Biological Sulphide Oxidation in Heterotrophic Environments

by

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Submitted in fulfillment of the requirements for the degree of

Master of Science

of

Rhodes University, Grahamstown

January 2002

Abstract

Acid mine drainage is a major environmental pollution concern associated with the mining of sulphide-containing ore bodies. Both physicochemical and biological options have been investigated for the treatment of acid mine drainage with recent interest in biological processes targeting low-cost and passive treatment applications. All acid mine drainage biological treatment processes are based to some extent on the activity of sulphate reducing bacteria, and their ability to reduce sulphate to sulphide in the presence of a range of carbon and electron donor sources. A portion of the sulphide produced may be consumed in the precipitation of heavy metals present in the mine drainage. Residual sulphide must be removed, not only due to its toxicity, but especially to prevent its re-oxidation to sulphate where salinity reduction is a target of the treatment process.

The partial oxidation of sulphide to elemental sulphur is an option that has received considerable attention and both physicochemical and biological options have been investigated. Biological processes have substantial potential cost advantages and run at ambient temperatures and pressures. However, the oxidation of sulphide to elemental sulphur is poised over a narrow redox range and process control to maintain optimum conditions remains a serious problem. In addition little has been reported in the literature on process control of sulphide oxidation to elemental sulphur, in the heterotrophic conditions prevailing in the reaction environment following sulphate reduction.

This study undertook an investigation of biological sulphide oxidation under heterotrophic conditions in order to establish the effect of organic compounds on biological sulphide oxidation, and to determine whether the presence of organics, and associated heterotrophic oxygen consumption, may be manipulated to maintain the defined redox conditions required for the production of elemental sulphur.

Biological sulphide oxidation under heterotrophic conditions was investigated in a series of flask experiments. Based on these results three different reactor configurations, a Fixed-Film Trickle Filter Reactor, Submerged Fixed-Film Reactor and a Silicone Tubular Reactor were used to investigate sulphur production.

The flask studies indicated that organics, and associated heterotrophic metabolism in the presence of excess oxygen in the sulphide oxidation reaction environment, did contribute to the poising of redox conditions and thereby enabling the production of elemental sulphur. While the Fixed-Film Trickle Filter Reactor was found to be redox unstable, probably due to excess oxygen ingress to the system, a reduced oxygen challenge in the Submerged Fixed-Film Reactor configuration was found to be more successful for production of elemental sulphur. However, due to the production of a predominantly filamentous sulphur producing microbial population, recovery of sulphur from the column was intermittent and unpredictable. Extended residence times for produced sulphur on the column increased the likelihood for its eventual oxidation to sulphate.

The Silicone Tubular Reactor was found to support a vigorous sulphide oxidising biofilm and produced elemental sulphur effectively. Electron microscopic studies showed that this occurred as both biologically produced sulphur and, probably mainly, as crystalline sulphur in the ortho-rhomic form. Given the linear extension of the sulphur production reaction environment it is was possible to investigate the sequence of the reaction mechanism in grater detail than is possible in mixed systems. Based on these findings a model explaining sulphur production under heterotrophic conditions has been proposed and is presented. The commercial implications of the development have also been noted.

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List Of Abbreviations

ALD	-Anoxic Limestone Drain
AMD	- Acid Mine Drainage
ANOVA	-Analysis of Variance
COD	- Chemical Oxygen Demand
DACST	- Department of Arts Culture, Science and Technology
DEA	- Diethanol Amine
EDX	- Energy Dispersive X-RAY MicroAnalysis
HPLC	- High Performance Liquid Chromatography
HRT	- Hydraulic Retention Time
OFS	- Orange Free State
OLC	- Open Limestone Channel
RSBR	– Reciprocating Sludge Bed Reactor
SEM	- Scanning Electron Microscopy
SOB	- Sulphide Oxidising Bacteria
SRB	- Sulphate Reducing Bacteria
TEM	- Transmission Electron Microscopy
TSS	- Total Suspended Solids
VFA	- Volatile Fatty Acids

Acknowledgements

I would like to acknowledge the supervision and guidance given by Professor P.D. Rose and thank him for the opportunity to carry out this work as well as his patience and encouragement throughout the duration of both the experimental work and writing of this thesis.

I would also like to thank the DACST Innovation Fund and the Water Research Commission for their financial support for this project.

Thanks to the staff and students of the Rhodes University Microbiology and Biochemistry Department and especially my colleagues in the Environmental Biotechnology Group.

Thank you to Mr. Robin Cross and Ms. Shirley Pinchuck of the Electron Microscopy Unit at Rhodes University for the invaluable help with the preparation, viewing and photographing of the large number of light and electron microcopy samples generated during this study.

To my family, thank you for your support and encouragement during the completion of this thesis.

Lastly, a very special thanks to Nikki for her ongoing love and support throughout the duration of the thesis.

Chapter 1
Biological Sulphide Oxidation as a Tool for Correcting Local Imbalances in the Sulphur Cycle

1.1 The Sulphur Cycle

Sulphur and its organic and inorganic derivatives are essential and dynamic components of the natural environment. Sulphur is the twelfth most abundant element in the earth's crust (Mathews, 1990) and about 1% of the dry mass of living organisms is composed of sulphur (Jorgensen, 1982). Acceptable levels of the various oxidised and reduced forms of sulphur are maintained within the environment by the sulphur cycle (Figure 1.1), which serves as a dynamic balance between large, relatively inert sulphur pools such as geological pyrite deposits, sulphate reserves present in the ocean, the volatile sulphur compounds in the atmosphere and the sulphur requirements of living organisms.

Disturbance of the natural sulphur cycle, often by human activity, results in the mobilisation of these sulphur compounds from these relatively inert sulphur pools and the accumulation of unacceptable levels of sulphur compounds in the environment. Sources of sulphur pollution resulting from disturbances of the sulphur cycle include SO_2 emission from the burning of fossil fuels (Kuenen and Robertson, 1992a) and the oxidation of pyrite by micro-organisms in disused mines resulting in Acid Mine Drainage (AMD) (Davison *et al.,* 1989). A bacterial community, referred to as the "Sulfuretum" is the ecological community of sulphide oxidising and sulphate reducing bacteria responsible for the continuous cycling of sulphur compounds and can be regarded as the coupling of living biomass formation and the subsequent decomposition and remineralisation of the biomass (Jorgensen, 1982).

Sulphate reducing bacteria reduce sulphur compounds in the presence of a suitable electron acceptor to produce sulphide as an end product and, sulphide oxidising bacteria oxidise sulphur products in the presence of a suitable electron donor to produce sulphate as an end product (Jorgensen, 1982).

It has been suggested that the bacteria of the sulphur cycle may be utilised in biotechnological applications to re-establish the balance in the sulphur cycle in areas

where human activity has resulted in the over accumulation of specific sulphur compounds (Kuenen and Robertson 1992a). Wastewaters high in sulphate may be treated using a process utilising sulphate reducing bacteria, shifting the sulphur cycle towards the sulphide side of the cycle. Sulphide, being a highly toxic substance must be removed from the water.

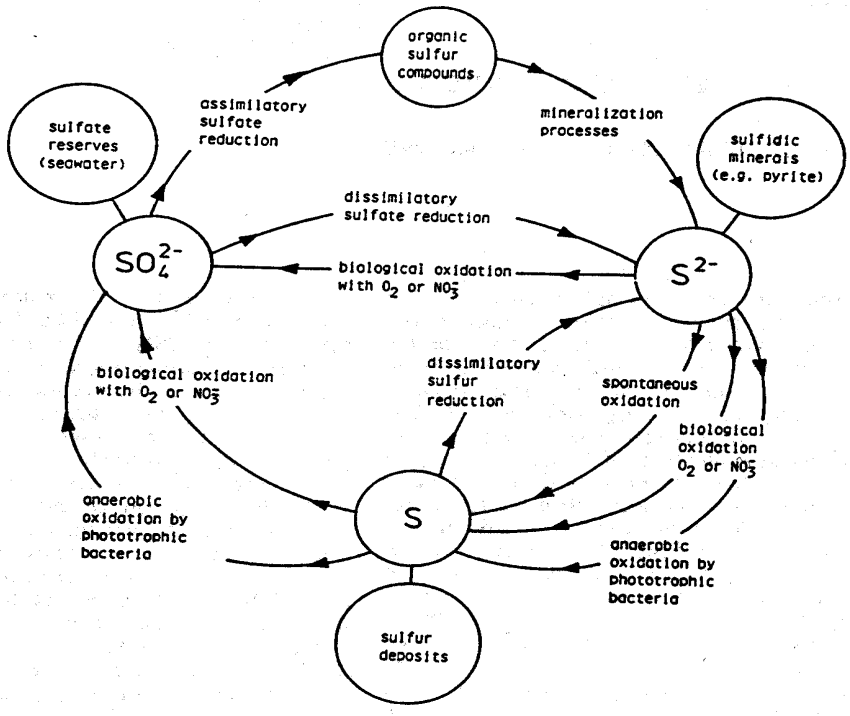


Figure 1.1 The Sulphur Cycle

1.1.1 Sulphide producing processes

Geothermally active areas e.g. volcanoes, hydrothermal vents and sulphur springs as well as microbial processes such as biological sulphate reduction represent primary sources of sulphide. Large bacterial communities able to utilise the sulphur compounds emanating from particularly hydrothermal vents and sulphur springs have been well documented (Muyzer *et al.,* 1995; McCollom and Shock, 1997).

As previously noted sulphur is an important component of a number of biologically active compounds e.g. proteins, vitamins and certain co-factors and the incorporation of sulphur compounds into these biologically active molecules is achieved through the processes of assimilatory sulphate reduction. Assimilated sulphate may be converted to sulphide through decay of sulphate rich organic matter.

Anaerobic sulphate reducing bacteria are able to obtain energy from the dissimilatory reduction of sulphate to sulphide (Jorgensen, 1982). Sulphate reducing bacteria are able to oxidise a number of organic acids such as acetate, propionate, lactate and hydrogen in the presence of sulphur, thiosulphate or sulphate which act as an electron acceptors (Widdel, 1988). In this process sulphate is utilised as the terminal electron acceptor for the oxidation of organic compounds. Sulphate reducing bacteria are divided into seven genera and include amongst others: *Desulfobacter*, *Desulfobulbus*, *Desulfonema*, *Desulfovibrio*, *Desulfotomaculum* and *Desulfomonas* (Jorgensen 1982). Sulphate and sulphur reducing bacteria play an important role in sewage systems and other anaerobic water treatment systems where a supply of a readily oxidisable carbon source is present in the presence of reducible sulphur compounds (Kuenen and Robertson 1992a).

1.1.2 Sulphide consuming processes

Sulphide may be removed from the environment by one of four processes (see figure 1.1):

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

4) Become biologically oxidised 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, tannery waste stabilisation ponds and on the surface of biological sulphate reducing reactors (Gilfillan, 2000), examples of these sulphur biofilms are shown in Figures 1.2 and 1.4.

Figure 1.2 Photograph of a floating sulphur biofilm present on the surface of hot springs in Namibia

Figure 1.3 Photograph of floating sulphur biofilm development on the surface of tannery waste ponds in Wellington, South Africa.

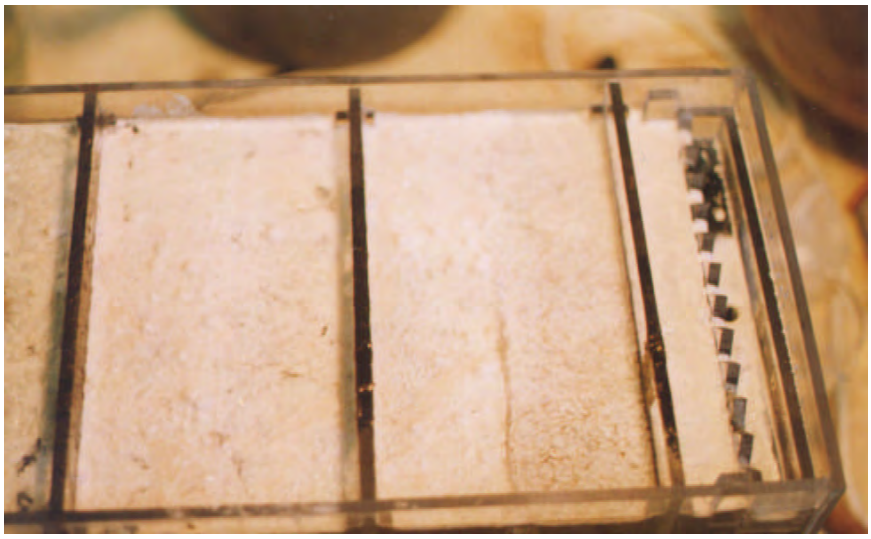


Figure 1.4 Photograph of well developed sulphur biofilm on the surface of a laboratory Reciprocating Sludge Bed Reactor (RSBR) at Rhodes University, South Africa

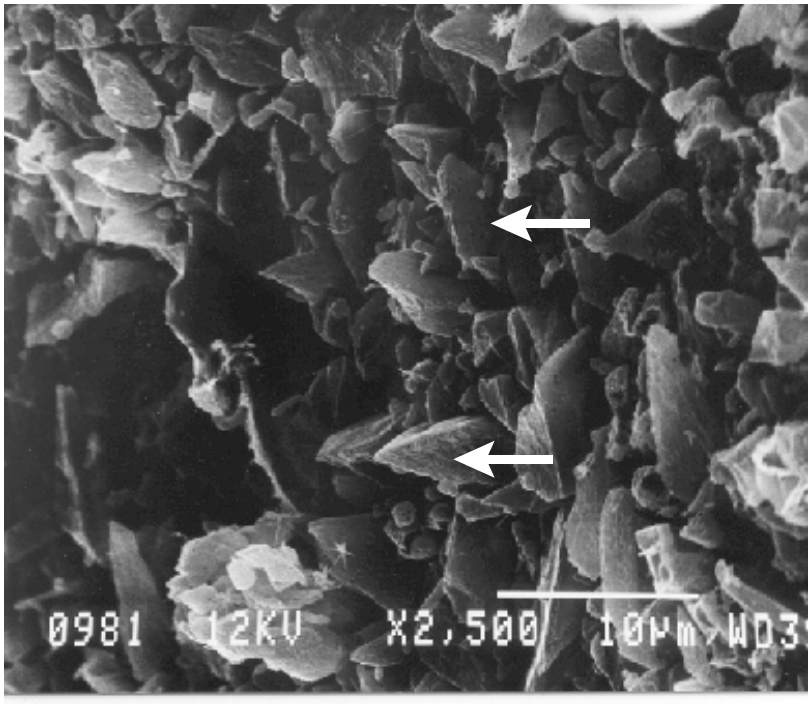


Figure 1.5 Electron micrograph of crystalline sulphur associated with floating biofilm formation on the surface of a sulphate reducing reactor from Gilfillan, 2000

1.2 Acid Mine Drainage

The human activity of mining has the potential to cause large local imbalances in the sulphur cycle and in South Africa the closure of gold mines on the East Rand of Gauteng is resulting in a serious sulphur-cycle related pollution problem (Scott, 1995). South Africa is the world's major producer of precious metals and minerals. The mining of coal and gold have been reported to account for 90% of the tonnage mined in South Africa (Henzen and Pieterse, 1978).

