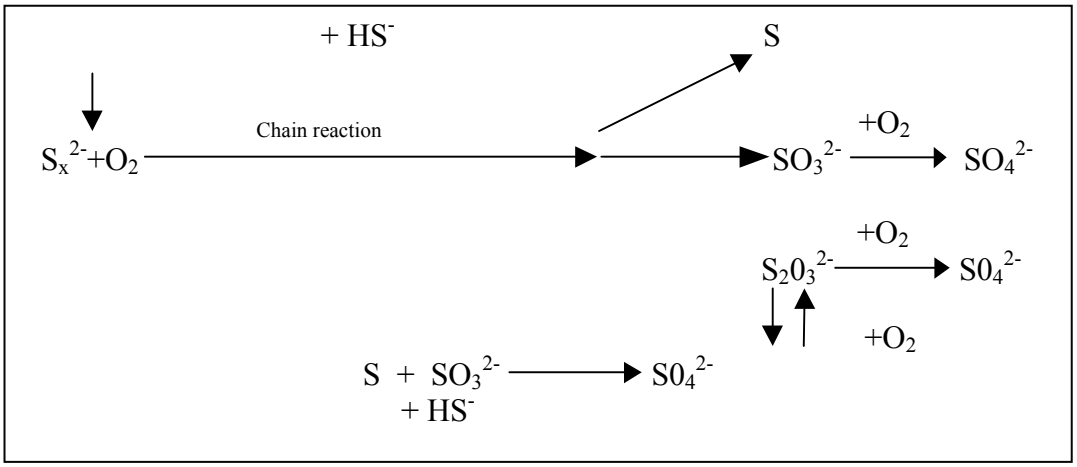


Figure 3.6 Mechanism of sulphide oxidation by oxygen as proposed by Chen and Morris (1972).

Chen and Morris (1972) suggested that the initial production of elemental sulphur, and subsequent reaction to produce polysulphides, was the rate-limiting step in sulphide oxidation. Furthermore they proposed that bacterial activity in this process functions to increase the rate of sulphide oxidation by increasing the rate of sulphur production.

3.4 Kinetics of Sulphide Oxidation

The kinetics of chemical oxidation of sulphide have been studied by various researchers. Chen and Morris (1972) found that sulphide oxidation by oxygen at pH 7.94 at 25°C could be described by the following equation:

$$R_I=k[S]^m[O_2]^n \quad (3)$$

Where:

[S] = Total sulphide concentration (M)

$[O_2]$ = Oxygen concentration (M)

m = 1.34

n = 1.56

k = 21.93

Chemical oxidation of sulphide with oxygen in a phosphate buffered system at pH 8 and at 20°C has been reported to be described by the following equation (Buisman *et al.,* 1990):

$$R_i=k[S]^m[O]^{n\log[S]} mg.L^{-1}.h^{-1} \quad (4)$$

Where:

R_i = initial oxidation rate ($mg.L^{-1}.h^{-1}$)

S = total sulphide concentration ($mg.L^{-1}$)

O = oxygen concentration ($mg.L^{-1}$)

k = the rate constant

m = the reaction order with respect to sulphide

n = the reaction order with respect to oxygen

Values for the rate constants m, n, k were experimentally determined to be 0.41, 0.39 and 0.57 respectively. Chemical oxidation of sulphide by oxygen is a relatively slow process at low oxygen concentrations allowing bacteria to compete kinetically with chemical oxidation (Kuenen, 1975; Jorgensen, 1982).

3.5 Thermodynamics of Sulphide Oxidation

An indication of the thermodynamic forces acting on a chemical system can be obtained from Pourbaix diagrams (Stumm and Morgan, 1995). These diagrams represent the equilibrium distribution of the domains of dominance of various chemical species at specific pH and pE (redox) values.

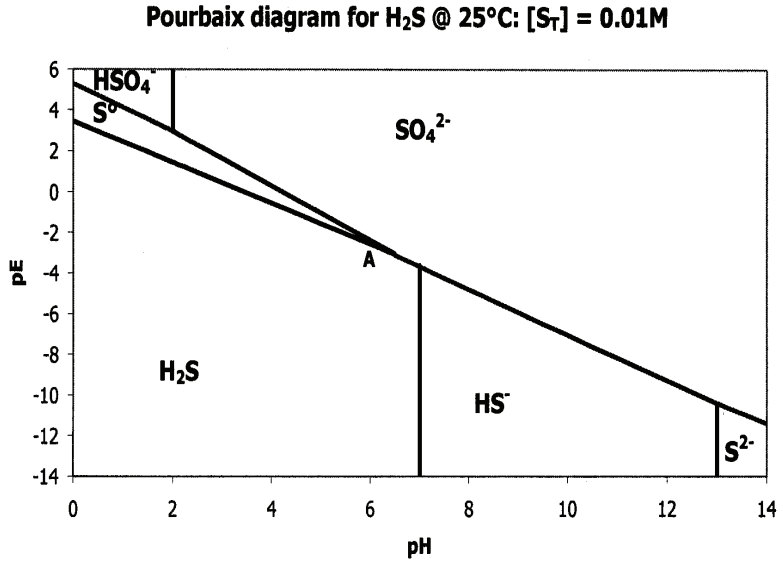


Figure 3.7 Pourbaix diagram for H_2S at 25°C for total S of 1M (Lewis *et al.,* 2000).

Figure 3.7 indicates that compared to the other oxidised forms of sulphur, elemental sulphur is formed in a narrow band of pE and pH conditions. Lewis *et al.* (2000) suggested that for a biological process, equilibrium thermodynamics have less of an influence on the major product of sulphide oxidation than kinetic considerations do. It is also possible that conditions in the bulk phase (those which are measured for chemical reaction process control purposes) are quite different from the intracellular conditions in living systems.

3.6 Characteristics of Biologically Produced Sulphur

In 1887 Winogradsky described the build up and disappearance of sulphur inclusions by *Beggiatoa*, depending on the presence or absence of H_2S in the aqueous medium (Winogradsky, 1887; Truper and Schlegel, 1964). The formation of this "elemental sulphur" has been reported for both phototrophic and colourless sulphur bacteria. The bacterially formed sulphur is in the form of transparent droplets that may be deposited intracellularly or extracellularly. These droplets reach diameters of up to 1μm in diameter and are at least

partially soluble in organic solvents such as acetone, chloroform, ethanol and carbon disulphide.

Biologically produced sulphur is hydrophilic in nature and is white to pale yellow in colour. The hydrophilic nature of this sulphur has been ascribed to the covering of the hydrophobic sulphur particles with an extended polymer layer. Biologically produced sulphur globules eventually convert to crystalline S_8 when allowed to stand. The polymer layer surrounding biologically produced sulphur particles has been described as most likely being composed of protein for sulphur produced by *Thiobacilli* (Janssen *et al.,* 1999). Prange *et al.* (1999) found the sulphur present in intact cells of phototrophic sulphur bacteria to be present in the form of sulphur chains with the structure R-S_n-R. The nature of the –R group was not established but the presence of sulphur rings, polythionates and anionic polysulphides was ruled out suggesting the presence of a long chain organic molecule.

Both SOB and certain SRB are able to utilise elemental sulphur. Studies have shown that *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* are able to interact with both crystalline and plastic sulphur but the effect on the two different forms of sulphur differed (Briand *et al.,* 1999). The interaction with crystalline sulphur resulted in surface smoothing indicating a superficial bacterial-sulphur interaction whereas bacterial interaction with plastic sulphur resulted in perforation of the sulphur bulk. Plastic sulphur (catenasulphur) was prepared by repeated melting and quick cooling of the sulphur liquid. Sloughing of outer membrane vesicles also referred to as “blebbing” has been proposed as a manner in which bacteria may overcome the hydrophobic barrier necessary for these bacteria to grow on elemental sulphur (Knickerbocker *et al.,* 2000

3.7 Treatment for Sulphidic Waste Streams

A considerable body of literature deals with both physico-chemical and biological treatment operations proposed for the removal of sulphide form waste streams. These have been extensively reviewed by Steudel (2000). Processes utilising chemoautotrophic sulphide oxidising bacteria have been considered here.

A number of studies have concentrated on the utilisation of known autotrophic colourless sulphur bacteria to oxidise sulphide to elemental sulphur. Buisman *et al.* (1989) and (1990a)

reported on the use of continuously stirred tank reactors innoculated with ditch mud and run under autotrophic conditions. They concluded that sulphate production could be minimised in favour of sulphur production by controlling the oxygen concentration within the reactor. Subsequent work (Buisman *et al.,* 1991a) described the kinetic parameters of this system. The influence of sulphide loading rate on growth yield and specific oxidation rate were investigated

Two types of bacteria were proposed to be present in these reactors: Sulphate producers (Type A) that were able to grow at sulphide loading rates up to 200 $mg.L^{-1}.h^{-1}$ (6.25 $mmol.L^{-1}.h^{-1}$) and (Type B) that grew at higher loading rates. Later work published by Janssen *et al.* (1995) suggested that it was unlikely that two different metabolic types of bacteria were present. They suggested that depending on oxygen availability, bacterial populations present were able to switch between various electron transport routes and therefore the same population would be able to switch from a predominantly sulphur producing to sulphate producing population very quickly. Upscale work on this system showed that these microbial populations could be immobilised on Pall rings and that a 90% sulphide removal efficiency could be obtained with a hydraulic retention time of 19 minutes in a 4 m^3 biorotor reactor (Buisman *et al.,* 1991b).

The presence of organic substrates such as acetate, higher fatty acids or glucose do not have a significant effect on the sulphide removal capability of a biotechnological process employing colourless sulphur bacteria in a fixed film upflow reactor (Buisman *et al.,* 1990b). The presence of these organic substrates did however encourage the growth of filamentous sulphide oxidising bacteria such as *Thiothrix.* Sulphide loading rates of greater than 105 $mg.L^{-1}.h^{-1}$ (3.28 $mmol.L^{-1}.h^{-1}$) were found to inhibit *Thiothrix* growth.
The growth of *Thiothrix* could represent a problem for two reasons:

1) *Thiothrix* accumulates sulphur intracellularly, making sulphur reclamation more difficult;
2) *Thiothrix* may cause serious sludge bulking problems. Thiothrix growth has also been found to foul groundwater systems (Brigmon *et al.,* 1997).

Various studies have concentrated on developing ways in which to accurately control the biological conversion of sulphide to elemental sulphur. Janssen *et al.* (1995) assessed how the relation between oxygen and sulphide consumption affected the type of product formed in a

sulphide oxidising reactor. At sulphide loading rates up to 75 $mg.L^{-1}.h^{-1}$ (2.33 $mmol.L^{-1}.h^{-1}$) both sulphur and sulphate may be formed at oxygen concentrations below 0.1 $mg.L^{-1}$. Furthermore under highly oxygen limited conditions oxygen/sulphide consumption ratios below 0.7 $mol.h^{-1}/mol.h^{-1}$ thiosulphate is the predominant oxidation product. Formation of easily settleable sulphur sludge from the above system was found to be inhibited by turbulence caused by aeration of the reactor. Janssen *et al.* (1997) described a reactor in which aeration of the medium and the oxidation of sulphide were spatially separated. In addition to this they investigated the biological and physicochemical properties of the formed sludge under both autotrophic and heterotrophic conditions. Under autotrophic conditions a well settleable sulphur sludge developed and a maximum sulphide loading rate of 583 mg $HS^{-}.L^{-1}.h^{-1}$ (17.6 mmol $HS^{-}.L^{-1}.h^{-1}$) was reached. Under heterotrophic conditions (with acetate and propionate present) the system performance deteriorated with increased sulphide accumulating due to the activity of sulphate reducing bacteria and the formation rate of the sulphur sludge declined as more sulphur was found to be washed out of the reactor.

Redox potential has been investigated as a controlling parameter for a biological sulphide oxidising system (Janssen *et al.,* 1998). A linear relationship between measured redox potential and hydrogen sulphide concentration has been shown to exist in natural environments. The optimal redox value for sulphur formation in a continuous flow gaslift reactor was found to be between –147 and –137 mV (H_2 reference electrode 30°C, pH 8).

3.8 Biological Sulphide Oxidation Utilising Heterotrophic Sulphide Oxidising Bacteria

Various studies have been conducted utilising the known heterotroph *Pseudomonas putida* in a biological sulphide oxidation process (Chung *et al.,* 1996a; Chung *et al.,* 1996b; Huang *et al.,* 1997). When immobilised with Ca-alginate beads, these heterotrophic bacteria were shown to be able to remove 97% of a 5 to 60ppm sulphidic gas stream at gas flow rates of between 36 and $72L.hr^{-1}$ in a bubble column reactor. The major products in this process were found to be sulphate, sulphide, sulphite and elemental sulphur and occurred in the following ratios 15%, 12%, 8%, 50% respectively. The researchers ascribed the 15% that was unaccounted for to assimilation as inorganic sulphur compounds.

Basu *et al.* (1995) reported on a novel process for the removal of sulphate and organic matter from wastewater. In this five-stage process sulphate reducing bacteria were utilised to reduce

sulphate and organic matter, and sulphide was removed by microaerophilic *Beggiatoa* species.

3.9 Interactions between Sulphide Oxidising Bacteria and Sulphate Reducing Bacteria

In natural environments sulphate reduction and sulphide oxidation processes occur within close proximity to one another. Interactions occur between the various types of sulphur utilising bacteria and investigations into these interactions have been reviewed by Overmann (2000). A complete sulphur cycle (oxidation/reduction) may exist within a vertical section of only 2000 μm (Okabe *et al.,* 1998, Yu and Bishop, 1998). Biofilms in contact with an oxygenated aqueous bulk phase (1-2 $mg.L^{-1}$ O_2) was found to be completely oxygen depleted within 300-500μm of the biofilm/water interface. Furthermore in a mixed population biofilm grown under aerobic conditions in a synthetic waste water having a chemical oxygen demand (COD) of 160 $mg.L^{-1}$ the redox potential decreased sharply (277 mV decrease over 50μm) over a very narrow spatial band. This was ascribed to stratification of microbial processes within the biofilm (Yu and Bishop, 1998).

The interactions between a sulphate reducing bacterium (*Desulfovibrio desulfuricans*) and a colourless sulphide oxidising bacterium (*Thiobacillus thioparus*) were investigated by van den Ende *et al.* (1997). During these chemostat experiments mixed cultures of these bacteria were grown in media supplemented with lactate as carbon and energy source and sulphate as electron acceptor under oxygen limiting conditions. Under increasing air flow (O_2 still limiting) total biomass increased with a simultaneous decrease in sulphide concentrations. When oxygen supplied to the reactor surpassed the amount required for complete oxidation of the sulphide present, both organisms washed out of the reactor; *Desulfovibrio* because of oxygen toxicity and *Thiobacillus* due to the lack of available sulphide. Cell count and cell sizing revealed that the numbers of *Thiobacilli* increased with increasing oxygen supply, but the increased biomass was largely due to increased numbers of sulphate reducing bacteria. This was attributed to the increased abundance of reduced sulphur intermediates produced by the *Thiobacilli* under the oxygen-limited conditions, which could be utilised by *Desulfovibrio.*

3.10 Sulphide Oxidation in Organic Rich Aqueous Environments

The majority of processes utilising colourless sulphide oxidising bacteria have been developed to treat relatively pure sulphide solutions that are virtually devoid of contaminating organics with reactors being run under autotrophic conditions (Buisman *et al.,* 1989).

From literature on these processes the following challenges may be expected in developing a biological sulphide oxidising process in which elemental sulphur is the major product:

1) Elemental sulphur is the major product of sulphide oxidation under very specific redox and pH conditions. Biotechnological processes need to be controlled rigorously to prevent complete oxidation of sulphide to sulphate (De Smul and Verstraete, 1999; Janssen *et al.,* 1998; Lewis *et al.,* 2000);

2) The presence of organics in a sulphidic environment encourages the growth of filamentous sulphur bacteria. These bacteria, and especially *Thiothrix,* accumulate sulphur intracellularly and oxidise it further to sulphate when redox conditions allow for this to occur (Buisman *et al.,* 1990b);

3) The presence of organics and partially oxidised and fully oxidised sulphur compounds (thiosulphate, sulphur and sulphate) and anaerobic conditions will encourage the growth of SRB. The presence of active bacterial sulphate reduction in a sulphide oxidising bioreactor is a disadvantage since the overall sulphur removal capacity will be decreased (Janssen *et al.,* 1997). Sulphate reduction has been shown to take place in aerobic biofilms (Okabe *et al.,* 1998, Yu and Bishop, 1998);

4) Biological sulphur is produced as amorphous sulphur covered in a layer of organic molecules. This organic layer renders the sulphur hydrophilic and tends to form stable colloidal sols (Janssen *et al.,* 1999). This makes recovery of the sulphur by settling difficult.

A need therefore exists to develop and evaluate a biotechnological approach to oxidation of sulphide to elemental sulphur in an organics rich environment. Research undertaken by the

Environmental Biotechnology Research Group at Rhodes University has focused on the development of a biological integrated treatment system. In addition to the evaluation and application of a number of carbon sources such as tannery effluent and algal biomass (Boshoff *et al.,* 1996 and Rose *et al.,* 1998) and sewage sludge (Whittington-Jones, 2000) for sulphate reduction, this research has included fundamental work on microorganisms responsible for the chemical reactions underlying these systems.

3.11 The Floating Sulphur Biofilm Reactor

Based on the observations of floating sulphur biofilms on sulphide-producing tannery ponds, Gilfillan (2000), Bowker (2002) and Molwantwa (Molwantwa et al., 2003 and Molwantwa, 2007) undertook the investigation and development of the floating sulphur biofilm reactor for the removal of sulphide by oxidation to elemental sulphur. The baffle reactor was developed as an experimental system in which the biology and process dynamics of the system could be studied under laboratory conditions (Figure 3.8 and 3.9). Anaerobic compartments underlying the surface of the reactor were shown to be responsible for the poising of the redox potential within the layer exposed to air and thus providing the conditions necessary for elemental sulphur production (Gilfillan, 2000).

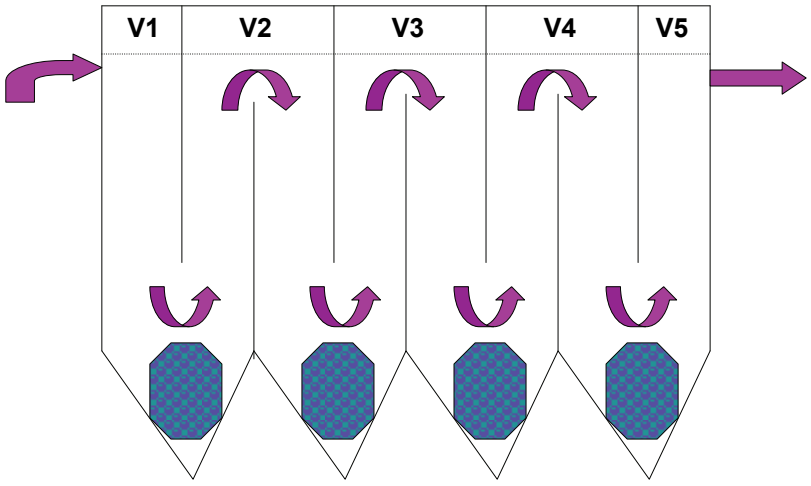


Figure 3.8. Baffle reactor developed for the production and study of sulphur biofilms under laboratory conditions.

Attempts were made to scale up the baffler reactor design (Figure 3.9) but harvesting proved to be difficult and led to the loss of the biofilm which took several days to reform. Based on

these findings, Molwantwa (2007) then undertook the development of the floating biofilm reactor in which the sludge bed was eliminated and the flow rate of the sulphide stream to be treated could be uncoupled from the biofilm harvesting process (Figure 3.10).

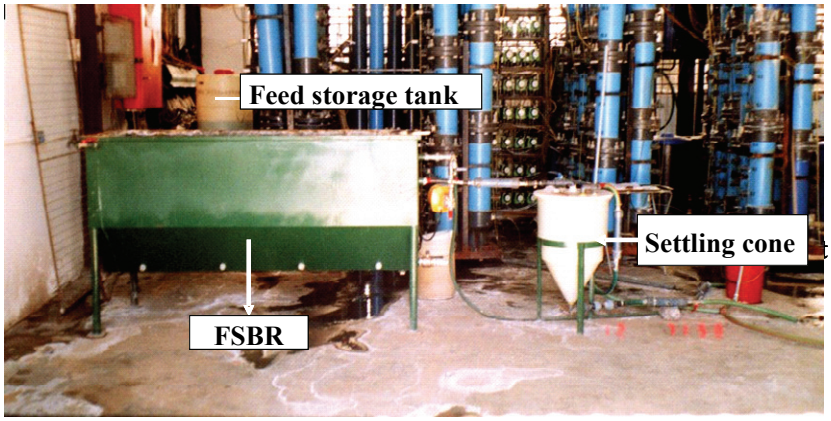


Figure 3.9. Scale up of the baffle reactor used for the production of sulphur biofilms from complex organic sulphidic waste streams. This was also known as the floating sludge bed reactor.

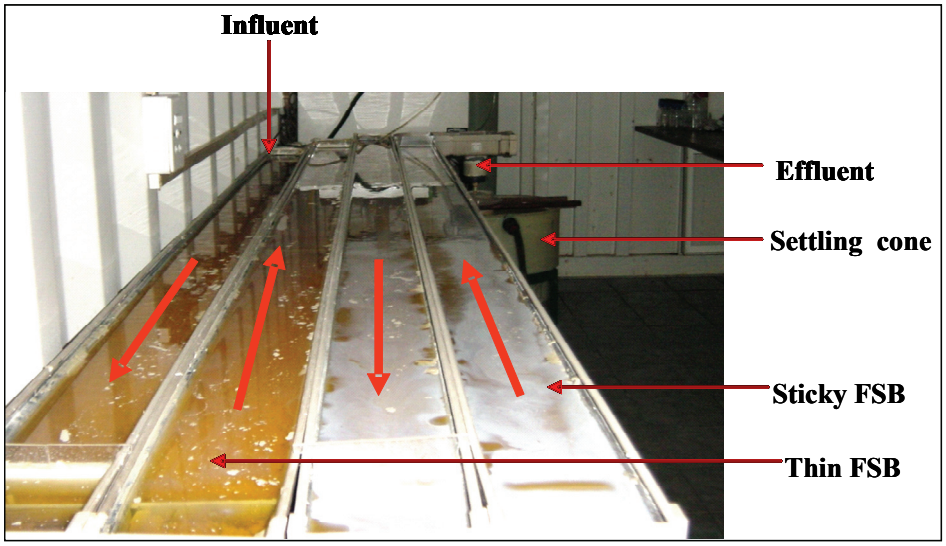


Figure 3.10 Four-channel linear flow channel reactor set up in a constant environment room operating at 25°C.