Gold has been mined on the Witwatersrand and surrounding areas since its discovery in 1886. Mining activities have concentrated on nine distinct gold mining areas (goldfields) in the greater Witwatersrand basin. The Witwatersrand basin is a large geological feature that extends from Johannesburg in the north, Klerksdorp in the west to the OFS goldfields in the south and Kinross in the east. Of the nine goldfields two of these areas namely the East Rand and The Central Rand areas are closely associated with the city of Johannesburg and its surrounding areas. Mining in the east rand region takes place in what is more specifically referred to as The Far East Rand Mining Basin and is a term used to describe the interconnected gold mine workings underlying all or portions of the municipal districts of Benoni, Brakpan, Springs and Nigel.

Mining in these areas has been historically difficult with intensive dewatering of mines being required to allow mining operations to continue. Up until the 1950's each mine was responsible for their own dewatering operations, but as mines came to the end of their working lives, economic considerations have left fewer mines to continue with the dewatering of the basin. By the early 1960's all pumping from the Far East Rand Basin was undertaken by only three gold mines, and as it currently stands dewatering of the whole basin is achieved by pumping at Grootvlei's No 3 shaft. This water is discharged to the Blesbok Spruit, a sensitive wetlands system, and Ramsar site. Apart from the increased volume, discharge of this water would not represent a major problem if the water quality was of a reasonable standard. The quality of water that is being pumped from the mine is very poor as a result of the production of acid mine drainage (AMD). Depending on the geological composition of reefs that are mined for gold the water may be characterised by a combination of the following: Low pH, high total dissolved solids (TDS), high sulphates, high levels of heavy metals particularly Iron (Fe), Manganese (Mn), Nickel (Ni), Zinc (Zn) or Cobalt (Co) (Scott, 1995).

During mining operations rock is removed in order to gain access to the ore body, creating a large system of well ventilated underground workings that expose rock that is usually under anaerobic conditions to aerobic conditions. If the rock contains pyrite, microbes are able to oxidise the pyrite in the presence of oxygen according to the following equation (Davison *et al.,* 1989):

$$FeS_2\,(s) + 7/2O_2\,(aq) + 10H_2O \rightarrow Fe^{2+} + 2SO_4^{2-} + 2H^+ \quad (1)$$

During mining operations water is actively pumped out of the mine, but when mining ceases the mine floods and water flows out carrying the products of pyrite oxidation namely iron, sulphate and acidity. Hence a large quantity of previously inert sulphide (in the form of pyrite) is mobilised in the form of soluble sulphate accompanied by acidity and quantities of metals, particularly iron. The resulting outflow of acidic, sulphate and metals rich water is referred to as Acid Mine Drainage AMD. This problem is not confined to South Africa or particularly to the gold mining industry. Similar problems have been reported on and treated in the UK from abandoned coal mines (Younger 1995) and in the USA from a variety of mining operations (Hedin *et al.*, 1989). Estimates for the East Rand Basin predict that once active dewatering of the mines ceases outflows in the order of 100 $Ml.day^{-1}$ of water with a pH of 5, high sulphate concentrations(>800$mg.L^{-1}$) and iron and manganese also at unacceptably high levels can be expected (Scott, 1995). AMD is a long term pollution problem where outflows from disused mines may may result in flows containing elevated concentrations of heavy metals and sulphate lasting from decades to centuries (Younger, 1997)

1.3 Options for the treatment of AMD

A successful treatment system for AMD should accomplish the following objectives:

1) Neutralise of acidity
2) Decrease heavy metal concentrations
3) Decrease salinity (sulphate) concentrations

In addition to these chemistry based objectives a treatment for AMD should be sustainable due to the long term nature of the AMD problem. Both physicochemical and biological approaches may be implemented in order to achieve these objectives. Physicochemical and biological approaches to the treatment of AMD may be further subdivided into active and passive treatment processes. Active processes are dependent on carefully controlled externally addition of chemicals or substrates whereas passive processes require little or no external additions or control once established.

1.3.1 Physicochemical Treatment Options

1.3.1.1 High Density Sludge Process

The high density sludge process is essentially a two step active process that deals particularly well with iron contaminated waters. South African AMD waters are characterised by high iron metal loads, with iron sulphates derived from bacterially assisted oxidation of iron sulphides being the chief pollutants (Henzen and Pieterse, 1978). Raw mine water pumped from the Grootvlei Mine on the East Rand of Gauteng in South Africa contains 264 mg. L^{-1} iron (Grootvlei Proprietary Mine, 1997). In the first step of the high density sludge process the AMD solution is aerated in order to oxidise Fe^{2+} to Fe^{3+} The resulting Fe^{3+}, which is relatively insoluble is hydrolysed and precipitates from solution: Equation (2) represents an example of one such hydrolysis reaction.

$$Fe^{3+} + 3H_2O \leftrightarrow Fe(OH)_3(s) + 3H^+ \qquad (2)$$

Lime or sodium hydroxide is added in order to neutralise acidity associated with the AMD as well as the hydrolysis reaction. Heavy metals are precipitated as a hydroxide sludge.

The high density sludge process is widely utilised in the mining and refinery industries (van Wyk and Munnik, 1997) and is currently being used at the Grootvlei Gold Mine on the East Rand. While this process is highly effective in neutralising acidity and precipitating heavy metals it has the drawbacks of producing a highly voluminous hydroxide sludge that requires special disposal, has high chemical consumption which detracts from the long term sustainability of such a treatment process and does not significantly reduce salinity levels of AMD.

1.3.1.2 Precipitation with Barium Salts

Barium sulphate has a very low solubility in aqueous solutions. Sulphate may therefore be precipitated from AMD as a barium salt. Barium precipitation has been successfully utilised in the treatment of boiler feed waters. A proposed treatment for AMD consists

of a two stage barium carbonate process followed by a four stage barium sulphide process (Maree *et al*., 1989). The raw materials barium carbonate and barium sulphide may be recovered by thermal processes with the production of by products such as sulphur, sodium bisulphide and heavy metals also possible. This process is ideally suited to the lower flow rates associated with industrial effluents but is probably not a sustainable option for treating large volumes associated with AMD due to the requirement of thermal regeneration.

1.3.1.3 Slurry Precipitation Recycle Reverse Osmosis (SPARRO)

This process was developed by the South African Chamber of Mines and is based on a seeded reverse osmosis (RO) concept (Chamber of Mines Research Organisation, 1988). This process should deliver very high quality water with gypsum as a by-product. The drawbacks of this process are the very high initial capital costs, high maintenance costs and the susceptibility of RO membranes to become fouled.

1.3.1.4 Passive physiscochemical approaches using limestone

A number of different configurations for passive treatment systems exist and include (EPA: Coal Remining BMP guidance manual, 2000)

1) Anoxic Limestone Drains (ALD);
2) Open limestone channels (OLC)

Limestone ($CaCO_3$) may be used as an alternative to lime. It has the advantage that it occurs naturally and can be incorporated into the above systems. These systems are able to neutralise acidic waters, precipitate metals and even partially remove sulphate in the form of a calcium sulphate precipitate (Maree *et al*., 1992). These system do have the disadvantage of being dependent on a clean limestone surface for neutralisation to occur any deposition of ferric hydroxide negatively impacts on the performance of these systems.

1.3.2 Biological approaches to treating AMD

A number of biological approaches to AMD have been investigated, reported on and reviewed by Kuenen and Robertson, (1992a); Gadd and White, (1993); Barton, (1995); Johnson (1995) and Rose *et al.,* (1998). All biological approaches are based to some extent on the activity of sulphate reducing bacteria. Biological sulphate reduction is applied in both active and passive biological processes for the treatment of AMD. During bacterial sulphate reduction, sulphate is reduced in the presence of a suitable electron donor and carbon source for bacterial growth. The sulphide produced may be partially consumed for the precipitation of insoluble metal sulphides. This may be described by the following equations (Christensen *et al.,* 1996):

$$SO_4^{2-} + H^+ + 2CH_2O \rightarrow HS^- + 2H_2O + 2CO_2 \quad (3)$$

$$Me^{2+} + HS^- \rightarrow MeS + H^+ \quad (4)$$

Biological processes for the treatment of AMD achieve all of the objectives for the treatment of AMD. Alkalinity is produced in the form of sulphide and carbonates which aids in neutralisation of the water, metals may be precipitated as sulphides or carbonates and sulphate is removed in the form of sulphide. The sustainability of biological processes is predominantly determined by the cost of carbon sources utilised to drive sulphate reduction. For this reason, a number of complex carbon sources have been investigated as cost effective carbon sources and electron donors. Complex waste carbon sources that have been evaluated include: sewage sludge (Burgess and Wood, 1961); algal biomass and tannery effluent (Boshoff *et al.,* 1996); animal waste slurries (Ueki *et al.,* 1988); and straw, hay and lucerne (Bechard *et al.,* 1993).

1.3.2.1 Active biological processes

An active biological process for the treatment of AMD has been developed and tested at pilot scale by the Rhodes University Environmental Biotechnolgy Group. The Rhodes BioSure process utilises a patented reactor design enabling the use of primary sewage

sludge as a carbon source for biological sulphate reduction (Corbett, 2001 and Whittington-Jones, 2000). The use of a waste carbon source such as primary sewage sludge significantly improves the sustainability of a biological treatment system.

1.3.2.2 Passive biological treatment of AMD

The large volumes of low pH, sulphate rich water that will decant from disused mines when active pumping of water ceases (Scott, 1995) and the long periods of time over which this may occur (Younger, 1997) demands that a treatment system that can run with as little human intervention as possible and be able to cope with as large a volume of wastewater as possible. Passive treatment systems have been proposed as a means of treating these outflows over an extended period of time and may be defined as "a system excluding processes and plants requiring the input of external energy sources such as electrical or mechanical power".

Wetland processes represent the most extensively implemented passive biological treatment technology and may be defined as passive systems as they rely on natural geochemical and biological processes (Gazea *et al*., 1996). A wetland system consists of an area through which AMD is allowed to flow. The area is prepared with a variety of specially selected plants and is seeded with a suitable carbon source. If constructed correctly natural processes such as ammonification, denitrification, methanogenesis and reduction of sulphur and precipitation of meavy metals occurs (Kalin *et al*., 1991). Wetland systems do have the disadvantage of requiring large surface areas and the long term deposition of heavy metals over a large surface areas has also been questioned. In order to improve the effectiveness of wetlands they may be combined with some form of passive physicochemical process such as ALD.

1.4 Problems associated with hydrogen sulphide

Hydrogen sulphide is a highly odorous air and water pollutant. Large amounts of hydrogen sulphide are produced in association with a number of industrial processes that include the petrochemical industry, wastewater treatment, food processing, photographic processing, tanneries and the paper and pulp manufacturing industry. The treatment of high sulphate containing waters using sulphate reducing bacteria results in the production of hydrogen sulphide.

Hydrogen sulphide is a pollutant that must be removed from the environment due to its toxicity, corrosive properties and its characteristic rotten egg smell. Hydrogen sulphide causes serious odour problems due to the low odour threshold of 0.01-0.03 ppm (Guidotti, 1996), paralysis of olfactory nerves at concentrations between 150-200ppm and results in immediate collapse due to a condition referred to as sulphide knock-down at concentrations exceeding 1000ppm. Sulphide is extremely toxic to both marine and fresh water organisms. Any water that is going to be discharged into the natural environment requires treatment to remove the sulphide. The target levels of unionised H_2S in aquaculture are less than 0.001mg $H_2S.L^{-1}$ with 0.002 mg $H_2S.L^{-1}$ being classified as a long term health hazard for fish (Department of Water Affairs and Forestry - South African Water Quality Guidelines, 1996).

The biotechnological treatment of acid mine drainage requires the development of a sulphide oxidation process that can yield substantial amounts of sulphur through the bacterial oxidation to elemental sulphur under heterotrophic conditions

1.5 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, 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):

$$2HS^- + O_2 \rightarrow 2S° + 2OH^- \qquad \Delta G°' = -129 \text{ kJ./mol } HS^- \qquad (5)$$

$$2HS^- + 4O_2 \rightarrow 2SO_4^{2-} + 2H^+ \qquad \Delta G°' -772.43 \text{ kJ/mol } HS^- \qquad (6)$$

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 1.6) for the oxidation of sulphide has been proposed by Chen and Morris, (1972).

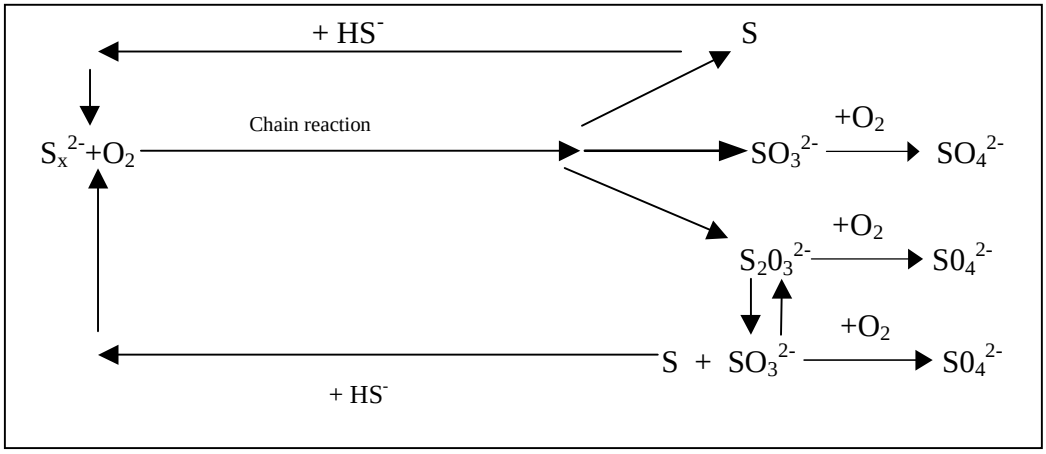


Figure 1.6 Mechanism of sulphide oxidation by oxygen as proposed by Chen and Morris, (1972)

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.

1.5.1 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:

$$R_I = k[S]^m[O_2]^n \quad (7)$$

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.*, 1990c):

$$R_i = k[S]^m[O]^{n\log[S]}\ mg.L^{-1}.h^{-1} \quad (8)$$

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 at low oxygen concentrations (Kuenen, 1975; Jorgensen, 1982).

1.5.2 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.

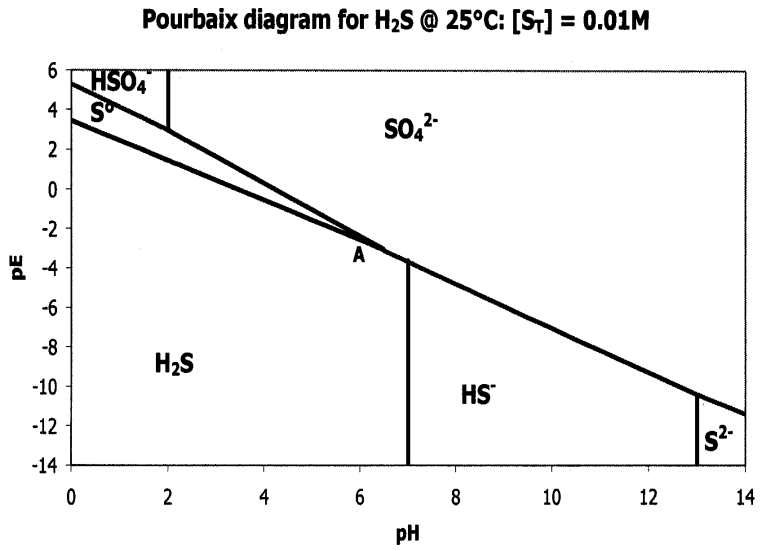


Figure 1.7 Pourbaix diagram for H_2S at 25°C for total S of 1M (Lewis *et al.*, 2000)

Figure 1.7 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.

1.6 Treatment options for sulphidic waste streams

1.6.1 Physicochemical methods for treating sulphide rich waste streams

1.6.1.1 Absorption and adsorption

Absorption and adsorption are often the first step in the treatment of sulphidic gas streams. Adsorption is based on the movement of sulphide onto the porous structure of an adsorbing material, whereas during absorption, H_2S is transferred to an alkaline scrubbing solution (Chwirka and Satchell, 1990). This sulphide rich solution can then be treated in either a biological or physicochemical treatment process.

1.6.1.2 Liquid redox processes

1.6.1.2.1 The Stretford Process

During this process sulphide in solution is converted into elemental sulphur in the presence of the catalyst vanadium (V) (Hammond, 1986). The process operates in the pH range 8.5-9.0 and is based on the catalytic oxidation of HS^- to S^o by V^v, during which V^{iv} is produced. The reoxidation of V^{iv} to V^v by molecular oxygen is enhanced by the addition of diethanolamine (DEA) or anthraquinone disulfonic acid (Steudel, 1996).

1.6.1.2.2 Lo-Cat™ and Sulferox™ processes

These are also referred to as iron chelate processes. In these processes chelate complexes of iron (III) are used as catalysts for the oxidation of HS^- to S^o. During this process the Fe^{3+} is reduced to Fe^{2+}. The Fe^{2+} is reoxidised to Fe^{3+} by the introduction of air into the system. In the Bio-SR™ process reoxidation of the Fe^{2+} to Fe^{3+} is performed by *Thiobacillus ferrooxidans* and has been found to enhance the reoxidation step by a factor of 200 000 (Rehmat *et al.*, 1997). A novel application of the concepts employed in these processes is the use of a silicone membrane to separate the sulphide containing water from the ferric iron solution (de Smul and Verstraete, 1999). This process is based on the observation that when a sulphide containing solution is pumped through a silicone cylinder immersed in a solution of ferric sulphate, a cloud of elemental sulphur is formed in the ferric sulphate if the pH of the sulphide solution is maintained below 8.5. The elemental sulphur formed subsequently sediments as orthorhombic α-sulphur particles.

1.6.1.2.3. Amine/ Amine plus Claus processes

These processes have been designed to treat large volumes of sulphide containing gas and are extensively utilised in the petrochemical and natural gas industries. H_2S is stripped into an amine or glycol containing solution at high pressure. The concentrated sulphide gas is then combusted with an oxygen bearing gas in a reaction furnace, in the presence of a catalyst such as bauxite. This combustion results in the production of SO_2 that reacts with the incoming H_2S to produce elemental sulphur. Elemental sulphur is recovered by condensation in a heat exchanger. Tail gas passes through a catalytic hydrogenation reactor where residual sulphur compounds are converted to H_2S and may be reintroduced at the beginning of the process.

Table 1.1 Physicochemical processes used to treat different sulphide loads

Sulphide treated per day	Type of process used
< 150kg.day^{-1}	Scavenging processes
150kg<HS^-<20tons.day^{-1}	Liquid redox, Amine, Amine + Claus, Amine + Liquid Redox
Sulphide>20tonnes.day^{-1}	Amine+Claus (Tail gas treatment if > 97% recovery required)

1.6.2 Biological sulphide oxidation

Physico-chemical methods for treating sulphide containing gas and liquid streams require large investments and high operating costs are involved due to the high pressures, high temperatures and speciality chemicals utilised in these processes. Microbial oxidation of sulphide is carried out at ambient temperature and pressure resulting in reduced energy costs and represents a feasible alternative to these physicochemical processes. In addition to lower energy costs, microbial oxidation is not dependent on the addition of hazardous chemicals, reducing the impact on the environment that these processes might have. Biological sulphide oxidation processes employ sulphide oxidising bacteria to oxidise sulphide.

In addition to sulphide, the *Thiobacilli* can oxidise elemental sulphur, thiosulphate and other reduced sulphur compounds that are common intermediates during sulphide oxidation in the natural environment (Jorgensen, 1982). Sulphide oxidising bacteria have to compete with the chemical oxidation of sulphide and are therefore often found in gradients at the interface between anoxic (sulphide-rich) sediments and aerobic waters or anaerobic waters and the atmosphere. At the lower oxygen concentrations the bacteria can effectively compete with the chemical oxidation of sulphide (Jorgensen, 1982). In natural environments complex interactions occur between photosynthetic sulphur bacteria, the colourless sulphur bacteria and sulphate reducing bacteria (Overmann and van Gemerden, 2000). Sulphide oxidising bacteria may be broadly divided into two groups:

1.6.2.1 Anaerobic phototrophic sulphide oxidising bacteria.

Phototrophic sulphide oxidising bacteria use sulphide as an electron donor for photosynthesis. Representative members of this group include *Chlorociacceae,*

Chloroflexaceae, Chromatiaceae and *Rhodospirilleae*. The general physiological characteristics of these bacteria are summarised in Table 1.2.

Table 1.2: Physiological types found amoung phototrophic sulphur oxidising bacteria (Kuenen *et al.*, 1985)

Family	Metabolic definition	Electron Donor
Chlorobiaccaea	Obligate phototroph. Facultative photoautotroph External S^o produced Anaerobe	S^{2-},$S_2O_3^{2-}$,S^o, H_2, organic acids
Chloroflexaceae	Facultative phototroph Facultative photoautotroph External S^o produced Thermophilic	S^{2-}, organic acids
Chromatiaceae	Facultative photoautotroph Internal S^o produced Facultative aerobe	S^{2-}, $S_2O_3^{2-}$,S^o, H_2, Organic acids
Rhodospirillae	Facultative photoautotroph External S^o produced	S^{2-}, $S_2O_3^{2-}$, organic acids

1.6.2.2 Colourless sulphur bacteria.

The colourless sulphur bacteria are a physiologically diverse bacteria that derive energy from the oxidation of sulphide. Oxygen (O_2), nitrate (NO_3), Mn(IV) or Fe(III) may be used as terminal electron acceptors for this process. These bacteria may be classified according to their physiological characteristics and range from obligate chemolithotrophs to chemoorganoheterotrophs.

Table 1.3 Physiological subtypes amongst the colourless sulphide oxidising bacteria (Kuenen and Robertson, 1992b). + indicates the ability of bacteria to grow under specified conditions, - indicates inability of bacteria to grow under the specified conditions.

Physiological Type	Carbon Source		Energy Source		Known Representative
	Inorganic	Organic	Inorganic	Organic	
Obligate Chemolithotroph	+	-	+	-	Many *Thiobacilli*, one *Sulfolobus*, *Hydrogenobacter*, all *Thiomicrospira*
Facultative Chemolithotroph	+	+	+	+	Some *Thiobacilli*, *Thiosphaera pantotropha*, *Paracoccus denitrificans*, certain *Beggiatoa* species
Chemolithoheterotroph	-	+	+	+	Few strains of *Thiobacilli*, Some *Beggiatoa*
Chemoorganoheterotroph	-	+	-	+	*Beggiatoa*, *Macromonas*, *Thiobacterium* and *Thiothrix*

1.6.3 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 $R\text{-}S_n\text{-}R$.

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).

1.6.4 Applications of microbial sulphide oxidation

Burgess *et al.,* (1961) reported on the possibility of producing elemental sulphur from sulphate enriched sewage sludge. They proposed that sulphide produced during the reduction of sulphate to sulphide could be subsequently oxidised to elemental sulphur.

Biological processes for the treatment of sulphide containing waters can be broadly divided into the following groups:

1) Processes in which nitrate (NO_3^{2-}) serves as the terminal electron acceptor for the oxidation of sulphide. *Thiobacillus denitrificans* is a bacterium often utilised in these processes (Sublette, 1989);

2) Processes utilising photosynthetic sulphide oxidising bacteria e.g. *Chlorobium limicola* (Cork., 1985);

3) Processes run under autotrophic conditions using Thiobacilli like bacteria (Buisman *et al.,* 1989);

4) Processes in which known chemoorganoheterotrophic bacteria e.g. *Pseudomonas* spp. are utilised under heterotrophic conditions (Chung *et al.*, 1996a).

1.6.4.1 Biological processes where nitrate is the terminal electron acceptor

Thiobacillus denitrificans has been utilised in a number of studies. It has been considered a good candidate for the treatment of sulphide containing gas streams due to its barotolerance and its relative tolerance of CS_2, COS and CH_3SCH_3, all common co-contaminants of natural gas (Sublette and Sylvester, 1987a). Pure cultures of *Thiobacillus denitrificans* are inhibited by high concentrations of sulphide and have been reported to be able to tolerate loading rates of 5.4mmol - 7.6mmol $H_2S.h^{-1}.g^{-1}$ biomass under anaerobic conditions (Cadenhead and Sublette, 1990). Under aerobic conditions *Thiobacillus denitrificans* is able to tolerate significantly higher loading rates of 15.1 - 20.9mmol $H_2S.h^{-1}g^{-1}$ biomass. The aerobic oxidation of sulphide by *Thiobacillus denitrificans* was found to consume significantly more caustic and produce less biomass per unit oxidation of H_2S compared to the corresponding anaerobic process (Sublette and Sylvester, 1987c).

The presence of heterotrophic bacteria in cultures of *Thiobacillus denitrificans* does not adversely affect oxidation of H_2S (Sublette and Sylvester, 1987b). Co-culture of *Thiobacillus denitrificans* with floc-forming heterotrophic bacteria has been investigated as a means of immobilising *Thiobacillus denitrificans* (Ongcharit *et al.*, 1989; Ongcharit *et al.*, 1990; Ongcharit *et al.*, 1991). This immobilised culture was later employed in a pilot scale $0.5m^3$ bubble column to treat sour gas. Immobilisation of autotrophic *Thiobacillus denitrificans* in this manner in a continuously stirred tank reactor (CSTR) had little or no effect on the stoichiometry and kinetics of sulphide oxidation. Although H_2S removal was constantly high, biomass yield per mole H_2S was considerably lower than values published for pure cultures of *Thiobacillus denitrificans*. The flocs were found to be stable over a period of four months and no organic supplementation was required to maintain the floc integrity. H_2S removal efficiency was found to decrease as the presence of mixed liquor suspended solid (an indication of floc disintegration) levels increased.

These cultures have also been used to successfully treat water containing 25mM ($825mg.L^{-1}$) sulphide (Lee and Sublette, 1993). Complete oxidation of sulphide to

sulphate was reported although small amounts of sulphur (3mM) were detected in the effluent stream as sulphide loading rate was increased. Once the loading rate stabilised, sulphur disappeared from the effluent stream. Simultaneous nitrate reduction and sulphide and acetate oxidation was reported on by Gommers *et al.,* (1988). Elemental sulphur was reported to form in this reactor when run under slightly aerobic conditions and when nitrate supply was limited.

In none of the processes reported here, where nitrate was utilised as a terminal electron acceptor for the oxidation of sulphide was elemental sulphur intended to be a major product of oxidation. Sulphate was the major intended product of these processes. Elemental sulphur was however observed as a product of sulphide oxidation under the electron donor (NO_3^{2-}) limitation conditions. Sulphur accumulation was accompanied by accumulation of nitrite (NO_2^-) or nitrous oxide (N_2O), both of which are products of the incomplete reduction of nitrate. (Sublette and Sylvester, 1987a; Gommers *et al.,* 1988; Lee and Sublette, 1993)

1.6.4.2 Biological sulphide oxidation utilising photosynthetic sulphide oxidising bacteria.

Photosynthetic oxidation of H_2S can be described by the following two equations (van Niel, 1931; Larsen, 1952)

$$2H_2S + CO_2 \rightarrow 2S^\circ + (CH_2O) + H_2O \quad (9)$$

$$H_2S + 2CO_2 + 2H_2O \rightarrow 2(CH_2O) + H_2SO_4 \quad (10)$$

These equations suggest that under conditions where sufficient light and CO_2 are available SO_4^{2-} is the major product of sulphide oxidation whereas under conditions of light and CO_2 limitation elemental sulphur is the major product.

A number of studies have been conducted on photosynthetic sulphide oxidising processes. The results of these investigations are summarised below in table 1.4.

Table 1.4 : Results of investigations on continuous sulphide removal using photosynthetic sulphide oxidising bacteria. Removal efficiency = (S^{2-}in –S^{2-} out)/S^{2-} in. Conversion efficiency = $S°_{out}/S^{2-}_{in}$ (Henshaw *et al.,* 1998)

Reference	Reactor Configuration	Volume (L)	S^{2-} loading rate ($mg.L^{-1}.h^{-1}$)	Removal efficiency	Conversion efficiency
Kobayushi *et al.*, 1983	Fixed film upflow, fixed film plug flow	8 0.1	0.59-1.27 102-125	81-92 100	0 8-12
Cork, 1984	Suspended growth, CSTR	0.8	62	100	
Cork *et al.*, 1985	Suspended growth, CSTR	0.8	109-174	100	93-95
Maka and Cork,1990	Suspended growth, CSTR	0.8	32-64	100	90-97
Kim *et al.*, 1990, Kim *et al.*, 1991	Suspended growth, CSTR	4	61	99.5	35
Kim *et al.*, 1992	Suspended growth, CSTR	4	64	100	63

Chlorobium limicola, a photosynthetic green sulphur bacterium has been investigated for its ability to oxidise sulphide present in a liquid stream to elemental sulphur (Henshaw *et al.,* 1998). These investigations concluded that the conversion of sulphide to elemental sulphur or sulphate is a function of the light radiated to the reactor and the sulphide feed rate.