These studies demonstrated that the floating film was a true biofilm, composed of a number of different microbial types, and differentiated both spatially and by physiological performance within the various functional compartments of the film. Heterotrophs at the bottom of the floating film were shown to be responsible for the Redox poising of the system and, by controlling oxygen ingress to the system, enabled sulphide oxidising forms to predominate with the formation of elemental crystalline sulphur (Figure 3.12).

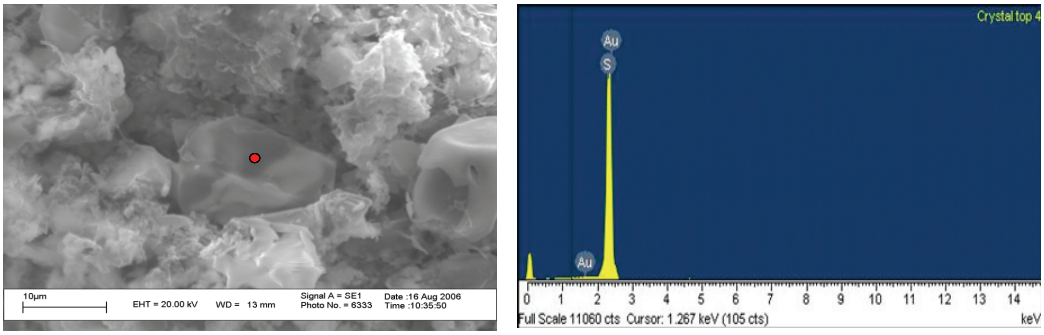


Figure 3.11 Scanning electron microscope micrograph and energy dispersive X-Ray spectrum of a crystal structure within the floating sulphur biofilm (area analysed indicated in red). (S=100%).

Molwantwa (2007) proposed a descriptive model to account for these processes (Figure 3.13) in which biologically produced sulphur catalyses the formation of polysulpide chains and thereafter the formation of crystalline sulphur.

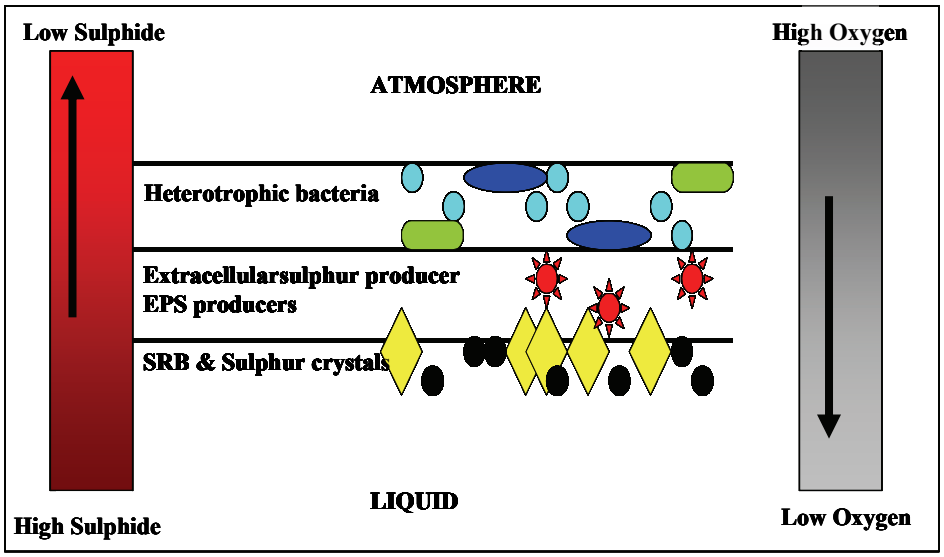


Figure 3.12 Summary illustration of the descriptive model integrating the various processes proposed to occur in the Floating Sulphur Biofilm. These occur against falling dissolved oxygen and Redox potential gradients and sulphide migrating upwards into the Floating Sulphur Biofilm (indicated by the arrows). Aerobic heterotrophic bacteria (blue dots and green rectangles) establish at the air/liquid interface and, in consuming oxygen diffusion into the strongly anaerobic system, establish steep DO and Redox gradients at the surface. Below this layer, anaerobic exopolysaccharide producers generate a copious slime layer which constitutes the matrix of the Floating Sulphur Biofilm (red stars). Within the correctly poised Redox window, both biological (black dots) and inorganic sulphur formation occurs and gives rise to large sulphur granules which characterise the Floating Sulphur Biofilm.

The potential of the floating sulphur biofilm for use in a bioprocess unit operation for sulphide removal in lignocellulose-based low-flow passive systems for acid mine drainage wastewater treatment was investigated. The linear flow channel reactor was scaled up and it was shown that the optimum sulphide removal of 74% and sulphur recovery of 60% could be achieved at 20°C. In a further scale up of the linear channel reactor, the floating sulphur

biofilm reactor was developed and operated. Sulphide removal and sulphur recovery of 65 and 56% respectively was measured in the process.

Process scale-up studies are underway to evaluate the application of the Floating Sulphur Biofilm Reactor in sulphide removal in AMD passive treatment operations. Problems encountered include the stability of the process under extended operation and its susceptibility to environmental perturbation in outdoor operating conditions. Given the complexity of the system it has also been difficult to establish a rigorous basis for engineering modelling of the system.

During the above studies, the production of elemental sulphur by sulphide oxidising biofilms was observed in silicone tubing through which sulphidic solutions were pumped. These observations suggested the possible application of tubular systems for process development.

4. THE TUBULAR FIXED FILM REACTOR

4.1 Introduction

As has been shown, biological sulphide oxidation occurs predominantly at liquid-air interfaces where the redox poising of this zone enables a microbially catalysed oxidation of sulphide to elemental sulphur (Jorgensen, 1982). The above observations had indicated that the inner surface of silicone tubing provided such a liquid-air interface suitable for sulphur biofilm formation.

Silicone is permeable to a number of chemical compounds including oxygen. The oxygen permeability of silicone has been reported to be 610 barrers (Koros *et al.,* 1987) (1 barrer = 10^{-10} cm^3 (STP) $cm.cm^{-2}.s^{-1}.cm^{-1}$ Hg). In addition to this, silicone membranes have been shown to be permeable to sulphide where they have been employed in chemical reactors and sulphide is oxidised by an acidic ferric solution to produce orthorhombic sulphur (De Smul and Verstraete, 1999). Silicone has also been used as a means of separating sulphate reducing bacteria from toxic metals solutions in the treatment of metal containing wastewater (Chuichucher *et al*., 2001)

The observations that sulphur biofilms developed on silicone tubing in which sulphidic solutions were pumped, and evidence that silicone type materials had been utilised in sulphide oxidation and biotechnological applications led to the conceptualisation of the silicone Tubular Fixed Film Reactor.

4.2 Materials and Methods

4.2.1. Reactor Configuration

The reactors in this investigation consisted of lengths of silicone tubing (13,2 m in length, 5 mm (ID) x 8 mm (OD)). This gave a total reactor volume of 272 mL, a HRT (hydraulic retention time) of 47 min at a flow rate of 5.8 $mL.min^{-1}$. The surface area of the reactor was calculated to be $2902cm^2$. A sulphide/sewage mix was fed to the reactor which was attached to a 2.5 m plastic mesh column for support. The reactor was fed from the top downward. A

photograph of the laboratory configuration of the silicone tube reactor is shown in Figure 4.1 and 4.2.

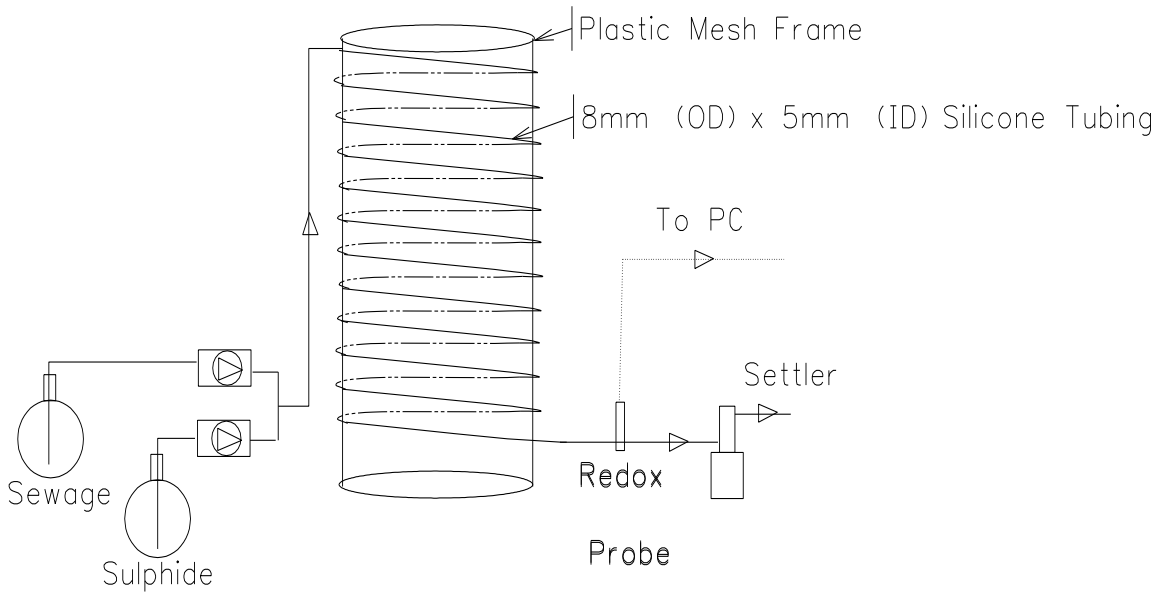


Figure 4.1 Diagrammatic representation of Silicone Tubular Reactor set-up

Figure 4.2 Photograph of the Silicone Tubular Reactor laboratory set-up. Silicone tubing was supported on a plastic mesh column

The reactor was attached to the mesh frame so that a continual downward angle was maintained along the length of the reactor. This aimed to prevent settling along the length of the reactor and possibly encourage movement of produced sulphur down and out of the reactor and into the settling unit.

4.2.2 Reactor Operation

4.2.2.1 Reactor Start-Up

During this investigation a sulphide/sewage mixture was pumped through a clean piece of silicone tubing. The aim was to determine how long it would take for a sulphide oxidising population to develop and how the development of this population affected the aqueous chemistry of the fluid passing through the reactor. During this investigation the flow rate of the sulphide feed was adjusted during the initial 16 hour period of feed.

4.2.2.2 Biofilm Harvesting

Biofilm that developed in the reactor was collected by removing the tubing from the mesh frame, sealing both ends and rolling over the tubing with a large roller. The collected biofilm was freeze-dried, a known mass was resuspended in acetone and the sulphur content determined by HPLC.

4.2.2.3 Reactor start-up after biofilm removal

The aim of this investigation was to compare how quickly a silicone reactor would begin to oxidise sulphide after the biofilm had been removed as described above. This investigation started directly after biofilm removal. The flow rate was maintained at 5.8 $mL^{-1}.min^{-1}$ throughout this investigation. In addition to this it was suspected that deterioration in the sulphide oxidising capacity of the reactor would occur as the biofilm thickness increased. A decrease in the efficiency of oxygen delivery to the biomass would result in less efficient oxidation of sulphide.