The use of photosynthetic bacteria in a sulphide oxidising bioreactor may be disadvantageous for the following reasons:

1) Cultures need to be maintained strictly anaerobically;
2) Cultures require a constant source of radiant energy. This increases the energy costs of running such a process, and the provision of this radiant energy becomes increasingly difficult due to turbidity as a result of the build up of biomass and elemental sulphur in the culture.

1.6.4.3 Biological processes utilising chemoautotrophic sulphide oxidising bacteria

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 200mg.L^{-1}.h^{-1} (6.25mmol.L^{-1}.h^{-1}) and (Type B) that grew at higher loading rates. The sulphide-loading rate is defined as the amount of sulphide introduced to the system per unit time per unit volume of the reactor. Type A bacteria were found to have a significantly higher growth yield (expressed as dry S/mol S) than Type B bacteria. Type A bacteria had a high affinity for sulphide and were inhibited at sulphide concentrations exceeding 10mg.L^{-1} (0.31mmol) whereas Type B bacteria had a lower affinity for sulphide and were not inhibited by sulphide but rather by the presence of oxygen. 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 4m^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} (3.28mmol.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 75mg.L^{-1}.h^{-1} (2.33mmol.L^{-1}.h^{-1}) both sulphur and sulphate may be formed at oxygen concentrations below 0.1mg.L^{-1}. Furthermore under highly oxygen limited conditions oxygen/sulphide consumption ratios below 0.7mol.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} (17.6mmol HS^-.L^{-1}.h^{-1}) was reached. Under heterotrophic conditions (with acetate and propionate present) the system performance deteriorated significantly with increased sulphide accumulating due to the activity of sulphate reducing bacteria and the formation rate of the sulphur sludge declined significantly as more sulphur was found to be washing 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 –137mV (H_2 Reference electrode 30°C, pH 8).

1.6.4.4 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.

1.6.4.4.1 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 2000µm (Okabe *et al.*, 1998, Yu and Bishop, 1998). Biofilms in contact with an oxygenated aqueous bulk phase (1-2mg.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 160mg.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.*

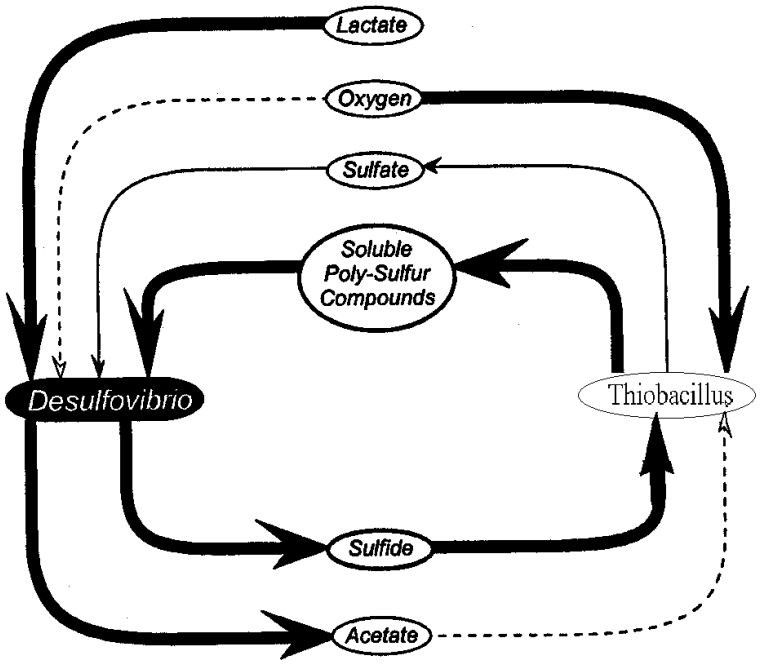


Figure 1.8: Interaction between sulphate reducing bacteria (*Desufovibrio*) and a colourless sulphide oxidising bacterium (*Thiobacillus).* The thickness of the arrows reflects the relative importance of the process in the microbial interaction (from van den Ende *et al.,* 1997)

1.7 Sulphide oxidation in organic rich aqueous environments

A biological sulphate reducing process to treat AMD will require the use of a waste complex carbon source due to the prohibitive costs of using a refined carbon source to treat large volumes of AMD. The use of a complex carbon source is likely to result in a high sulphide organic rich effluent stream. Due to the extremely toxic nature of sulphide a process to remove sulphide from the liquid stream will be required.

Removal of the sulphide by oxidation to elemental sulphur is the most preferable option since this reduces the total sulphur pollution in the water and the sulphur may be recovered and either sold as a value-added product or used in processes such as bio-leaching (Tichy *et al.,* 1994).

As already noted, a number of physico-chemical methods have been developed to convert sulphide to elemental sulphur. These processes are often energy intensive and require strict process control, require the addition of potentially polluting chemicals and often produce noxious secondary sludges that require special disposal. Biological sulphide oxidation, which was discussed in the previous section, presents a viable alternative to these physicochemical processes. Biological sulphide oxidation occurs at ambient temperatures and pressures. Biological processes utilising both phototrophic and colourless sulphide oxidising bacteria have been developed. Processes utilising phototrophic sulphide oxidising bacteria have the disadvantage of requiring a constant radiant energy source that is difficult to supply under turbid culture conditions. Colourless sulphur bacteria have been used in biological sulphide oxidation processes.

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 have historically needed 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 sulphide 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 this sulphur 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 over the last few years 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. In addition to this the Environmental Biotechnology Research Group at Rhodes University has been a key participant in a Department of Arts, Science, Culture and Technology (DACST) Innovation Fund project in asssociation with Pulles,Howard and de Lange to develop passive systems for the treatment of contaminated mine and

industrial effluents. The investigations on biological sulphide oxidation undertaken here focussed on complementing the body of knowledge on integrated biological water treatment systems and in the development of a passive system for the treatment AMD.

1.8 Hypothesis

Elemental sulphur is produced as a product of sulphide oxidation in a very narrow thermodynamic window. The organics present in an aqueous sulphidic environment act to buffer redox changes and to poise the redox conditions such that sulphur is the predominant product of sulphide oxidation. This redox poising capacity will reduce the stringency of control required to obtain sulphur as the predominant product of microbial sulphide oxidation.

1.9 Objectives of the study

1) To investigate chemical parameters determining biological sulphide oxidation under heterotrophic conditions;

2) To evaluate reactor configurations for the biotechnological oxidation of sulphide to elemental sulphur in organic-rich aqueous environments.

3) To establish optimal sulphide and oxygen loading conditions for sulphur production in organic-rich aqueous environments within sulphide oxidising bioreactors.

Chapter 2

Materials and Methods

2.1 Materials

Chemicals used during all investigations were of analytical grade. Sulphide solutions were made by dissolving $Na_2S.9H_2O$ (Merck) in distilled water. The pH of this solution was adjusted using 32% H_3PO_4. The sewage used in all experiments was the supernatant from the primary settling tanks at the Grahamstown Municipal Sewage Works.

2.2 Analytical Methods

2.2.1 Sulphide

1mL of sample was added to 1mL 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 (1975)

2.2.2 Sulphate

Sulphate concentrations were determined by ion chromatography (IC) using a 15mm x 4.1mm Hamilton PRP-X100 column, 4mM p-hydroxybenzoic acid, 2.5% methanol, pH 8.5 as the mobile phase,Waters 510 pump flow rate 1mL.min^{-1} and detection by Waters 430 conductivity detector. Prior to ion exchange chromatography, samples filtered through 0.45μm nylon filters and passed through a 25mg C_{18} Isolute® solid phase extraction column to remove contaminating organics.

2.2.3 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 150mm x 4.6mm C18 column, 95:5 Methanol: H_2O mobile phase at a flow rate of 2mL.min^{-1}. 1mL of sample was centrifuged at 13200rpm for 10 minutes and the resulting pellet was

resuspended in 1mL of HPLC grade acetone, either filtered through a nylon 0.45µm filter or recentrifuged before being run on the HPLC system.

2.2.4 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.

2.2.5 pH

pH was determined using a Cyberscan 2000 pH meter.

2.3 Microscopy

2.3.1 Transmission Electron Microscopy

Samples for Transmission Electron Microscopy (TEM) were prepared as described by Cross, (1979). 2mL 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 Eppendorf tube for TEM according to the procedures described below.

Following primary fixation in glutaradehyde, the samples were washed in 0.1M 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 (30minutes), followed by Reynold's

lead citrate (5 minutes). For TEM, ultrathin sections were examined using a JEOL JEM 100 CXII transmission electron microscope.

2.3.2 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 3mm x 3mm 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 0.1M phosphate buffer) overnight. The fixative was decanted off washed twice for fifteen minutes with cold 0.1M 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 dessicator. Samples were observed in JEOL JEBM V120 scanniing electron microscope.

2.3.3 Light Microscopy

A small amount of reactor effluent, reactor influent, or biofilm present in the reactor would be transferred to a microscope slide using a flame-sterilised loop. The sample was spread with the loop and slowly heat fixed over a Bunsen burner flame. Samples to be stained were then immersed in methylene blue for 30s to one minute, washed with distilled water and allowed to air dry. Dry samples were observed using a Nikon phase contrast light microscope.

Chapter 3

Sulphide Oxidation in a Heterotrophic Environment: Flask Studies

3.1 Introduction

Biological processes employing sulphate reducing bacteria have been proposed as cost effective means for treating acid mine drainage containing high levels of sulphate and heavy metals. The biological oxidation to elemntal sulphur of sulphides produced in the sulphate reduction process has been proposed as a mechanism for the final removal of sulphur from the aqueous system.

Very little work has been reported on the biological oxidation of sulphide to produce elemental sulphur in organic-rich environments. The effects of glucose and volatile fatty acids (VFA), acetate and propionate have been reported not to have any marked effect on biological sulphide oxidation in a reactor employing *Thiobacilli* under predominantly autotrophic conditions with the bacteria still able to carry out efficient sulphide oxidation (Buisman *et al.*, 1990b). The presence of these compounds did encourage the growth of the filamentous sulphide oxidising bacteria *Thiothrix* (Buisman *et al.*, 1990b). Janssen *et al.*, (1997) found that the presence of these VFA did have a detrimental effect on the running of an autotrophic biological sulphide removal process. The presence of these compounds resulted in poor settling properties of the produced sulphur sludge. The known heterotroph *Pseudomonas putida* has reportedly been utilised in a process to remove sulphide from sulphide containing gas (Chung *et al.*, 1996a; Chung *et al*., 1996b; Huang *et al.*, 1997), and although sulphur was reported to be a product of this sulphide oxidation, little was said about the optimal conditions for sulphur production under heterotrophic conditions.

Development of a biotechnological process that produces elemental sulphur as the primary product of sulphide oxidation is dependent on whether a sulphide oxidising population may be selected for under strongly mixotrophic conditions (high-organics and high-sulphide). The determination of key chemical parameters of chemical (abiotic) and

biological (biotic) sulphide oxidation in a high organics environment as well as the effects that the organics present would have on these processes are fundamental aspects required to be known in the development of such a process.

Prior to commencing reactor studies a series of flask studies were undertaken. A fixed-film trickle filter reactor was identified as a possible reactor design, representing a reactor configuration that would potentially fulfil the objective of being integrated as a stand-alone module into a passive system for the treatment of AMD. Knowledge gained from comparing oxidation of sulphide in flasks inoculated with sterile and non-sterile domestic sewage respectively would be used to design and assess the performance of a fixed-film trickle filter reactor.

3.2 Materials and Methods

3.2.1 Flask experiments

Triplicate flasks of sterile and non-sterile sewage were prepared and the results reported reflect the mean of analytical results for each of the flasks at each sampling time. Sterile sewage was prepared by autoclaving 2L of sewage at 121°C for 20 minutes. 150mL of sterile and non-sterile domestic sewage were added to six sterile 500mL conical flasks. The flasks were stoppered with cotton wool and covered with aluminium foil. The flasks were allowed to stand overnight and 150mL of 300mg.L^{-1} HS^- solution, (pH 8.5) was added to each of the flasks. The pH of the sulphide solution was adjusted to 8.5 using 32% H_3PO_4. Sulphide, sulphate, elemental sulphur, pH and redox were monitored according to methods explained previously in Chapter 2. Experiment 1 and experiment 2 differed only in the sampling regime employed. During experiment 1 sampling was carried out at 0, 2, 4, 6, 8, 10, 12 and 24 hours respectively, whereas during experiment 2 sampling was carried out at 0, 4, 8, 12, 16, 20 and 24 hours respectively

3.2.2 Analytical methods

Sulphide, sulphur, sulphate, pH and redox were determined according to methods previously described.

3.2.3 Statistical Analysis

Analysis of variance (ANOVA) and the students t-test were performed on data obtained from experiments 1 and 2 using Microsoft Excel data analysis tool.

3.3 Results

Comparisons of sulphide, sulphate and sulphur concentrations, pH and redox for experiment 1 are reported in Figures 3.1a, to 3.5a and the same comparisons for experiment 2 are reported in Figures 3.1b to 3.5b.

3.3.1 Sulphide Removal

The sulphide removal profiles differed between non-sterile and sterile flasks in both experiment 1 and experiment 2 (see Figures 3.1a and 3.1b). Profiles for non-sterile flasks in experiments 1 and 2 were comparable and the same applied to the sterile flasks. In both experiments the most profound differences were found to occur between 12 and 24 hours of the experiment and may be observed to occur from as early as 8 hours in experiment 2. The presence of a bacterial population in the non-sterile flasks appears to have increased the overall sulphide removal with all sulphide being removed from the non-sterile flasks with sulphide still present in the sterile flasks after 24 hours. During experiment 2 more sulphide was removed from the non-sterile flasks after 16 hours with significant sulphide still being present in the sterile flasks at this time (n=3, P = 0.00019). Significantly more sulphide was removed from the non-sterile flasks compared to the sterile flasks in both experiments 1 and 2 (Exp. 1, n=3, P = $7x10^{-5}$; Exp. 2, n=3, P=0.0002)

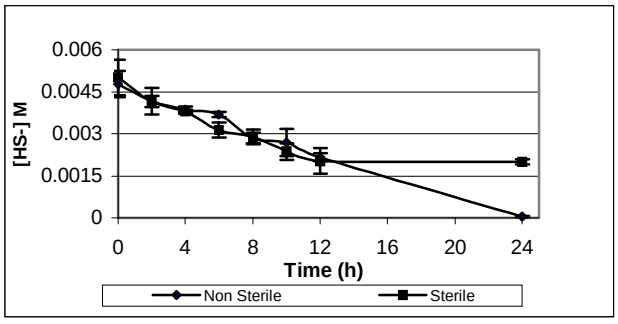


Fig. 3.1a

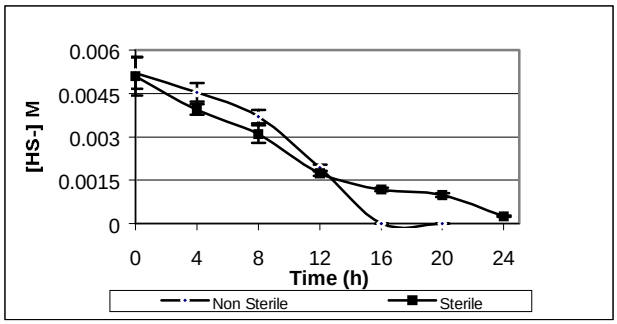


Fig. 3.1b

Figure 3.1 (a) and (b). Sulphide concentration profiles over a 24 hour period during experiments 1 and 2 respectively

3.3.2 Sulphate Production

The sulphate removal profiles were found to differ between non-sterile and sterile flasks in both experiment 1 and experiment 2 (see Figures 3.2a and 3.2b). Profiles for non-

sterile flasks in experiments 1 and 2 were comparable and the same applied to the sterile flasks. During experiments 1 and 2 sulphate production was greater in the sterile flasks during the first 10 hours of the experiment, with significantly more sulphate present in sterile flasks compared to non-sterile flasks at this point (Exp. 1, n=3, P = 0.00025; Exp. 2, n = 3, P = 0.0025). After 12 hours sulphate production increased in the non-sterile flasks with significantly more sulphate being present in the non-sterile flasks compared to the sterile flasks after 24 hours (Exp. 1, n = 3, P = 0.00025; Exp. 2, n=3, P=0.000457).

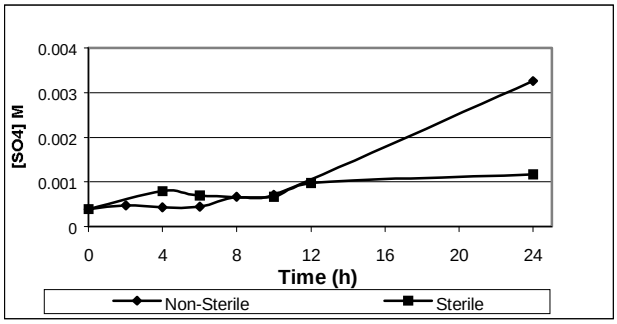


Fig 3.2a

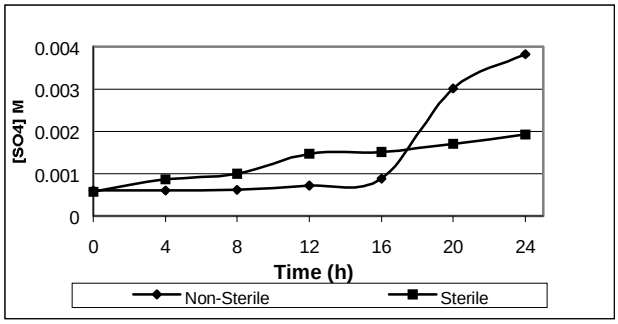


Fig 3.2b

Figure 3.2 (a) and (b). Sulphate concentration profiles over a 24 hour period obtained during experiment 1 (a) 2 (b) (ANOVA df=6, F>F-crit, P=1.14 x 10^{-21}) respectively

3.3.3 Sulphur production

Sulphur was only detected in the non-sterile flasks during experiments 1 and 2 (see Figures 3.3a and 3.3b) although the sulphur concentration profile during experiments seemed to differ during the two experiments with sulphur increasing steadily between 10 and 24 hours in experiment 1 and sulphur appearing between 16 and 20 hours before disappearing again at 24 hours in experiment 2.

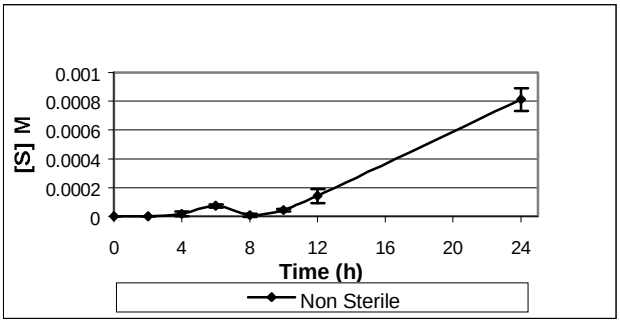


Fig.3.3a

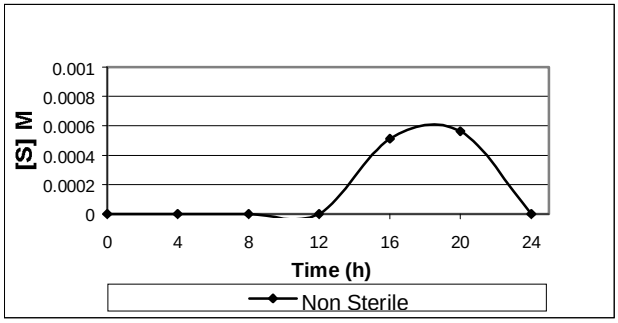


Fig3.3b

Figure 3.3 (a) and (b). Sulphur concentration profiles over a 24-hour period during experiment 1 (a) and 2 (b)

3.3.3 pH Profiles

Overall pH profiles differed between non-sterile and sterile flasks during both experiments 1 and 2 (see Figures 3.4a and 3.4b). Profiles for non-sterile flasks in experiments 1 and 2 were comparable and the same applied to the sterile flasks. Significant differences between the profiles only became evident after 8 hours. (Experiment 1: ANOVA on 0 – 8 hours data yields F<F-crit, P = 0.19 and ANOVA on 8-24 hours data yields F>>F-crit, P = 3.17×10^{-10}) with a similar trend being observed in experiment 2. The pH of the non-sterile flasks ended up significantly lower than that of the sterile flasks in both experiment 1 and experiment 2 (Exp. 1, n=3, P = 0.00288; Exp. 2, n=3, P=0.00196)

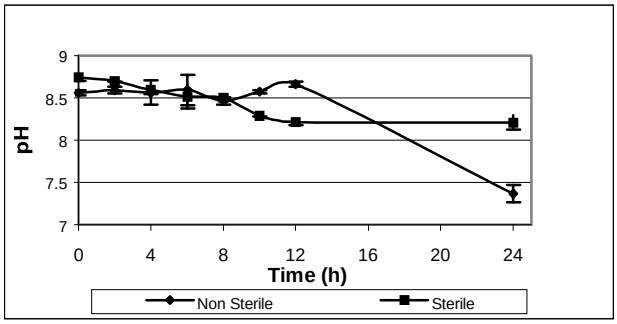


Fig. 3.4a

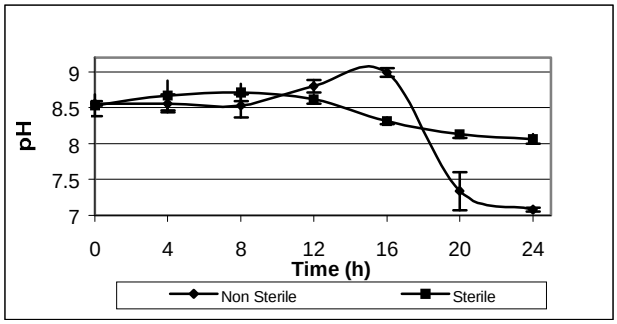
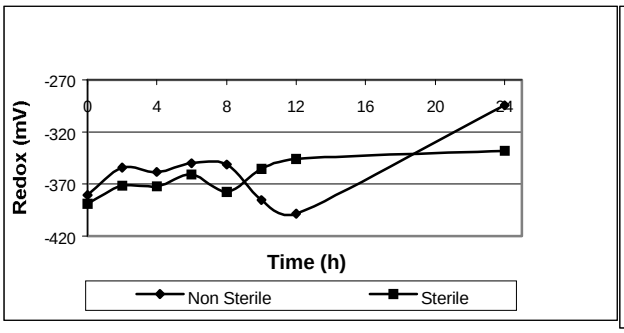


Fig. 3.4b

Figure 3.4 (a) and (b). pH profile over a 24-hour period during experiments 1(a) and 2 (b)

3.3.4 Redox Profiles

The measured redox profiles for non-sterile and sterile flasks were distinctive during experiment 1 and experiment 2 (see Figures 3.5a and 3.5b) with the measured redox in the non-sterile flasks showing a negative trend between 8 and 12 resulting in the redox being significantly lower at 12 hours in the non-sterile flasks compared to that in the sterile flasks (Exp. 2, n=3, P=0.00095). The drop in the measured redox in the non-sterile flasks was followed by an increase in the measured redox at 24 hours where measured redox was significantly higher in the non-sterile flasks than the sterile flasks at 24 hours (Exp. 1, n=3, P = 0.00288; Exp. 2, n=3, P=0.00196)



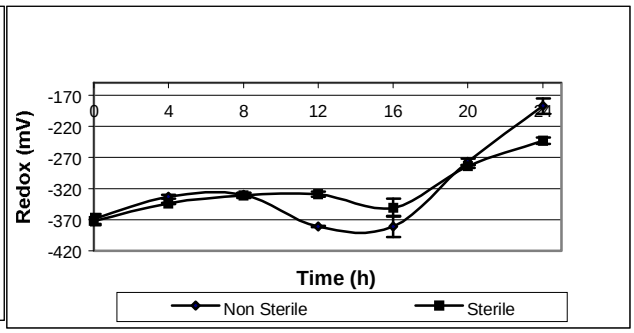


Fig 3.5a **Fig 3.5b**

Figure 3.5 (a) and (b) Redox profiles over a 24-hour period obtained during experiments 1 (a) and 2 (b). (ANOVA, df= 6, F>F-crit, P=1.17x 10^{-10})

3.4 Discussion

The results of the flask experiment reported here show that the bacterial populations normally present in domestic sewage apparently act to affect the oxidation of sulphide in an organic environment. The presence of normal bacterial population in domestic sewage affects sulphide oxidation in the following way:

The rate of sulphide disappearance was significantly faster in the flasks containing non-sterile domestic sewage. This increased rate could possibly be ascribed to the activity of a sulphide oxidising bacterial population present in sewage. Sulphide was undetectable in the flasks containing non-sterile sewage after 16 hours during experiment 1 and no sulphide being detectable after 24 hours during experiment 2. Not all sulphide was oxidised during experiment 1 in the sterile flasks and complete disappearance of sulphide only occurred at 24 hours in the sterile flasks during experiment 2. A possible explanation for the complete removal of sulphide in the non-sterile but not the sterile flasks in experiment 1 is that due to the sampling protocol used during experiment 1(during which no sampling occurred between 12 and 24 hours) all oxygen present in the head space was utilised between 12 and 24 hours of the experiment. This may have resulted in the cessation of chemical oxidation in the sterile flasks. In the non-sterile flasks it is possible that the bacterial population that developed was able to utilise an electron donor other than oxygen (e.g. nitrate) to maintain the oxidation of sulphide in the absence of oxygen.

Complete disappearance of sulphide from the sterile flasks during experiment 2 may be explained by the fact that oxygen was present in the headspace throughout the experiment due to introduction during sampling. The observation that significant oxygen ingress may have occurred during sampling also suggests that a portion of the sulphide may have been lost in both sterile and non-sterile flasks to the headspace, although every effort was made not to disturb the headspace while sampling.

The presence of the bacterial population present in sewage resulted in the production of elemental sulphur as a product of sulphide oxidation. No sulphur was detected in the sterile flasks in experiment 1 or experiment 2 at any point during the 24-hour study. Sulphur was detected in non-sterile flasks during both experiments. The sulphur concentration profiles obtained in the non-sterile flasks for experiments 1 and 2 were different and could be explained by the different sampling regimes employed during experiments 1 and 2.

Sulphate profiles for the sterile flasks during experiments 1 and 2 showed a slow but steady increase during the 24 hour period, mostly attributable to chemical oxidation of sulphide by oxygen, whereas sulphate profiles for non-sterile flasks are characterised by slow increases initially, due to predominantly chemical oxidation followed by dramatic increases at later stages of the experiment as a sulphide oxidising population developed. Of particular importance and relevance to the development of a biotechnological process is the observation that sulphur appears in the non-sterile flasks before the appearance of sulphate during both experiments. This suggests that sulphur may be produced preferentially to sulphate under heterotrophic conditions. The rate of formation of sulphate in the first 10 hours of the experiment was slower in the non-sterile flasks compared to the sterile flasks suggesting that background heterotrophic metabolism decreases the amount of oxygen present for chemical oxidation of sulphide, thereby favouring biologically mediated oxidation of sulphide to sulphur. The background heterotrophic metabolism decreases the oxygen concentration in the liquid thereby decreasing the rate of chemical sulphide oxidation. The rate of chemical oxidation increased with increasing oxygen concentration as determined by equations (7) and (8).

Sulphur was present at 24 hours in the non-sterile flasks during experiment 1, but was observed to appear and disappear in non-sterile flasks during experiment 2. Sampling at 12, 16 and 20 hours during experiment 2 allowed sufficient oxygen to enter the flasks and allowed for oxidation of accumulated sulphur. During experiment 1 however, oxygen limited conditions were maintained between 12 and 24 hours, preventing further oxidation of sulphur resulting in sulphur remaining detectable at 24 hours. The lag phase period between sulphide disappearance was probably due to chemical oxidation predominating during the initial 8 hours during which time a sulphide oxidising bacterial population developed. Minimum doubling times of 2.85 hours have been reported for autotrophic *Thiobacilli* (Stefess 1993). This suggests that a new sulphide oxidising population did not develop within the flasks but rather that the population already present was able to switch to a sulphide oxidising physiological state. In addition to this, the initial sulphide concentrations may have been too high for bacterial activity and the reduction of sulphide concentration to a suitable level (at between 8 and 12 hours) due to chemical oxidation may have enabled the population present to become metabolically active.