4.2.2.4 Particulate collection

These investigations aimed to determine how much of this sloughed material could be collected under normal flow rate conditions, what percentage of the sloughed material was indeed elemental sulphur and if the sloughing process could be enhanced by periodically increasing the flow rate. The reactor was run until a well established film was present in the reactor. Particulate matter was collected in a flow-through cell over a period of 6 days. Particulate matter collected over a twenty four hour period was filtered through a dry Whitman GFC filter of known mass, dried at 60°C overnight and the mass calculated by

difference. Elemental sulphur presence was determined by cutting up the filter, and placing it in a suitable volume of acetone overnight. The concentration of elemental sulphur was determined by HPLC as described previously. The reactor was run for 6 days at the normal flow rate of 5.8 $mL.min^{-1}$ in order to determine the baseline amount of particulate matter in the effluent. After this six day period the reactor was run for the following 6 days under the following conditions: a programmable pump was used to increase the flow rate to maximum (125 $mL.min^{-1}$) for 1 min every 3 HRT. This also meant that half of the hydraulic volume of the reactor would be replace with fresh feed every three HRT. The redox was measured and plotted during these investigations to determine how the reactor reacted to the upset conditions.

4.2.3 Electron Microscopy

The bacterial population present in the reactor was investigated by scanning electron microscopy. Sections from points at various lengths of the reactor were investigated. Six sections were removed and prepared from the reactor at 2-2.2 m intervals. The aim of these investigations was to determine whether any bacterial morphological differences were present in the microbial population along the length of the reactor. The reactor was run for 14 days until a thick biofilm was present. The reactor was then sacrificed and small sections of silicone were removed at approximately 2 m intervals along the reactor. These pieces were prepared for SEM as previously described.

4.2.4 Analytical Methods

4.2.4.1 Sulphide

1 mL of sample was added to 1 mL of zinc acetate. This was further diluted to give a final dilution of 1000X. Total sulphide in solution was then determined according to methylene blue method Truper and Schlegel (1964)

4.2.4.2 Sulphate

Sulphate concentrations were determined by ion chromatography (IC) using a 15 mm x 4.1 mm Hamilton PRP-X100 column, 4 mM p-hydroxybenzoic acid, 2.5% methanol, pH 8.5 as the mobile phase, Waters 510 pump flow rate 1 $mL.min^{-1}$ and detection by Waters 430

conductivity detector. Prior to ion exchange chromatography, samples were filtered through 0.45µm nylon filters and passed through a 25 mg C_{18} Isolute® solid phase extraction column to remove contaminating organics.

4.2.4.3 Sulphur

Elemental sulphur concentrations were determined using the modified procedure of Mockel (1984). Elemental sulphur was quantified using reversed phase High Performance Liquid Chromatography (HPLC) using a Phenomenex® Luna 150 mm x 4.6 mm C18 column, 95:5 Methanol: H_2O mobile phase at a flow rate of 2 $mL.min^{-1}$. 1 mL of sample was centrifuged at 13 200 rpm for 10 minutes and the resulting pellet was resuspended in 1 mL of HPLC grade acetone, either filtered through a nylon 0.45 µm filter or recentrifuged before being run on the HPLC system.

4.2.4.4 Redox

The oxidation/reduction potential of the solution was determined using an Endress + Hauser ® ORP probe connected to a custom built data collection system. The data collection system sent data to a PC where it could be logged. This system was custom built by the Physics and Electronics Department at Rhodes University.

4.2.4.5 pH

pH was determined using a Cyberscan 2000 pH meter.

4.2.4.6 Transmission Electron Microscopy

Samples for Transmission Electron Microscopy (TEM) were prepared as described by Cross (1986). 2 mL of reactor effluent was spun down in Eppendorff tubes at 13000rpm for 10 minutes. The pellets were pooled and spun down again at 13000rpm for 10 minutes. The resulting pellet was prepared in the Eppendorff tube for TEM according to the procedures described below.

Following primary fixation in glutaradehyde, the samples were washed in 0.1 M phosphate buffer followed by post fixation for 90 minutes in 1% phosphate buffered osmium tetroxide. Following two further buffer washes the samples were dehydrated through a series of

ascending concentrations of ethanol (30-100%). This was followed by two washes in propylene oxide and transition to a resin medium through three propylene oxide:epoxy resin mixtures (75:25, 50:50, 25:75) and finally to pure epoxy resin. Samples were then transferred to pure epoxy resin and polymerisation was allowed to take place over 36 hours at 60 °C. Ultra thin sections of the resin embedded cells were cut using a LKB 111 ultramicrotome and collected on alcohol washed grids. The sections were then stained with 5% aqueous uranyl acetate (30 minutes), followed by Reynold's lead citrate (5 minutes). For TEM, ultrathin sections were examined using a JEOL JEM 100 CXII transmission electron microscope.

4.2.4.7 Scanning Electron Microscopy (SEM)

Immobilisation media (PVC or Silicone) with attached biofilm were removed from the respective reactors. Immobilisation medium and attached biofilm were carefully cut into small squares approximately 3 mm x 3 mm with a sharp blade. These were prepared according to the method of Cross (1979). These biofilm containing pieces were then placed in cold buffered fixative (2.5% glutaraldehyde in 0.1 M phosphate buffer) overnight. The fixative was decanted off washed twice for fifteen minutes with cold 0.1 M phosphate buffer. The samples were then subjected to a step-wise increasing ethanol gradient (30% ethanol – 100 ethanol) at 4°C for 10 minutes at each ethanol concentration. The 100% ethanol step was repeated twice. The 100% ethanol was decanted off and the samples were placed in 75:25 ethanol:amyl acetate solution. The samples were eventually suspended in 100% amyl acetate via 50:50 and 25:75 ethanol: amyl acetate steps. The samples were placed in specially designed critical point drying baskets and were transferred, submerged in 100% amyl acetate, to the critical point drying apparatus. Samples then underwent critical point drying, were mounted on stubs and coated with gold. Samples that were not going to be observed immediately were stored in a desicator. Samples were observed in JEOL JEBM V120 scanning electron microscope.

4.3 Results

4.3.1 Reactor Start –Up

Figure 4.3 shows the concentrations of influent sulphide, effluent sulphide and effluent elemental sulphur during the start-up of a clean silicone tube reactor. The measured pH of the influent and effluent are shown in Figure 4.4. During the first 28 hours of this experiment the

reactor was fed a sewage/sulphide mixed solution of sulphide concentration varying between 2.5 and 4 mM (81-133 mg.L^{-1}), pH 8.5 and a flow rate of 2 mL.min^{-1}. During this stage the effluent contained 0.7-1.7 mM (23-54 mg. L^{-1}) HS^- at a pH of above 8.5. No elemental sulphur was detected in the effluent during the first 28 hours of operation. Between 28 and 44 hours the flow rate was increased to 3.8 mL.min^{-1}. At this point the concentration of sulphide in the effluent decreased dramatically, the effluent sulphide concentration at 36 hours was 0.8 mM (6 mg. L^{-1}). During this time the effluent sulphur concentration increased to a maximum of 1 mM (32 mg. L^{-1}). This corresponded to a decrease in the effluent pH to a minimum of 7.5. Sulphate concentrations were not determined during this start-up investigation.

Between 48 and 60 hours the reactor was run at 4.4 mL.min^{-1}. During this time the sulphide concentration in the effluent remained low, the effluent sulphur concentration remained constant between 0.6 and 0.8 mM (19-25.6 mg.L^{-1}) and the pH increased to 8. From 68 hours onwards the reactor was run at 5.8 mL.min^{-1}. Between 72 and 90 hours the elemental sulphur concentration in the effluent remained constant around 0.7 mM (20 mg.L^{-1}) and the pH around 8.5, the effluent sulphide concentration remaining low until the sampling at 110 hours.

From 90 hours onwards sulphide was again present in the effluent and the sulphur concentration in the effluent decreased to below 0.5 mM (16 mg. L^{-1}). The increase in the effluent sulphide concentration coincided with an increase of the effluent pH to 9. The maximum sulphide oxidising rate during this start-up investigation was calculated to be 1.07 x 10^{-3} M.h^{-1}.

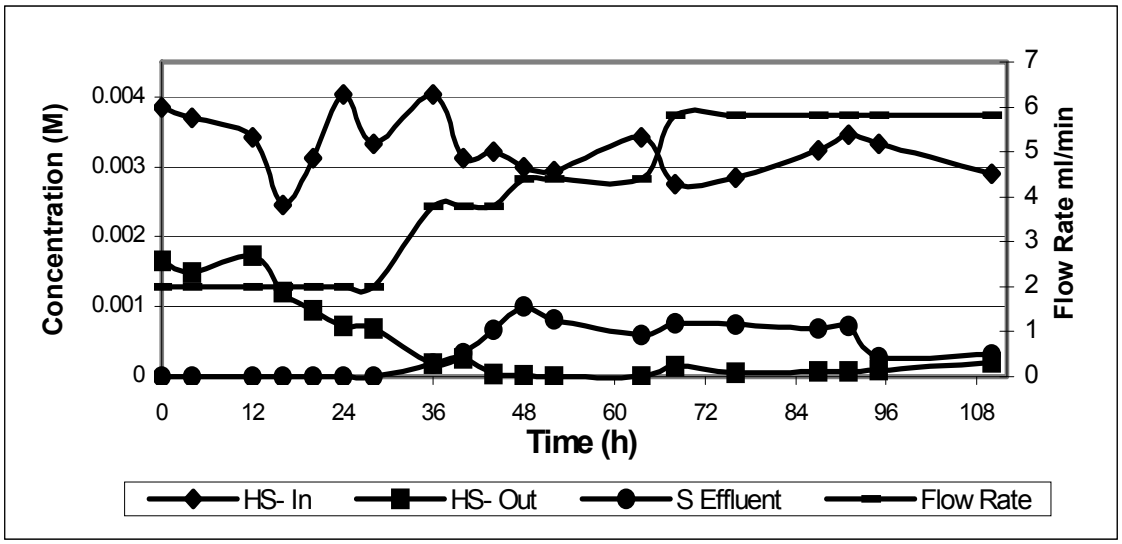


Figure 4.3 Sulphide influent and effluent concentrations, sulphur effluent concentration and liquid flow rate for a silicone Tubular Fixed Film Reactor on start-up using a fresh length of tubing.

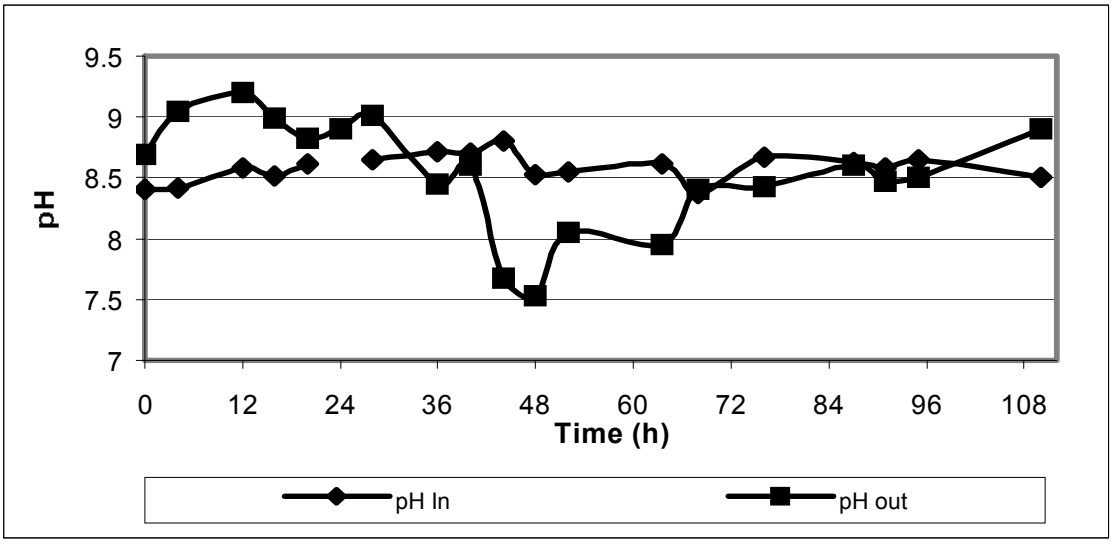


Figure 4.4 Influent and effluent pH measurements during reactor start-up with a fresh length of silicone tubing

A thick white biofilm was observed to have developed after 10 days of continuous reactor operation (Figure 4.5). After 10 days of operation, the biofilm was harvested. 5.54g of total mass was harvested from the reactor of which 1.16g (21%) was elemental sulphur. The freeze dried biofilm had a powdery off-white appearance (Figure 4.6).

igure 4.5 Photograph of silicone Tubular Fixed Film Reactor showing white biofilm development within the tubing (top tube) compared to a fresh length of silicone tubing attached below each turn for illustrative purposes.