The pH profiles obtained can be explained using equations (5) and (6) as well as

$$2S^{\circ}+3O_2+2H_2O \rightarrow 4H^{+} + 2SO_4^{2-} \qquad (11)$$

The appearance of sulphur in the non-sterile flasks was preceded at 12 hours in both experiments 1 and 2, by an increase in the measured pH in the flasks (see Figures 3.4 and 3.9). The increase in the pH was due to the microbial production of elemental sulphur according to equation (6) (Kuenen 1975). The increase in pH does not correspond directly with detection of sulphur and is possibly due to at least part of the sulphur being involved in reactions with sulphide to form polysulphides according to the following general equation:

$$HS^{-} + (x-1)\,S^{\circ} \rightarrow S_x^{2-} + H^{+}$$
$$x = 2\text{-}5 \text{ (pH dependent)} \qquad (12)$$

This explanation is further supported by the appearance of a yellow-green colour in the solutions in the non-sterile flasks, coinciding with the increase in pH. This

yellow-green colour is characteristic of the presence of polysulphides (Steudel, 2000). A decrease in the pH coincided with sulphate appearance in the non-sterile flasks, the decrease in pH being attributable to overall equation (6), which yields H^+ ions. However in the case of the non-sterile flasks (in experiment 2 in particular) equation (11), which is also a H^+ generating reaction, is likely to represent the mechanistic explanation for the pH decrease because sulphide is not present in the non-sterile flasks when the pH decrease and sulphate increase occurred. This lends further evidence to the assertion that sulphur formation by bacteria is a faster reaction than sulphate formation, a mechanism that allows bacteria to remove harmful sulphide at higher rates (Buisman *et al*., 1991a).

The redox profiles of the sterile and the non-sterile flasks differed significantly from one another (see Figures 3.5 and 3.10). Redox has been used as a parameter for the control of biotechnological sulphur producing processes and it has been shown that measured redox values, affected by both the sulphide concentration and the pH of the solution, needs to be taken into account when interpreting measured redox values. Janssen *et al*., (1998) reported that a linear relationship exists between the mV reading of a platinised redox electrode at a constant pH and sulphide concentration. At pH 8.1 in a buffered phosphate solution this relationship was found to be 35mV/p(HS^-). This implies that for a 0.005M decrease in sulphide concentration ($\Delta p(HS^-) = -2.3$), at pH 8.1, an 81 mV increase in the measured redox could be expected. Furthermore they reported that at a low sulphide concentration (7.8mg.L^{-1}) a relationship of -14.4 mV/pH existed for a polished redox electrode. Although these values cannot be applied directly to the investigations here the following trends should be noted: Decreasing sulphide concentrations result in increased measured redox values and increasing pH results in a decrease of the measured redox value. Changes in measured redox need to be interpreted in terms of the rates of the various acidity generating/consuming processes as well as the rates of change of the redox affecting components of which sulphide is the most important species.

Bearing this in mind the measured redox, may be interpreted as follows: The redox increased slowly during the first 8 hours in both sterile and non-sterile flasks during experiment 1 and 2 and was due to the relatively slow disappearance of sulphide, predominantly due to chemical sulphide oxidation by oxygen. The redox then

decreased in the non-sterile flasks as a result of the increasing pH and possibly due to the interaction of polysulphides with the probe surface. The decrease in the measured redox in the non-sterile flasks was followed by a dramatic increase in pH as all sulphide disappeared and the pH falls as a result of sulphate formation. The redox measurement attained in the sterile flasks was significantly lower than that for the non-sterile flasks and is attributable to the fact that the pH did not drop as low in the sterile flasks as it did in the non-sterile flasks. In both experiment 1 and experiment 2 an amount of residual sulphide is present in the sterile flasks. The slower changes in the measured redox in the sterile flasks were due to the lower overall oxidation that occured in the sterile flasks.

In an attempt to determine how much of the initial sulphide and sulphate that were present could be accounted for, a mass balance calculation was done on the measured sulphur species at the end of the 24-hour period. The percentage sulphur species recovery was calculated as follows:

$$\{([HS^-]_{final} + [SO_4^{2-}]_{final} + [S^\circ]_{final})/([HS^-]_{initial} + [SO_4^{2-}]_{initial}) \} \times 100$$

All concentrations are in molar units.

The percentages sulphur species recovery was 61% and 25.4% for sterile flasks in experiment 1 and 2 respectively, and 80.5 and 61% for non-sterile flasks in experiments 1 and 2 respectively. Greater losses were encountered during experiment 2 during which the flasks were disturbed every four hours between 12 and 24 hours. The larger losses may be explained by headspace losses during sampling. Future experiments on a similar system could possibly be carried out in closed containers with a sampling system developed that avoided such disturbances.

It has been proposed that bacteria increase the rate of sulphide oxidation by increasing the rate-limiting step of initial polysulphide formation (See Figure 1.2). The results obtained during these flask studies would tend to support this assertion, with the interaction of sulphide and elemental sulphur to form polysulphides playing a key role in biological sulphide oxidation.

3.5 Conclusions

1) A bacterial sulphide oxidising population present in domestic sewage became metabolically active in the presence of both organics and sulphide;

2) The presence of this bacterial population resulted in a larger sulphide removal potential and faster sulphide removal rate compared to flasks in which only abiotic sulphide oxidation occurred;

3) Elemental sulphur was an intermediate of sulphide oxidation where this bacterial population was present whereas under abiotic conditions sulphur was not detectable as a product or intermediate of sulphide oxidation;

4) Elemental sulphur presence preceded sulphate appearance, lending support to the assertion that the rate of bacterial sulphur formation is faster than its oxidation to sulphate;

5) The presence of the organics inhibited abiotic sulphide oxidation in a non-sterile environment, due probably to background heterotrophic metabolism decreasing the overall oxygen concentration;

6) Maintenance of an oxygen limited environment encouraged sulphur production whereas the presence of excess oxygen allowed for the further oxidation of the sulphur to sulphate;

7) A mixed bacterial population capable of oxidising sulphide may use electron donors other than oxygen should oxygen be completely depleted.

8) The measured redox potential in an aqueous sulphidic environment is a cumulative indication of changes in predominantly sulphide concentration but also in pH. An inverse relationship exists between sulphide concentration and the measured redox and proportional relationship exists between the measured redox and pH.

The conclusions from these flask studies have the following implications for the design and operation of a sulphide oxidising bioreactor producing elemental sulphur:

1) The reactor will have to be run under oxygen limited conditions to prevent complete oxidation of sulphide to sulphate, and the oxidation of elemental sulphur to sulphate.

2) The reactor should be designed such that a decreasing oxygen concentration gradient exists down the length of the reactor to prevent further oxidation of sulphur produced at the top of the reactor being further oxidised down the length of the reactor.

3) The presence of organics will serve to decrease the overall oxygen concentration within the reactor due to background heterotrophic metabolism; this will further suppress the contribution of chemical oxidation and possibly also decrease the stringency with which oxygen supply to the reactor needs to be controlled. This would be an important consideration in large-scale bioreactor design.

Three reactor configurations were evaluated including a Fixed-Film Trickle Reactor, a Submerged Fixed-Film Trickle Reactor and a Silicone Tubular Reactor.

Chapter 4

Sulphide Oxidation in a Fixed-Film Trickle Filter Reactor

4.1 Introduction

The insights obtained during flask studies on sulphide oxidation were utilised to conceptualise reactor configurations that could be investigated at laboratory scale for biological sulphide oxidation and sulphur production under heterotrophic conditions. The results from the flasks studies reported in chapter 3 suggested that a reactor in which a sulphide oxidising bacterial population had been selected needed to be run under oxygen limited conditions, and that the presence of the organics would decrease the contribution of chemical oxidation to the overall process due to lowering of the overall oxygen concentration by background heterotrophic metabolism.

Trickle filter reactors are the most widely utilised fixed film bioreactors and have been widely utilised to treat domestic wastewater and have been applied in these processes in full-scale applications since the late 19th century (Grady 1983). Trickle filters have also found applications in more specialised applications that include removal of organic pollutants from groundwater (Langwaldt and Pubhakka, 2000), nitrification of aquaculture recirculation water (Greiner and Timmons, 1998) and cyanide removal from gold milling effluent (Evangelho *et al*., 2001). Trickle filter-type reactors utilised for traditional waste-water applications have the following potential benefits in terms of biological reactor configuration:

1) Rapid initial start-up;
2) Ability to withstand shock loadings;
3) Rapid restart after long shutdowns;
4) Elimination of mechanical mixing/ biomass recycle;
5) Good stability and efficiency;
6) Low operational costs.

These potential benefits suggest that if a Fixed Film Trickle Filter type system could be developed for biological sulphide oxidation it could meet the criteria of a free-standing module in a passive system for the treatment of AMD.

As a reactor configuration for biological oxidation of sulphide to predominantly elemental sulphur, a trickle filter system was developed and tested in which the oxygen partial pressure within the reactor was maintained at a low levels within the reactor. No air was introduced and excessive ingress of air was prevented by nitrogen sparging into the reactor. The low oxygen concentrations created by the nitrogen sparging would tend to inhibit chemical oxidation, allowing for biological oxidation to dominate, resulting in significant oxidation of sulphide to elemental sulphur. Although nitrogen sparging would not be feasible on a large scale, where oxygen ingress could be controlled by design this method was chosen as the simplest way in which to control oxygen ingress in a laboratory scale unit.

It was envisaged that once a microbial population had developed steady state conditions could be attained during which sufficient oxygen would be supplied to the population so that sulphur would be the principal product of sulphide oxidation. Also that a significant portion of this would be present in the reactor effluent from where it could be recovered. The presence of background heterotrophic metabolism was predicted to decrease the overall oxygen availability, thereby poising the dissolved oxygen concentration and the redox in the reactor, encouraging sulphur formation within the reactor. Elemental sulphur was envisaged to be a component of the effluent stream where it could be collected by settling.

4.2 Materials and Methods

4.2.1 Reactor Operation

A Fixed Film Trickle Filter Reactor (Figure 4.1) was operated over a period of 45 days, initially to allow a bacterial population to develop, and then over a 15 day period of steady state operation to determine if elemental sulphur could be produced as the collectable product of biological sulphide oxidation and under what conditions this could be achieved.

4.2.1.1 Liquid delivery

Sulphide (300mg.L^{-1}) pH 8 – 8.5 (pH adjusted using H_3PO_4) primary settled sewage supernatant were stored separately in two 5L round-bottomed flasks. The four streams of the two solutions were pumped at 4 mL.min^{-1} by two Watson Marlow 504S peristaltic pumps into a plastic T-piece in which mixing would occur. This resulted in the sulphide sewage feed being supplied to the reactor at 8 mL.min^{-1}. From the T-piece the solution (150mg.L^{-1} sulphide pH 8-8.5 and ½ diluted sewage) was pumped via four tubes to the trickle filter. This system of four separate inlets was employed to increase the surface area that the sulphidic solution came into contact with. The tubing used throughout the reactor was Tygon® R-3603 1.6mm (ID) oxygen impermeable tubing. Tubing for the peristaltic pump heads was Watson Marlow Marprene tubing also selected for its oxygen impermeability. The flow rate for the duration of the experiment was 8 mL.min^{-1}.

4.2.1.2 Fixed Film Trickle Filter Reactor configuration

A laboratory- scale Fixed-Film Trickle Filter Reactor was constructed and consisted of a tank 600mm (h) x 150mm (l) x 150mm (b) giving a total void volume of 13.5L see (Figure 4.1). The reactor was constructed from 6mm thick glass. The reactor was filled to within 50mm of the top with 30m of 10mm lengths of 12mm ID PVC tubing. This tubing served as an immobilisation medium for the sulphide oxidising biofilm. Effluent was prevented from pooling at the bottom of the reactor by a sloped floor and was actively pumped out, via a flow-through cell into which a redox probe could be placed, by a Watson Marlow 505S peristaltic pump. The trickle filter had four sets of three ports drilled at various levels on the reactor. The holes were filled with rubber septa and

allowed for the introduction of gases (in this case nitrogen into the reactor). Nitrogen sparging was employed in order to decrease the oxygen partial pressure within the reactor. Nitrogen was introduced to the reactor through one of the septa near the bottom. The nitrogen flow rate was controlled using a precision gas flow controller and nitrogen flow rate was accurately determined using a film-flow burette.

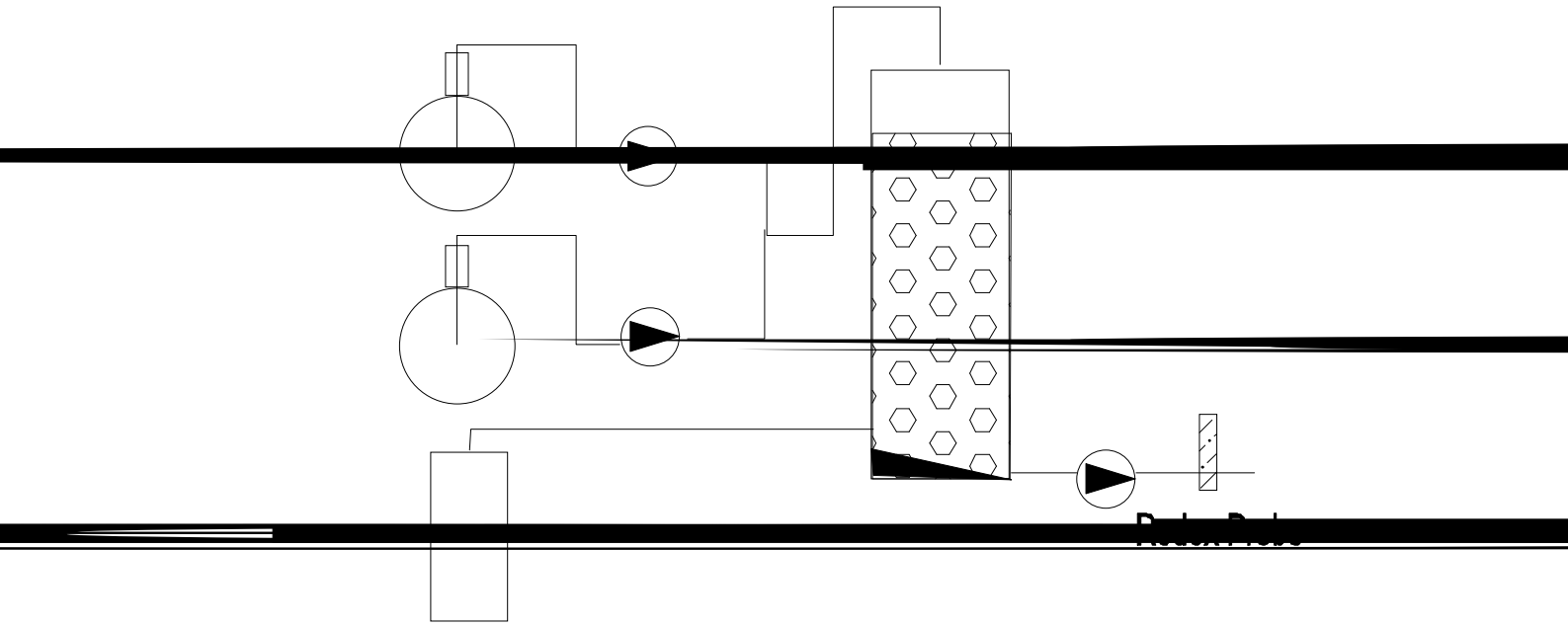


Figure 4.1: Diagram of trickle filter reactor supplied with nitrogen to control oxygen tension within the reactor

4.2.2 Analytical Methods

Sulphide, sulphate and sulphur concentrations, pH and redox levels were determined by methods described previously in chapter 2.

4.2.3 Electron Microscopy

PVC tubing on which biofilm growth was evident was removed from the reactor, small sections were carefully cut using a clean sterile blade and prepared for scanning electron microscopy as previously described in Chapter 2.

4.3 Results

The concentration of sulphur species in reactor effluent and reactor influent are reported in Figure 4.2. The reactor was run for a total of 45 days with the results shown represent 15 days of running once a bacterial population had developed within the reactor. The influent sulphide concentration was maintained between 0.005 and 0.006 M (165-200mg.L^{-1}). During the first three days, when the nitrogen flow rate to the reactor was low (10.8mL.min^{-1}), sulphate was the predominant oxidised sulphur compound in the reactor effluent. Sulphide was present in the effluent at low concentrations of 0.39mM

At no stage during this investigation were large concentrations of elemental sulphur detected in the reactor effluent with the highest concentration being detected on Day 3 (0.39mM), almost an order of magnitude lower than the sulphate present in the effluent. Increasing the nitrogen flow rate to the reactor on day 4 from 10.9mL.min^{-1} to 109.5mL.min^{-1} decreased the sulphate concentration present in the effluent, although this increased again to around 3mM over the following 2 days (days 5 and 6). Over the following 6 days the nitrogen flow rate was maintained at a constant level of between 110 and 115 mL.min^{-1}. During this time the sulphate concentration showed a downward trend and sulphide began to appear in the effluent. Further increases in the nitrogen flow rate on days 11-15 resulted in decreased sulphate and increased sulphide concentration in the effluent. Significantly more sulphide was present in the effluent on days 13, 14 and 15 compared to days 1, 2 and 3 ($n=3$, $P = 0.03$) the inverse was true for sulphate with sulphate concentrations being significantly lower towards the end of the investigation ($n=3$, $P = 0.018$).

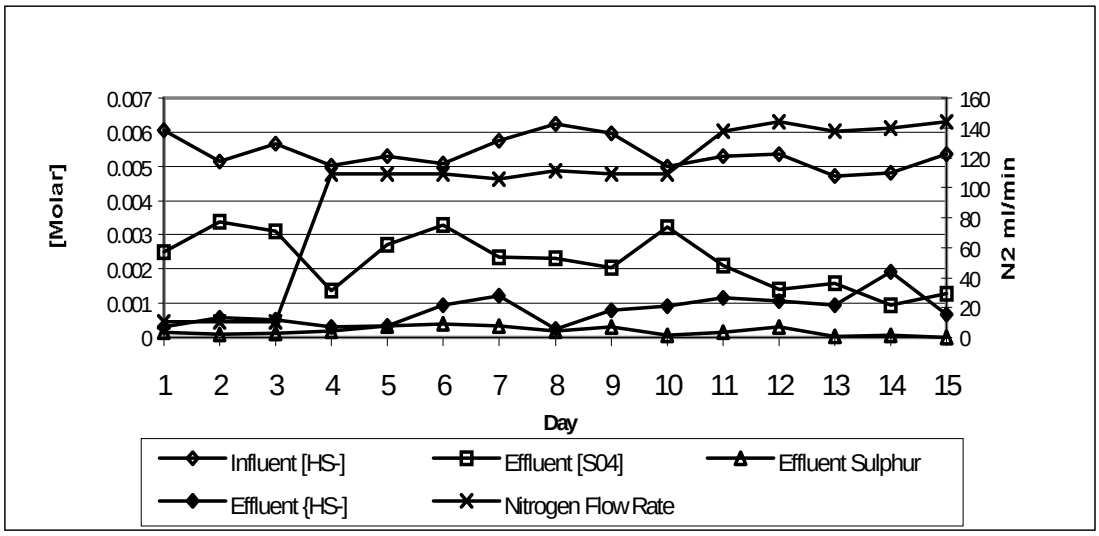


Figure 4.2 Sulphur species profile of trickle filter reactor over 15 days as nitrogen flow rate was increased (oxygen partial pressure decreased). (Effluent sulphate = $[SO_4^{2-}]_{effluent} - [SO_4^{2-}]_{influent}$)

The pH and redox profiles are reported in Figure 4.3. The influent pH to the reactor was maintained between 8.3 and 8.5 for the duration of the investigation. Increasing the nitrogen flow rate to the reactor had the general effect of increasing the effluent pH from below 6.5 at a nitrogen flow rate of 10.5 mL.min^{-1} on day 2, to above 8.5 on days 14 and 15 at nitrogen flow rates of 138 and 144 mL.min^{-1} respectively. The measured redox potential of the reactor effluent was observed to drop throughout the investigation from –360 to –470mV between days 1 and 15 of the experiment.

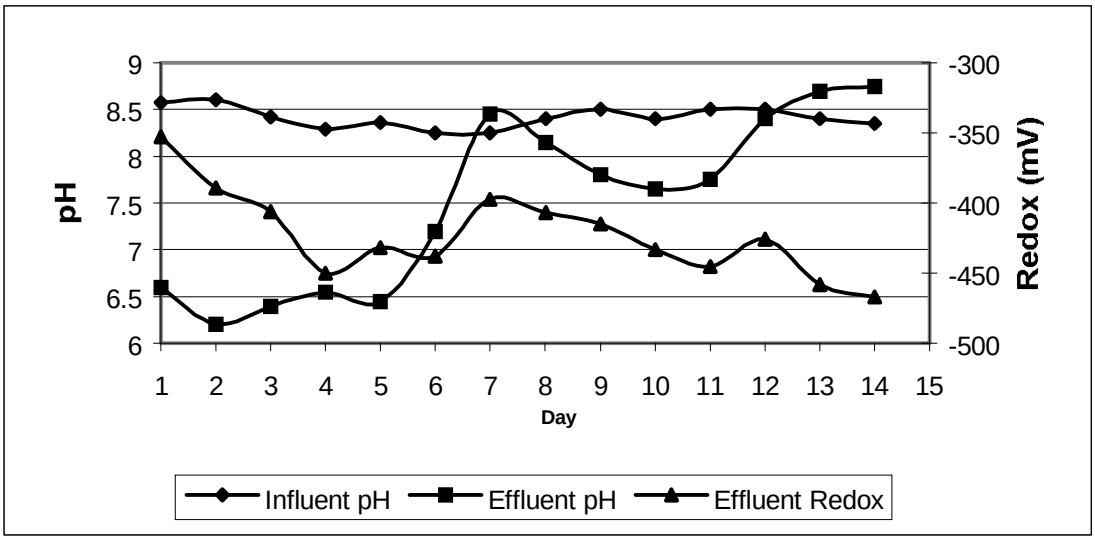


Figure 4.3 Influent pH, effluent pH and effluent redox during the running of a trickle filter as nitrogen flow rate was increased (oxygen partial pressure decreased)

Figure 4.4 shows the percentage recovery of the influent sulphide that could be accounted for during the 15 day period. The percentage sulphur species recovery was

calculated as the sum of the molar concentrations of SO_4^{2-}, HS^-, and S° in the effluent as a percentage of the molar concentration of HS^- in the influent. The percentage sulphur species recovery that could be accounted for was generally low with a 100% percentage sulphur species recovery not being obtained at any stage during the investigation. The inability to account for all the sulphur could be ascribed to accumulation of elemental sulphur within the reactor and losses due to a small amount of stripping of sulphide gas from the liquid stream. Increasing the nitrogen flow rate had the general effect of decreasing the percentage sulphur species recovery.

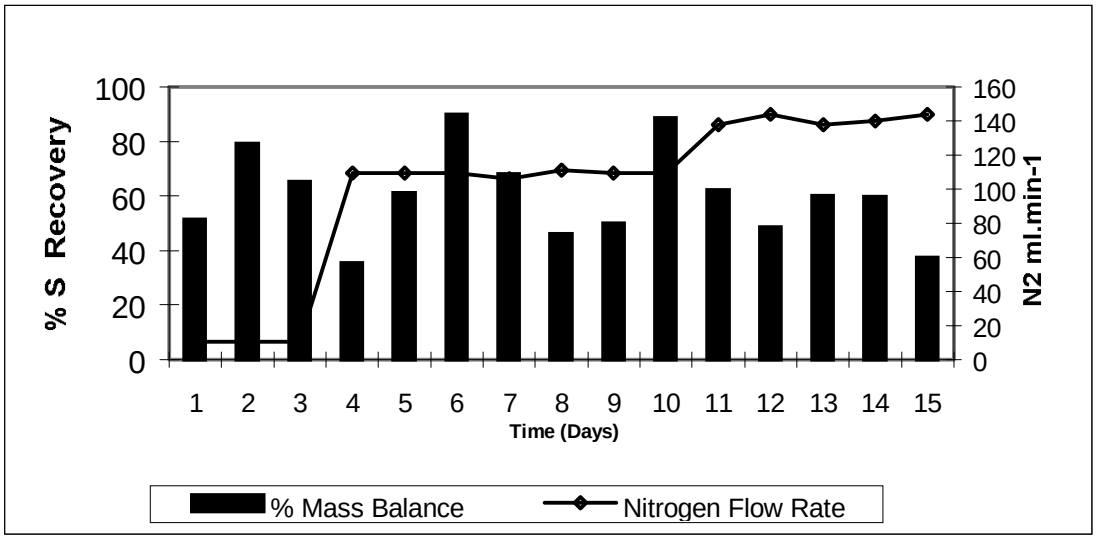


Figure 4.4 Percentage sulphur species recovery calculated as nitrogen flow rate was increased (oxygen partial pressure decreased). Percentage sulphur species recovery = ($[HS^-]_{influent}/[SO_4^{2-}]_{effluent} + [S]_{effluent} + [HS^-]_{effluent}$) x 100

The effluent on days 12 - 15 of the experiment had a distinctive yellow-green colour. A photograph of the effluent collected on days 12-15 of the investigation is shown in Figure 4.5.

Figure 4.5 Photograph of reactor effluent collected on days 12-15 of the investigation. The green-yellow colour is indicative of the presence of polysulphides.

A white biofilm was found to have covered some of the PVC packing material within the reactor. Electron microscopy studies of biofilm attached to the PVC surface revealed uniform covering of crystalline sulphur (Figure 4.6) had developed in some areas of the reactor. The attached biofilm was characterised by filaments attached to the PVC surface. A dense population of bacillus-like bacteria was attached to these filamentous organisms.

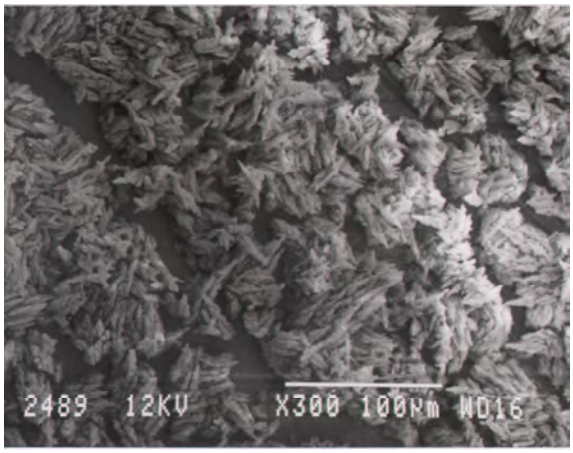


Figure 4.6 Scanning electron micrograph of surface of biofilm attached to PVC tubing. The crystalline deposits were suspected to be elemental sulphur.

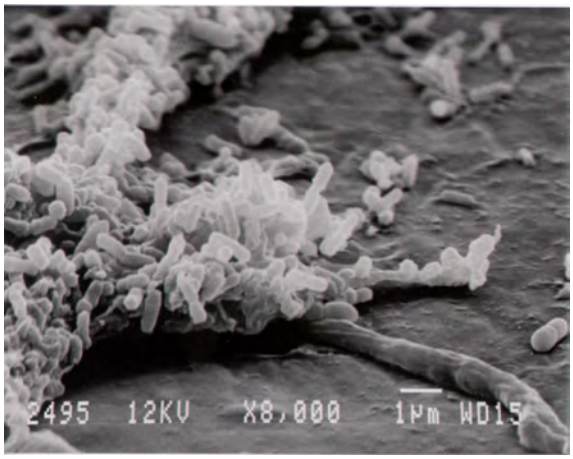


Figure 4.7: Scanning electron micrograph of the fixed-film showing bacterial adhesion to the PVC surface.

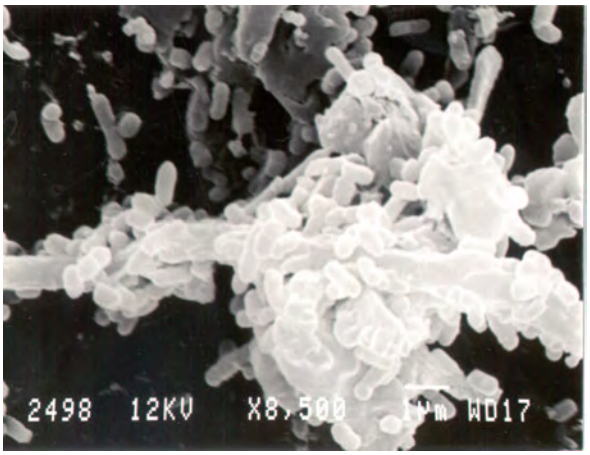


Figure 4.8 Scanning electron micrograph of the fixed-film showing both filamentous and bacillus-type bacterial growth.

4.4 Discussion

Decreasing the oxygen partial pressure in the reactor did not have the anticipated effect of decreasing sulphate formation in favour of elemental sulphur yield in the effluent. Sulphate was always detected as a product of sulphide oxidation during the investigation, with the concentration increasing as the oxygen partial pressure in the reactor was increased.

The evidence obtained during this investigation does suggest that sulphur production was taking place within the reactor, both the increase in the pH and the presence of polysulphide in the effluent point to this. The elevated pH measurements on days 7, 14 and 15 suggest that sulphur production was occurring within the immobilised biomass. Sulphur production is accompanied by the production of OH^- ions as described in equation (5). The increased pH that was observed cannot be regarded as an absolute

indicator of sulphur production since other factors could have contributed to the observed increase in pH. Stripping of carbon dioxide from the liquid stream by the nitrogen flow may have contributed to the increased pH measurements on days 14 and 15 when the nitrogen flow rate was at its highest, but the fact that a pH increase was noted when a change to a lower nitrogen flow rate was made (on day 7) would suggest that this was not a major contributor to the increased pH, and that sulphur production was the major contributor. This suggests that sulphur within the system only being mobilised by reaction with sulphide to form soluble polysulphides. The immobilised sulphur could possibly be removed by vigorous back-flushing of the reactor, but operational procedures such as this would preclude a reactor of this type being utilised in a passive treatment system.