Figure 4.6 Photograph of dried sulphur biofilm harvested from Silicone Tubular Reactor.

4.3.2 Reactor Operation after Biofilm Harvesting

Influent sulphide, effluent sulphide, elemental sulphur and the produced sulphate (Produced sulphate = $[SO_4^{2-}]_{effluent} - [SO_4^{2-}]_{influent}$) were determined during the start-up of the reactor immediately after harvesting of a previous biofilm as shown in Figure 4.7. The influent and effluent pH for the same period is shown in Figure 4.8 and recovery of sulphur species in Figure 4.9.

Re-starting the reactor after harvesting the previous biofilm at a flow rate of 5.8 mL.min^{-1} at a sulphide concentration of 3 mM (100 mg.L^{-1}) resulted in immediate removal of all sulphide from the effluent. The first determination of sulphur species in the effluent was carried out after 1 HRT and the effluent sulphide concentration was below 1.8 mM HS^- (10 mg.L^{-1}). At t = 0 (after 1 HRT) very little sulphate was detectable in the effluent and only a small amount of elemental sulphur detectable, although most of the sulphide had been removed. The sulphate concentration in the effluent then rapidly increased corresponding with a sharp drop in the effluent pH at 8 hours; a drop in the effluent sulphate and elemental sulphur concentrations and a small increase in the effluent sulphide concentration followed this.

Between 24 hours and 72 hours of operation a steady state seemed to be established. During this period sulphate produced (effluent-influent sulphate concentration) ranged between 1.3 and 1.7 mM (125 and 163 mg.L^{-1}), effluent elemental sulphur ranged between 0.5 and 0.8 mM (16 and 26 mg.L^{-1}), and effluent pH was lower than the influent pH at about pH 8. During this stage, above 60% of the predicted percentage sulphur species recovery could be accounted for in terms of sulphate, elemental sulphur and sulphide. After 72 hours the pH of the effluent began to rise to above 8.5, sulphate in the effluent decreased dramatically, effluent sulphur began to decrease and more sulphide began to appear in the effluent. At 96 hours the sulphate concentration in the effluent again increased, but with no major decrease in the effluent pH.

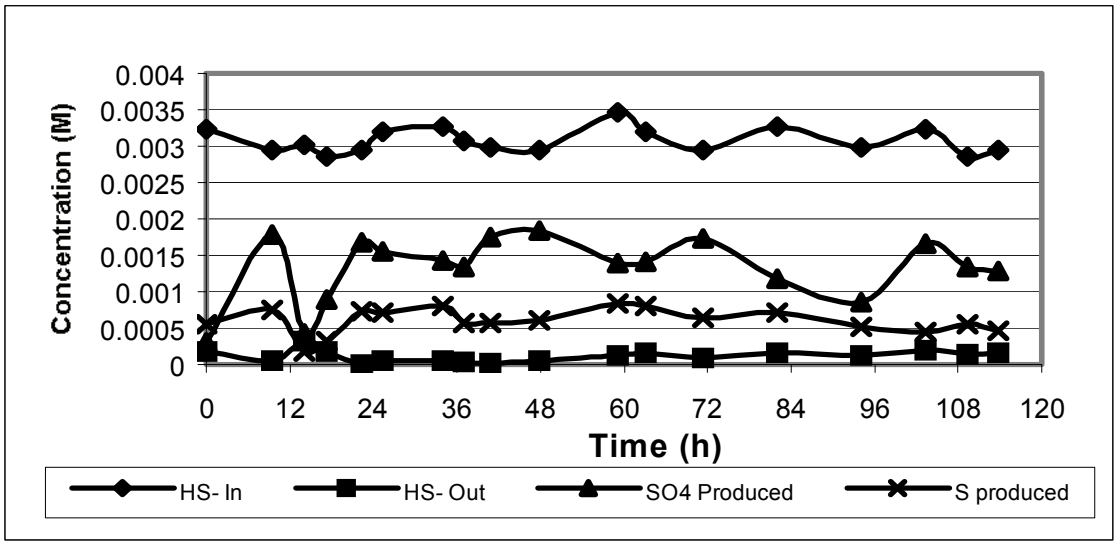


Figure 4.7 Influent and effluent sulphide concentrations, produced sulphate and effluent sulphur concentrations for the silicone Tubular Fixed Film Reactor started up directly after harvesting of a previous biofilm.

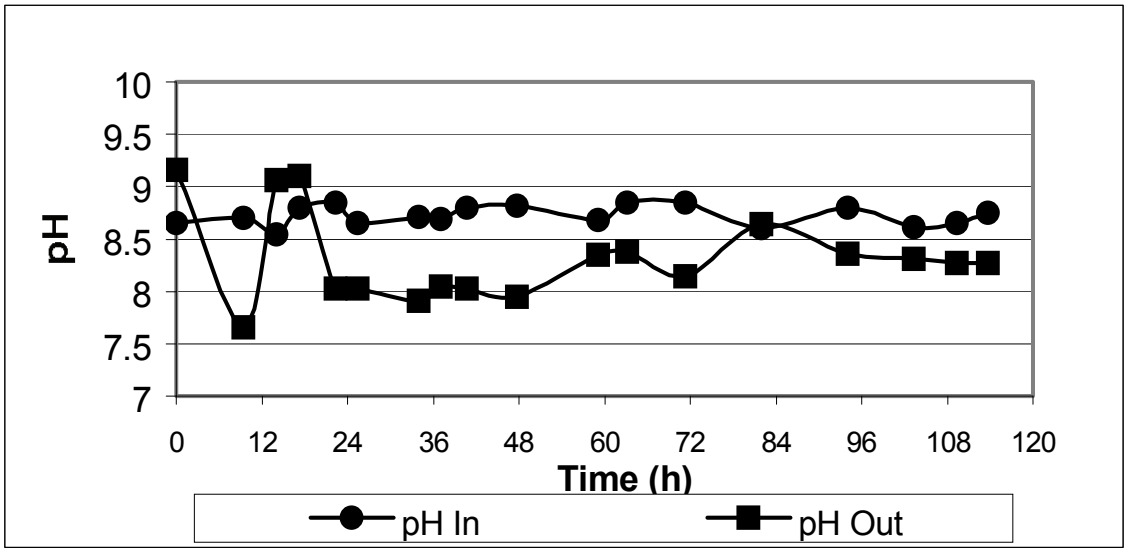


Figure 4.8 Influent and effluent pH measurements during operation of the silicone Tubular Fixed Film Reactor started up directly after removal of a previous biofilm.

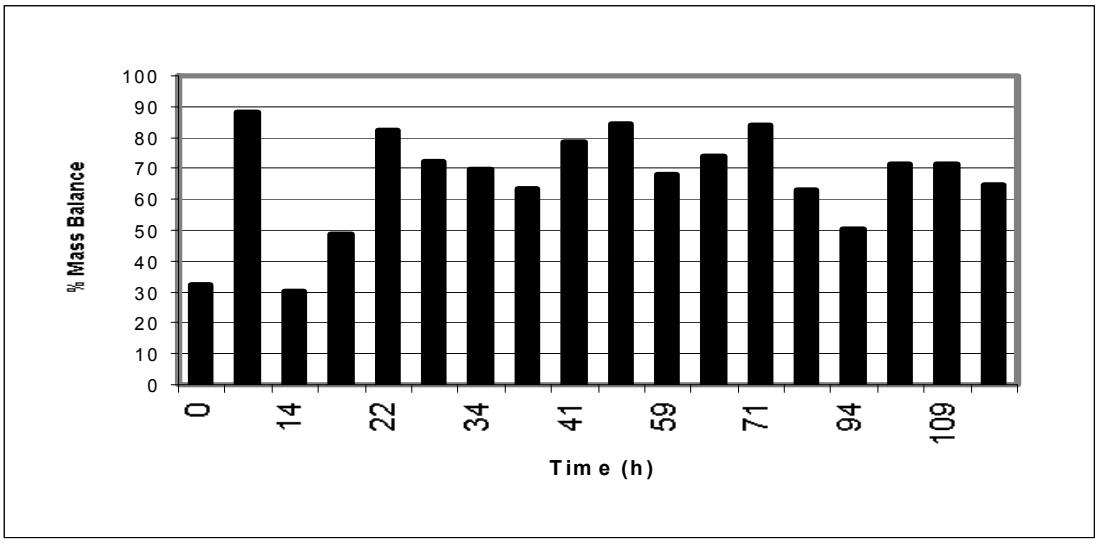


Figure 4.9 Percentage sulphur species recovery obtained during operation of a silicone Tubular Fixed Film Reactor started up directly after harvesting of a previous biofilm. Percentage S species recovery = $[HS^-]_{if}/[SO_4^{2-}]_{elf}$ $[S]_{Fe} + [HS^-]_{Fe}) \times 100$

4.3.3 Particulate collection

The mass of particulate matter collected over two six day periods as well as the portion present as elemental sulphur is shown in Figures 5.10 and 5.11 respectively. The amount of particulate matter collected from the reactor at a flow rate of 5.6 mL.min^{-1} during the first six days of the experiment ranged between 9 and 46 mg (average 26 +/- 14.7 mg) and the elemental sulphur present ranged between 1 and 6.5 mg (average 4.3 +/-1.9 mg) (Figure 6.9). The total sulphide load per day (assuming a constant sulphide concentration of 3 mM HS^-, 100 mg.L^{-1}) at 5.6 mL.min^{-1} was 806 mg.day^{-1}.

The amount of sulphur collected at 5.6 mL.min^{-1} in the particulates from the reactor represented a very small portion of the total percentage sulphur species recovery. Changing to a purge operation where the flow rate was increased to 125 mL.min^{-1} for 1 minute every 3 hydraulic retention times resulted in a large amount of material being collected during the first 24 hours after changing to this operating regime. In the first 24 hours 3912 mg of particulate material was collected of which 1137 mg was determined to be elemental sulphur. On the following four days an average of 53+/-24.8 mg of which 10.6+/-7.25 mg was determined to be elemental sulphur. On the last day 550 mg of particulate matter was collected of which 110 mg of elemental sulphur was determined to be elemental sulphur.

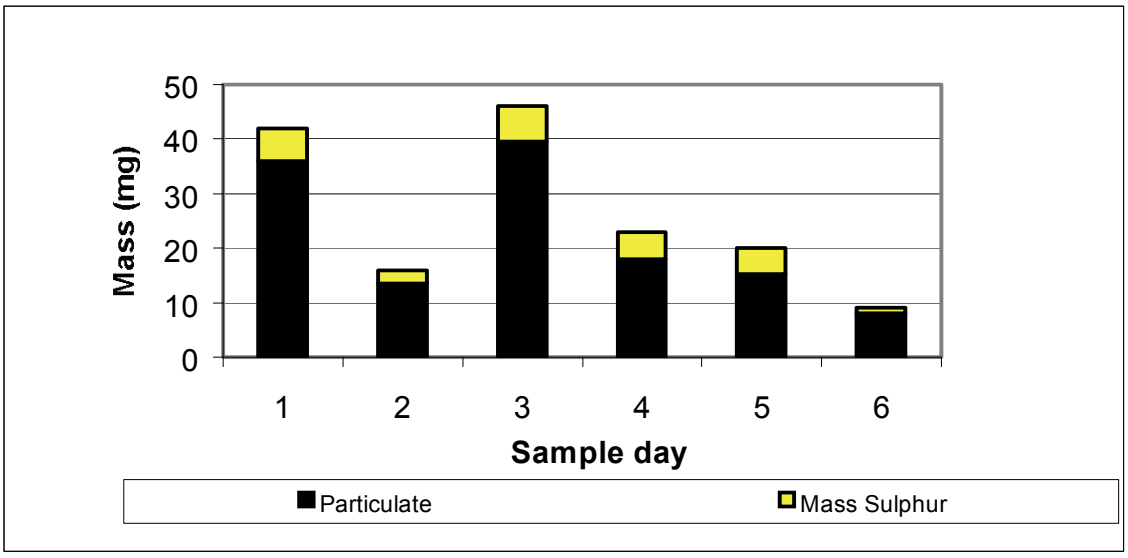


Figure 4.10 Daily mass of particulates collected from effluent and the proportion of the mass that was made up by sulphur with the reactor being run at 5.6 mL.min^{-1}.

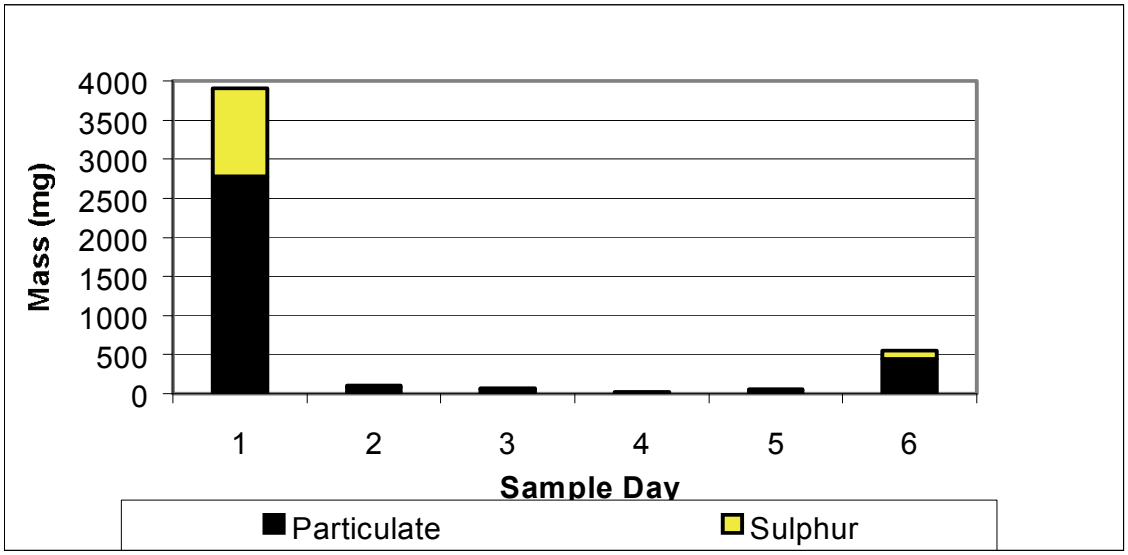


Figure 4.11 Daily mass of particulates collected from effluent and the proportion of the mass that was made up by sulphur with the reactor being run with purging ever 3 hydraulic retention times

4.3.4 Redox Changes during Purge Experiments

The redox of the effluent was logged using an in-line redox probe during the particulate collection experiments. Results of the data collected when a 50% reactor hydraulic volume purge was employed every 3 hydraulic retention times, are shown in Figures 4.12 and 4.13. Figure 4.12 shows that throughout a 13 hour period the measured redox dropped whenever the reactor was purged but returned to its previous level quite quickly. Closer examination of the redox profile after 1 purge event shows that the redox dropped from –382 to –415 mV quickly as the flow rate and sulphide loading were increased (at 5.35 hours) but returned to the previous level of -382 mV after 0.592 h (35.52 minutes) (see Figure 4.13). The increased sulphide load did not affect the oxidising capacity of the biomass once the load had washed out of the reactor.

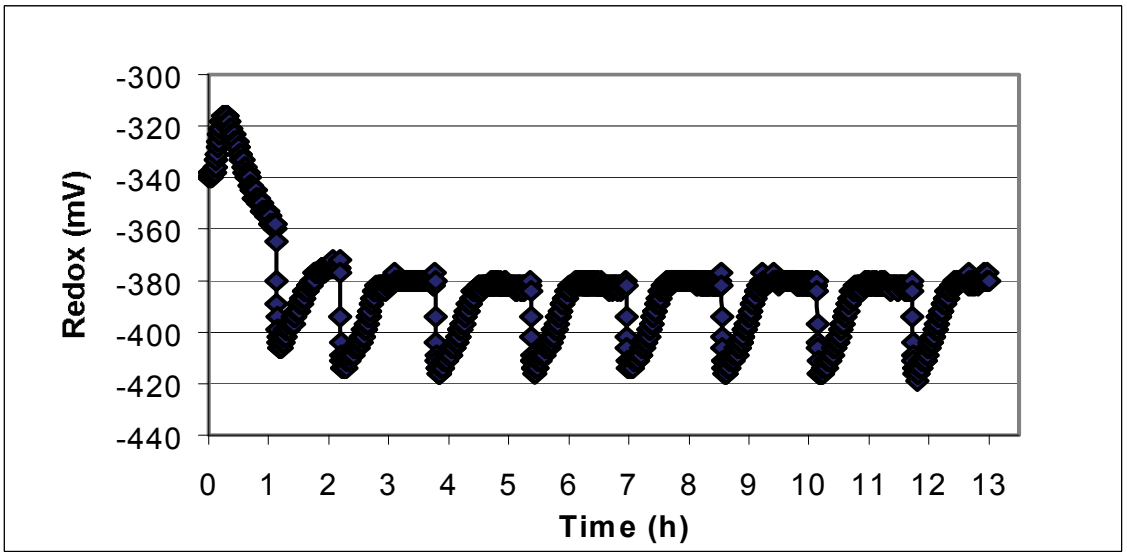


Figure 4.12 Measured redox potential over a 13 hour period with the silicone Tubular Fixed Film Reactor being run with purging every 3 hydraulic retention times.

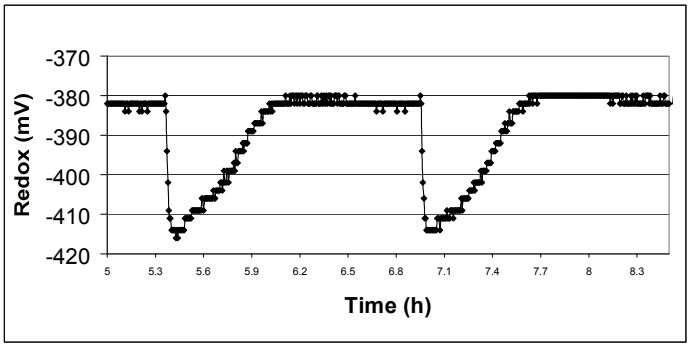


Figure 4.13 Measured redox potential between 5 and 8.3 hours of the same experiment shown in Figure 4.12, highlighting the time taken for the reactor to return to previous condition after purging.

4.3.5 Microscopy

Scanning electron micrographs of the attached bacterial population present in the silicone Tubular Fixed Film Reactor are shown in Figures 4.14 to 4.20. The following was noted:

1) A diverse bacterial biofilm had developed on the tube wall and the presence of coccoid, bacilliary and filamentous organisms were noted;

2) The biofilm contained large amounts of a matrix assumed to be exopolysaccharide (Figure 4.14);

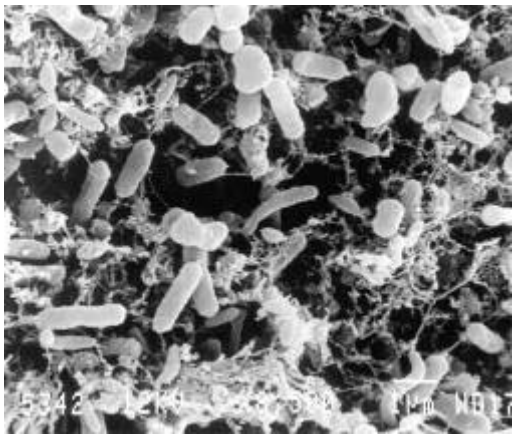
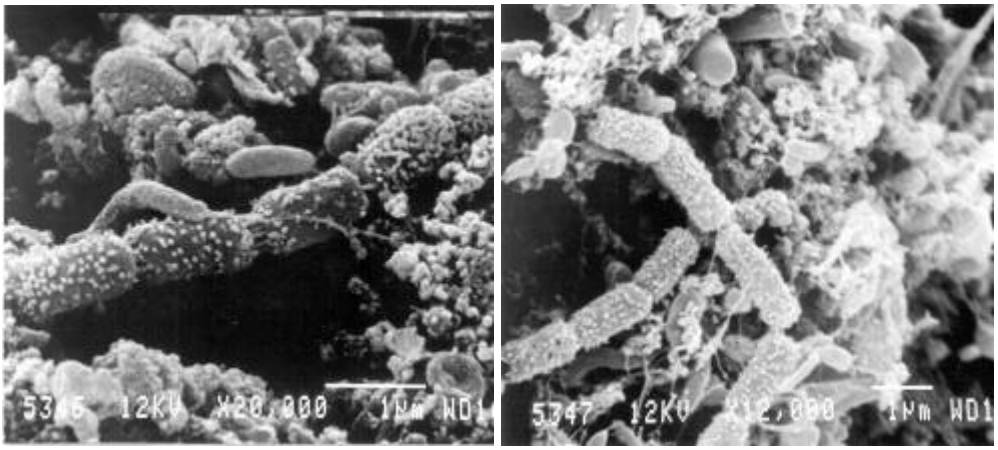


Figure 4.14 Scanning electron micrograph of attached bacterial population from the uppermost section of the reactor. Evidence of polymeric exopolysaccharide production and a diverse bacterial population are shown.

3) Microbiologically produced elemental sulphur was observed only in sections from the first four meters of the reactor (Figure 4.15 and Figure 4.16). The population present in this area was varied with a variety of bacteria exhibiting extracellular sulphur globules;



Figures 4.15 and 4.16 Scanning electron micrographs of the attached bacterial population from the second portion of the reactor that was sampled. Evidence of bacterial sulphur production is shown with both filamentous and coccoid bacteria producing elemental sulphur

4) Areas of apparently single microbial morphology were noted (Figure 4.17);

Figure 4.17 Scanning electron micrograph of attached bacterial population from the third section of the reactor that was sampled. A relatively dense, but morphologically uniform bacterial population seems to have developed

5) Large crystals of elemental sulphur were observed as part of the biofilm (Figures 4.18 and 4.19). This was based on work by Molwantwa (2007) and shown in Figure 3.7.

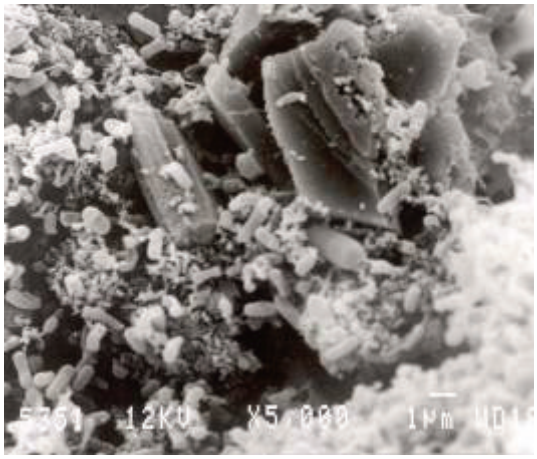


Figure 4.18 Scanning electron micrograph of bacterial population from the fourth section of the reactor that was sampled. Apart from the bacterial population, large crystalline structures of sulphur were observed.

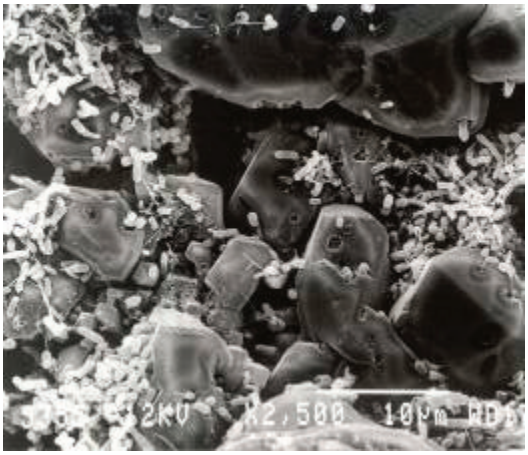


Figure 4.19 Scanning electron micrograph of the bacterial population from the fifth section of the reactor that was sampled. Crystalline structures of elemental sulphur were observed. Bacterial interaction with this crystalline sulphur is also observed

6) Filamentous bacteria were not observed as a large component of the bacterial population in the reactor. Filamentous bacteria were observed near the end of the reactor (Figure 6.20). This is where the sulphide loading rate was lowest.

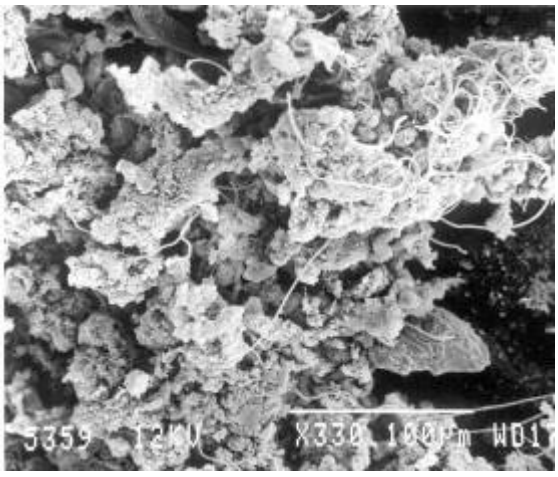


Figure 4.20 Scanning electron micrograph of the attached bacterial population from the sixth section of the reactor that was sampled. Evidence of the development of a filamentous population was observed in this section.

4.4 Discussion

4.4.1 Reactor Start-up

The inability of the reactor to oxidise all the sulphide feed during the first 28 hours of operation using a clean section of silicone tubing was probably due to a lack of an attached biofilm. The rate of sulphide oxidation during the first 12 hours was 4.32 x 10^{-4} $M.h^{-1}$ (8.1

$mg.L^{-1}.h^{-1}$) which compares well with the predicted initial chemical oxidation rate of a 100 mg. $L^{-1}.h^{-1}$ HS^- at an oxygen concentration of 3 $mg.L^{-1}$. Silicone is hydrophobic in nature and this hydrophobicity needs to be overcome before attachment of a biofilm could take place. The disappearance of sulphide from the effluent at 28 hours is indicative of the development of an attached microbial population able to oxidise sulphide. Previous observations would suggest that the decrease in pH was associated with an increase in the sulphate concentration of the reactor effluent and that sufficient oxygen was being delivered to the biomass for the complete oxidation of sulphide to sulphate.

These observations can be explained in a summarised form as follows:

During the initial 36 hours sulphide oxidation was inefficient and took place as a result of chemical oxidation. Initial colonisation of the silicone tube surface was slow due to the hydrophobic nature of the silicone surface. This has been reported to be overcome by the formation of a conditioning film prior to the adhesion of the arriving micro-organisms (Gristina, 1987). This conditioning film masks the physico-chemical properties of the substrate surface (Schneider *et al.*, 1994, van Dijk L.J., *et al*., 1988)

Between 36 and 48 hours sulphide disappeared from the effluent, elemental sulphur concentration in the effluent increased and the pH of the effluent decreased. This was probably due to the establishment of a sulphide oxidising biofilm on the silicone surface and a sulphide loading rate of the reactor which allowed for delivery of sufficient oxygen to the biomass so that sulphate could be produced (explaining the decrease in effluent pH). The loading rate applied during this stage of reactor running resulted in a steady increase in the effluent sulphur concentration and should be noted for future reference. The following is proposed as a meaningful expression of sulphide loading rate for a silicone tubular reactor and is expressed for a reactor with wall thickness of 1.5 mm:

HS^- loading rate = Molar HS^- x (Flow Rate.Reactor $volume^{-1}$.Reactor Surface $Area^{-1}$)

This expression takes into account that the reactor performance is dependent on the sulphide loading per unit length in relation to the reactor surface area. The reactor surface area plays a critical role in determining the oxygen transfer capability to the developed biomass.

At a feed concentration of 3.5 mM HS^-, at 3.8 $mL.min^{-1}$ in a reactor of length 1320cm ID 5 mm and OD 8 mm the loading rate is 7.5 x $10^{-7} mol.L^{-1}.h^{-1}.cm^{-2}$.

4.4.2 Reactor start-up after biofilm harvesting

Starting up the reactor immediately after removal of a previous biofilm resulted in sulphide being virtually undetectable in the effluent after the first hydraulic retention time. This could be explained by the presence of small amounts of residual biofilm that was not completely removed during the biofilm harvesting process. The residual bacterial population present in the unremoved biofilm was able to immediately begin oxidation of the sulphide. In addition to this the reactor was probably able to develop a new biofilm faster than fresh tubing due to the presence of an attached polymer layer (also referred to as a conditioning film) that was not removed during the biofilm harvesting. These polymers decreased the hydrophobicity of the silicone tubing and aided in the attachment of suitable organisms from the reactor feed. It was also possible that a small amount of residual sulphur was present in the tube. The presence of this sulphur could react with the sulphide to produce polysulphides according to equation 5.

$$HS^- + (x\text{-}1)\ S^\circ \rightarrow S_x^{2-} + H^+ \qquad (5)$$

x = 2-5 (pH dependent)

It is probable that the reactor was initially operating under non-steady state conditions in terms of microbial population, with the following parameters contributing to the selection of the predominant bacterial population in any given area of the reactor:

1) Sulphide loading rate {mol $HS^-.L^{-1}$ (unit reactor volume). h^{-1} (time). cm^{-2} surface area};
2) Organics concentration;
3) Type of organics present;

Towards the end of this investigation sulphide again began to appear in the reactor effluent. This would suggest that the amount of oxygen available to the biomass for oxidation had decreased. This could possibly be due to deposition of elemental sulphur within the biofilm and an increase in overall biofilm thickness and reducing oxygen diffusion. This would explain the increase in pH during this stage of the reactor operation. The increase in sulphate

concentration in the effluent could possibly be due to development of a new sulphide oxidising biofilm within the reactor.

Conceptually a sulphide loading rate needs to be determined above which autotrophic bacteria have a selective advantage over their heterotrophic sulphide oxidising counterparts and oxygen needs to be supplied to this population at a molar O_2: HS^- consumption ratio above which reduction of oxidised sulphur species is inhibited and below which sulphate is a major product of sulphur oxidation.

The sulphide loading rate for reactor of this type is determined by the oxygen concentration which is supplied chiefly by diffusion of oxygen through the reactor wall.

Oxygen permeability (P) is defined as

$$P = DS$$

D = Diffusion coefficient

S = Solubility coefficient

Oxygen flux may be calculated according to the following equation:

$$J = -DS\ (\Delta c/d)$$

Where DS = permeability coefficient

Δc = concentration difference on either side of the membrane

d = membrane thickness

Since the membrane thickness is constant for the length of the reactor, the oxygen flux into the reactor will predominantly be determined by the concentration of oxygen within the biofilm at the biofilm/silicone interface. The maximal amount of oxygen which may be supplied to the reactor will be determined by the surface area of the reactor, the surface area of the reactor will be determined by the length of the reactor multiplied by the average circumference of the reactor tube.

4.4.3 Particulate Collection

The profile of collected particulates suggests that sloughing events do occur with large sections of the biofilm being displaced from the reactor wall from time to time. Interestingly the ratio of sulphur mass to total particulate mass of the material collected seemed to be quite stable at 1:5, indicating that the biomass associated with the biofilm has a maximum elemental sulphur holding capacity. This maximum capacity may be determined by cycling of sulphur compounds within the biofilm and between the biofilm and the bulk phase**.**

4.4.4 EM and Light Microscopy

These studies showed that possibly two general types of elemental sulphur were present in the reactor namely biologically produced sulphur associated with bacterial growth in the upper sections of the reactor and crystalline sulphur present in the middle regions of the reactor. The presence of these extracellular sulphur globules was taken as an indication of autotrophic metabolism. Autotrophic metabolism can be considered to be a selective advantage at high sulphide loading rates. The highest sulphide concentrations are expected to occur within the upper sections of the reactor and hence this part of the reactor selects for an autotrophic population. It is possible that the biologically produced sulphur from the upper regions of the reactor acted as a catalyst for sulphur crystallisation further down the reactor. This could be determined by the relative amounts of sulphur species at different lengths along the reactor.

4.5 Conclusions

Pumping a non-sterile organics and sulphide containing solution through silicone tubing results in the selection of an attached bacterial biofilm capable of oxidising the sulphide, with sulphur being a major component of the oxidation product. Evidence to suggest that the oxidation is bacterially mediated was the lag time between reactor start-up and the time at which all sulphide was removed from the liquid stream. This is consistent with the development of a bacterial population on the reactor wall. The rate of sulphide oxidation after reactor start-up was significantly quicker than that predicted for chemical oxidation. The highest sulphide oxidation rate was 1.07×10^{-3} $M.h^{-1}$ (35 $mg.L^{-1}.h^{-1}$), which is (4 X) higher than the predicted chemical oxidation rate (Buisman *et al.,* 1990a).

Start-up of the reactor was significantly quicker when a tube was used from which the previous biofilm had recently been removed. This was ascribed to the presence of a polymeric layer on the tube surface enabling bacterial attachment, incomplete removal of previous bacterial biofilm, and presence of elemental sulphur promoting the formation of polysulphides.

An autotrophic bacterial population was demonstrated to have developed in discrete areas of the reactor (see Figures 4.14 and 4.15). The autotrophic population was observed by scanning electron microscopy close to the top of the reactor where the highest sulphide-loading rate and highest sulphide concentrations occur. In addition to this another form of sulphur possibly orthorhombic crystalline sulphur was observed further down the length of the reactor suggesting that biological sulphur production may enhance sulphur crystallisation at a point further along the reactor. Light microscopy evidence also suggested that elemental sulphur production occurred within discrete areas of the biofilm itself.

Although the investigations carried out here represent preliminary studies, they do suggest that a reactor based on tubular silicone does offer potential as a configuration for the biotechnological removal of sulphide as elemental sulphur from treated AMD. Biological sulphur production is dependent on the provision of very specific conditions that demand that strict process control be employed. Strict process control measures such as those based on maintaining a predetermined redox set point would not be applicable in a passive treatment system. The chemical characteristics of silicone and its oxygen permeability in particular, in addition to the bacterial growth that occurs on these silicone surfaces seem to be able to provide an environment in which this strict control is not required. This suggests that a reactor that meets the criteria of a passive treatment system may be developed using tubular silicone. Development of such a system will be dependent on determining the optimal relationship between sulphide load and reactor volume in relation to the silicone tube wall thickness and the development of strategies for the harvesting of sulphur from the reactor that fit the definition of a passive system.

5. SCALE-UP EVALUATION OF THE FIXED FILM BIOREACTOR SYSTEM

5.1 Operating System

Given the budgetary constraints of the Consultancy Contract, existing plant, equipment and analytical facilities available at EBRU were used in this study as far as possible. The experimental layout used is outlined in Figure 5.1.

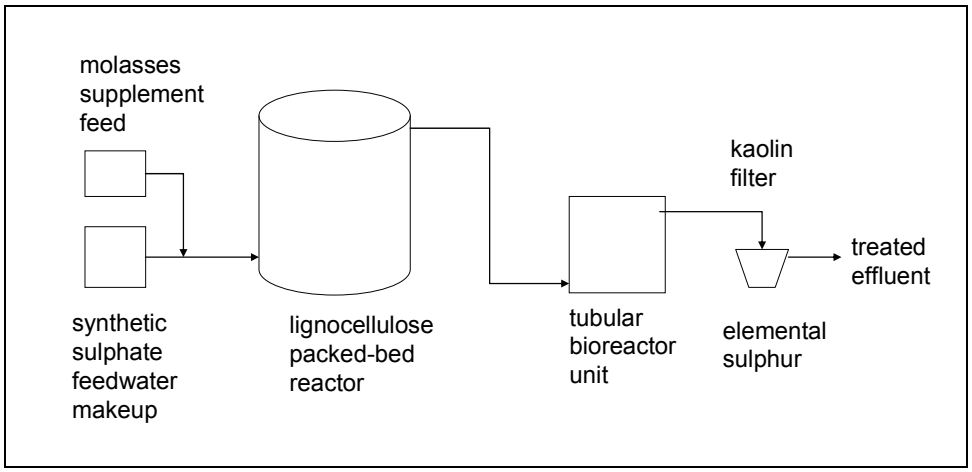


Figure 5.1 Experimental system used to evaluate the immobilized fixed tubular sulphur biofilm reactor. A synthetic sulphate mine drainage water was enriched by molasses addition and fed to a 10 m^3 lignocellulose degrading packed bed reactor where sulphate was reduced. The resulting sulphide-enriched stream was passed to the tubular reactor unit operation. The various materials and configurations of the tubular system evaluated were fitted in this unit. The harvested sulphur biofilm product was separated from the treated effluent on a kaolin filter.

In preparing a sulphide-enriched solution for use in the tubular immobilized biofilm studies, a synthetic minewater feed was made up to contain ~2000 mg/L sulphate and pumped to a 10 m^3 lignocellulose degrading packed bed reactor. The construction of this unit was based on the principles described by PHD (2002) in the development of the IMPI systems technology. The feed was supplemented with molasses in order to ensure enhanced sulphate reduction as described for the lignocellulose packed bed system by Molwantwa *et al*. (2003). The sulphide-enriched stream was then fed directly to the tubular bioreactor unit operation where the evaluation of the various configurations and materials was undertaken.

5.2 Tubular Reactor

The process development studies undertaken for the purposes of this investigation followed an empirical approach in the evaluation of the potential of the tubular reactor as a configuration for the operation of an immobilized sulphur biofilm system. Previous observations had indicated that the regulation of oxygen transmission across the tube wall was an important factor in the establishment of the necessary redox gradients (~Eh -150 mV) required for the growth of the sulphide oxidizing biofilm in tubular systems. Molwantwa (2007) has furthermore shown that the poising of this system between aerobic and anaerobic component populations of the biofilm occurs across a layer no more than 100 μ in thickness.

With this picture in mind, the assumptions to be tested in this study were identified as follows:

1. The oxygen permeability of the material from which the tube is made determines the delivery of oxygen to the inside surface of the tube;
2. The thickness of the tube material may play a role in controlling the mass transfer of oxygen across the tube wall;
3. The oxygen scavenging components of the biofilm population and of the bulk liquid would play a role in the redox poising of the system;
4. Addition of a small surplus of readily biodegradable COD in the form of molasses may be used to control the oxygen-scavenging functions of the biofilm and the bulk liquid microbial populations;
5. In addition to the above, flow rate and length of tube could also be used to control biofilm formation, rate of formation and mass of sulphide oxidation to elemental sulphur.

5.3 Harvesting

It has been observed that with the build-up of the sulphur-containing biofilm, it becomes brittle with time and peels away from the inner surface of the tube. This natural sloughing of the sulphur results in largish flakes together with a smaller fraction of fine particles passing out of the tube with the effluent. An elementary schmutzedeke-type kaolin filter was constructed and located to receive the effluent stream in order to capture the product and quantify the performance of the system.

Given a number of observations that the natural sloughing of the biofilm may occur at some time past the sulphide-sulphur conversion optimum, a form of tube pigging was also developed for harvesting the film prior to the natural sloughing event. A bullet-shaped pig was made from wood and sized to fit snugly into the particular tube being tested. The tubular reactor was disconnected from the feed at the inlet, the pig was inserted and blown through the tube using compressed air. Both the pig and the biofilm were then recovered on the kaolin filter. However, this method resulted in the complete fracturing of the biofilm and the production of a suspension of sulphur fine sulphur particles.

5.4. Results

The system, as described above, was set up at EBRU labs in Grahamstown and operated over a period of 5 months in early 2008. Given the objective of the study being to develop and evaluate an experimental system, and make recommendations preparatory to a more intensive follow-up study, the primary focus of the project was on operationalising the experimental system following a broadly empirical approach. Once the sulphide delivery system had been operationalised, the sulphide-enriched stream was fed to the tubular bioreactors which were maintained at 25^{o}C in a constant environment chamber. Here the various tubular materials were inserted and evaluated.

5.4.1 Sulphur Harvesting

Analytical studies confirmed that the sulphur recovered from the tubular biofilm was comparable in nature to that produced in the floating sulphur biofilm system.

While the schmutzedeke-type kaolin filter constructed for this study was able to remove a large fraction of the sulphur produced, it was not entirely effective in dealing with the fine colloidal sulphur particles that resulted, in particular, with the use of the piging system for harvesting the biofilm. Given the short-term and preliminary nature of this phase of the project, and the associated budgetary constraints, it was not possible to acquire a more effective sulphur harvesting device for determining accurate mass balances for these studies. Thus performance outcomes were evaluated on a somewhat subjective assessment of sulphur production but relied more accurately on the measurement of sulphide breakthrough.

Decreasing concentrations of sulphide would be measured in the tube effluent during the period that the biofilm was in the process of establishment. Once sulphide removal had stabilized at a breakthrough of <5 mg/L, the period of optimum performance was considered to have commenced. After a period of time the sulphide concentration would begin to rise again in the effluent. This was thought to be associated with the maximum desirable thickness of the biofilm being reached on the inside of the tube, and where oxygen transfer across the tube wall and biofilm would become limiting. Where the system was allowed to continue for a period of time until natural sloughing of the biofilm occurred sporadically, sulphide levels would rise in the effluent and a substantial breakthrough of sulphide would be seen.

However, where the pigging system for biofilm harvesting was used, the point to commence the process could be determined quite accurately depending on the rise in sulphide concentration following steady state operation. A number of studies were undertaken to determine the optimum number of pigging cycle repeats that would give the maximum sulphur yield but also ensure the lowest time of return to steady state operation. It was found that between 1 and 2 pigging cycles would produce the best results. Increasing the pigging cycles beyond this number would result in a substantial increase in sulphur yield but at the expense of recovery time which could be extended by several days. During this interval sulphide would pass directly through the system largely un-oxidised. This correlated with the previous laboratory studies where the presence of a conditioning film was found to be necessary for rapid establishment of biofilm. An optimal harvesting regime should thus aim to leave this layer as undisturbed as possible.

The need to evaluate and develop effective low-cost systems for sulphur harvesting following the tubular reactor was thus identified as one of the key factors that would need to be considered in any follow-up study. This not only to enables accurate analytical measurement of the system but also to provides an effective harvesting unit operation where full-scale implementation was undertaken.

6. PATENT AND LITERATURE SEARCH

The intellectual property associated with the work described in this report was made available in the public domain with the registration of US Patent 7285216 "Water Treatment" Rose PD and Rein NB, 23 October 2007. At this time a full literature search was undertaken and the results are reported in Table 6.1. No prior art was discovered which might invalidate the patent claim.

It is of importance to note that the use of the term 'floating sulphur biofilms' on the Google search engine, resulted in the first 8 hits being related directly to the WRC/EBRU work described above including WRC-owned US Patent 7285216 (Rein and Rose, 2007). For 'fixed sulphur biofilms' 2 out of the first 8 and for 'immobilised sulphur biofilms' 3 of the first 8 hits related directly to EBRU reports. This observation of the leading position EBRU publications occupy in the field of sulphur biofilm research was largely confirmed using more detailed academic search engines including Science Direct, Biological Abstracts, JSTOR, Scopus and Pub Med.

The overall conclusions that can be drawn from the literature search include:

1. The review of Molwantwa (2007) remains the most up to date published report on the topic of 'sulphur biofilms' and including 'immobilised', 'fixed biofilm' and 'tubular reactor' extensions of the sulphur biofilm term;
2. Very little has been published in the field generally outside the papers, theses and patent produced by EBRU within WRC-funded research programmes;
3. The concept 'immobilised, fixed film, tubular sulphur biofilm reactor' appears not to have been described yet and remains a novel concept at this time;
4. With both the IP secured and the positive results emerging from these studies, it can be strongly recommended that the WRC proceed to commercialisation of these processes either alone or in whatever form of partnership that might work. Prior claim will reside in this patent until approximately 16 May 2023

Table 6.1 Literature search undertaken to determine prior art relating to the findings published in this report. Implications have been graded N for no implication and Y for a possible implication.

Reference	Implication on prior art
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7. CONCLUSIONS AND RECOMMENDATIONS

The work reported in this study was undertaken to evaluate, at the pre-feasibility level, the proposition, based on observations by EBRU researchers over several years, that the immobilised sulphide oxidising fixed film system may be considered for the treatment of sulphide containing wastewaters. The passive treatment application was targeted in these studies where sulphide removal presents a severe technological bottleneck in the development of these treatment systems. In the laboratory-scale studies that were undertaken the following conclusions may be drawn:

- The feasibility of the sulphide oxidising tubular fixed film system for sulphide removal from wastewaters has been demonstrated;
- This has been demonstrated at the pre-feasibility scale and provides sufficient evidence on which to base larger-scale engineering scale-up studies;
- The results acquired appear to warrant investment in further development of the process;
- Patent and literature searches confirm the originality of the work and that intellectual property residing with the WRC (Rose and Rein 2007) provides a satisfactory base for the protection of the rights of any further investment in development of the process;
- The intellectual property rights would be protected until approximately 16 May 2023 if the registration is kept current;
- Passive treatment of AMD is certain to grow in importance as the gold and coal mining industries in South Africa reach maturity and large scale mine closure ensues. Biological treatment offers one of the few environmentally sustainable technology options and this is crucially dependent on effective technology for the removal of sulphur species from the treated water;
- It is thus strongly recommended that the patent be maintained and that further work be undertaken to establish an engineering scale-up and implementation of the process at industrial scale.

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