The increasing sulphide concentrations in the reactor effluent could be explained by a decreased oxidative capacity of the immobilised biomass due to the decreased partial pressure of oxygen in the reactor, or possibly the occurrence of microbial sulphate reduction occurring within the reactor. Sulphide oxidation and sulphate reduction have been shown to occur simultaneously in a stirred continuous culture under oxygen limiting conditions, with soluble polysulphide compounds being implicated as the major chemical link between these two bacterial populations (van den Ende *et al.*, 1997).

The reactor was only run for a total of 60 days and it is possible that over an extended period of steady state operation the immobilised biomass, along with a portion of the immobilised sulphur would begin to slough from the packing material. This remains a possibility that may be investigated in the future.

Sulphate production as a result of either sulphide or sulphur oxidation would be expected to decrease the effluent pH due to H^+ according to equations (6) and (11) respectively. The fact that sulphur was never detected as a major component in the effluent may be explained by the following:

1) Sulphur accumulation on the column (see Figure 4.6)
2) Loss as polysulphides (see Figure 4.5), or other partially oxidised intermediates, although no thiosulphate was detected by ion chromatography.

These explanations would also explain the generally low percentage sulphur species recovery that was obtained throughout the investigation.

The fact that sulphate was always detectable during the experiment suggests that at all times sufficient oxygen was being supplied to at least parts of the reactor to enable complete oxidation to sulphate to take place. This observation highlights one of the major drawbacks of a trickle filter as a bioreactor configuration for this process.

The overall process of biological sulphide oxidation is dependent on the oxygen supply to the biomass, and the predominant product of oxidation is determined by the rate of oxygen supply to the biomass. Oxygen transfer from the gaseous phase to the liquid phase within the reactor is dependent on parameters such as oxygen concentration, flow rate of the liquid within the reactor and turbulence of both the liquid and gaseous phases within the reactor.

The chemical results suggest that large differences in these parameters may have occurred at different areas within the reactor. This suggests that a reactor configuration suited to biological sulphide oxidation to elemental sulphur should have a more uniform distribution of oxygen to the sulphide oxidising biomass.

4.5 Conclusions

A Fixed Film Trickle Reactor offers a variety of advantages in terms of the simple mode of running and the low initial capital costs in setting up such a reactor. These advantages make a reactor of this type an ideal reactor configuration as a module in a passive treatment system.

The investigations conducted here showed that the process would require strict control of the oxygen partial pressure within the reactor. Lack of strict control resulted in complete oxidation of sulphide to sulphate in areas of the reactor where the partial pressure is too high and incomplete oxidation of the sulphide in areas of the reactor that became anaerobic. Elemental sulphur that was formed within the reactor was retained with little chance of being able to recover the product. Due to the nature of the gas and liquid flow dynamics within the trickle filter a predictable, overall oxygen to sulphide consumption

ratio could not be maintained within the reactor. The strict control of oxygen delivery in a reactor of this configuration was found to be difficult to achieve. The variability of the chemical results suggested that oxygen was being supplied at different rates to various regions of the reactor. Interpretation of the observations and results obtained during this investigation suggest that a reactor in which the transfer of oxygen to the liquid phase (and therefore the biomass) is more predictable and influenced less by diffusion of oxygen in a large space would present a simpler system to control.

A Submerged Fixed Film Reactor was proposed as a solution to the disadvantages of running a fixed-film trickle filter. In a submerged configuration with counter current liquid and air flow, sloughing of sulphur produced within the reactor was proposed to be enhanced, with the mechanical action of gas flow aiding in this process. Flushing options to encourage sloughing of entrapped sulphur could be more practically implemented in a submerged reactor. Poising of the dissolved oxygen concentration and therefore the redox within the reactor was expected to be more predictable in a submerged configuration.

Preliminary investigations using a simple trickle filter showed that elemental sulphur was not the major product of sulphide oxidation in this type of reactor under oxygen-limited conditions. This was due to an inability to supply the correct O_2:HS^- consumption ratio throughout the reactor. Supplying the correct ratio throughout a reactor of this configuration was found to be difficult to implement practically as this rate is dependent on a number of variables. The variables that may affect this rate include gas diffusion in the reactor, mass transfer of oxygen between gas and liquid phases and mass transfer between liquid phase and the biofilm. These factors would make a process such as this difficult to control in a trickle filter configuration.

Chapter 5

Sulphide Oxidation in a Submereged Fixed-Film Reactor

5.1 Introduction

The difficulties encountered in the operation of a sulphide oxidising trickle filter for elemental sulphur production led to the investigation of a Submerged Fixed-Film Reactor down flow reactor as a possible reactor configuration for the biological conversion of sulphide to elemental sulphur. Reactors of this configuration have been investigated and applied in biological nitrification processes, for the bacterial oxidation of ammonia to nitrate (Fdz-Polanco *et al*., 2000). In these nitrifying reactors both influent liquid and oxygen were fed to the bottom of the reactor resulting in co-current flow of oxygen and the water to be treated. Distinctive zones of bacterial activity were reported in reactors such as these with heterotrophic populations developing at the bottom of the reactor where organic and oxygen concentrations were highest, and nitrifying activity predominating further up the reactor where organics had been depleted.

The reactor to be tested in the investigations on sulphide oxidation here would be run with counter-current liquid (sulphide and organics from the top) and oxygen (from the bottom) flows. A mechanism analogous to that observed in the nitrifying reactors, would be investigated in which heterotrophic bacterial metabolism would consume oxygen and organics creating a suitable environment for the oxidation of sulphide to elemental sulphur.

Run in this configuration, oxygen supply to the bacterial population would predominantly be determined by mass transfer between gaseous and liquid phases, and between the liquid and biofilm within the reactor. This would make oxygen supply to the bacterial population more predictable thus overcoming some of the problems encountered with running the conventional Fixed-Film Trickle Filter Reactor.

5.2 Materials and Methods

5.2.1 Reactor Configuration

Figure 5.1 shows a diagrammatic representation of the reactor configuration and Figure 5.2 shows a photograph of the laboratory set-up of the Submereged Fixed-Film Reactor. The reactor consisted of a Perspex tube 800mm (h) x 35mm (r). This gave the reactor a total hydraulic volume of 1.680L a hydraulic retention time of 3.5 hours at a feed flow of 8 mL.min^{-1}. The reactor was filled with Poraver® expanded glass which served as the immobilisation matrix for the bacterial population.; 1.680L a hydraulic retention time of 3.5 hours at a feed flow of 8 mL.min^{-1}.

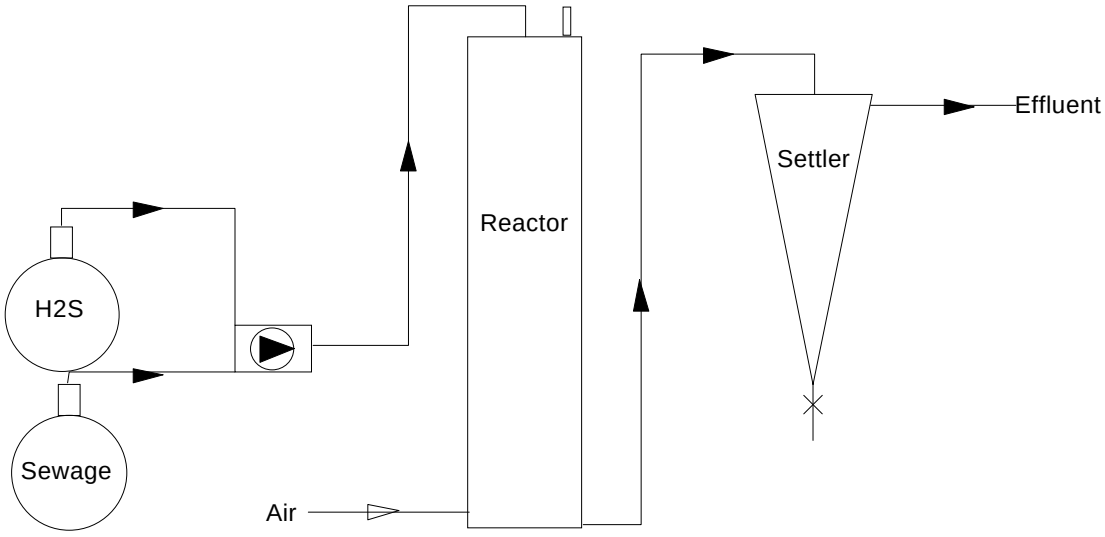


Figure 5.1: Diagramatic representation of the Submerged Fixed-Film Reactor.

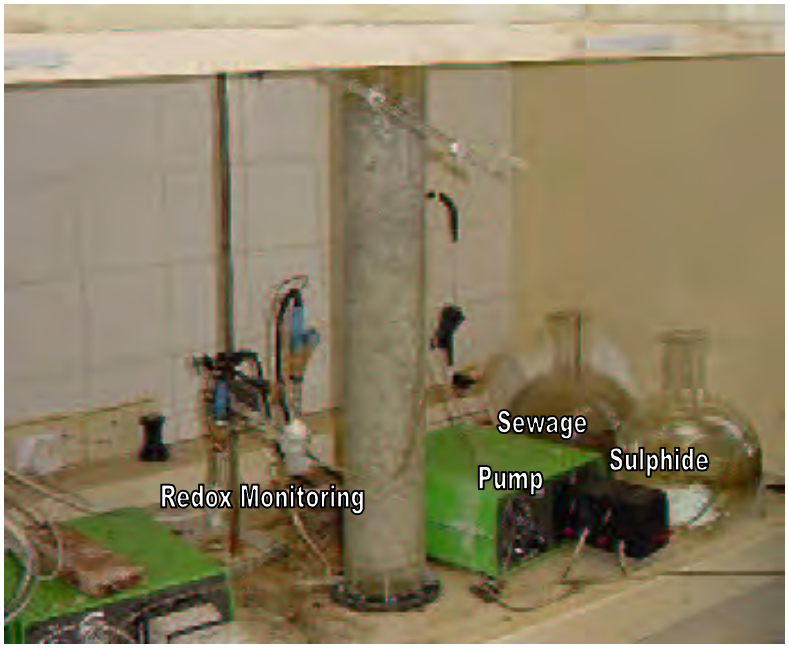


Figure 5.2: Photograph of laboratory set-up of drowned trickle filter

The feed consisted of a 1:1 ratio of primary settled sewage supernatant and a concentrated sulphide solution to give a final sulphide concentration of 150-200mg.L^{-1} (4.44 - 6.06mM). The sewage and sulphide solutions were stored separately and mixed by pumping with a Watson Marlow 504S peristaltic pump into a plastic T-piece and then via a single tube into the reactor. The tubing used for liquid delivery to the reactor was Tygon® R-3603 1.6mm (ID) oxygen impermeable tubing. Marprene® tubing was used in the peristaltic pump heads.

Air was delivered at the bottom of the reactor through an air stone from a compressed air cylinder. Airflow to the reactor was controlled using a precision gas flow controller. Airflow was accurately determined using a film flow burette. The possible presence of sulphide in the effluent gas stream was determined. Sampling ports were located down the length of the reactor. These consisted of 5mm (ID) x 35mm (length) Perspex tubes. The 35mm length allowed for sampling of liquid present in the middle section of the reactor. These sampling ports were placed at 130, 400 and 680mm from the top of the reactor respectively. Effluent from the reactor was directed to a height similar to that of the reactor feed. This maintained the hydraulic level within the reactor without the need for pump control of the effluent. The effluent was passed through a settler unit consisting of a 1L Imhoff cone to collect settleable solids that may have passed out of the column.

The reactor was operated over a period of 45 days initially, to allow a bacterial population to develop. Following this start-up period, the reactor was run under varying conditions over a 15-day period, during which sulphide oxidation and elemental sulphur production were monitored. Sampling was carried out at least 5 hours after a change to the reactor running conditions was made. This allowed at least 1.5 hydraulic retention times (HRT) for the microbial population present in the reactor to acclimatise to the new conditions.

The air flow rate and the sulphide loading rate of the reactor were varied during the running of the reactor. The air flow rates and sulphide loading rates employed during the investigation are shown in Table 5.1.

Table 5.1: Summary of reactor running conditions for each of the days of the reactor operation.

	Air Flow Rate (mL. min^{-1})	Sulphide loading rate ($mg.L^{-1}.h^{-1}$)
Days 1+2	12	32,33
Days 3+4	32	32,28
Days 5-13	72	27,31,33,39,34,42,49,45

5.2.2 Analytical Methods

Sulphide, sulphate and elemental sulphur concentrations, pH and redox were determined as described previously. TEM samples were prepared as described previously.

5.2.3 Energy dispersive X-ray Microanalysis

Energy dispersive X-Ray Microananalysis (EDX) analysis was performed on samples from the reactor effluent in order to determine whether white intracellular inclusions observed during TEM investigations were elemental sulphur. Embedded cross-sections were prepared as described for TEM except that the cross sections were cut slightly thicker and no staining procedures were carried out on the cross sections. TEM-EDX investigations were carried out at the University of Port Elizabeth Physics Department. The cross sections were viewed under TEM suspected inclusions were subjected to EDX analysis.

5.3 Results

5.3.1 General reactor operation

Figure 5.3 shows the concentration of sulphur species in the effluent related to the air flow rate to the reactor. The effluent pH and redox potential of the reactor effluent are shown in Figure 5.4. Figure 5.5 shows the percentage sulphur species recovery on different days during the investigation. The percentage sulphur species was calculated according to the following equation:

$$\text{Percentage Sulphur Species Recovery} = ([HS^-]_{inf}/[SO_4^{2-}]_{eff} + [S]_{eff} + [HS^-]_{eff}) \times 100$$

At no stage was a substantial sulphur concentration detectable in the reactor effluent. High concentrations of sulphide 0.001M (27-31$mg.L^{-1}$) were detected in the reactor effluent on days 1 and 2. During the first two days less than 50% of the influent sulphide

was accounted for in percentage S species recovery. Sulphide losses as a result of loss in the sparge gas were ruled out since no white precipitate was detected in the zinc acetate trap during the investigations.

An increase in the airflow rate to the reactor on day 3 resulted in the disappearance of sulphide from the reactor effluent and a small increase in the effluent sulphate concentration. A small increase in the percentage sulphur species recovery was noted on these two days. Effluent pH remained elevated. The increase of the air flow rate to the reactor on day 4 resulted in the following observations: a large increase in the effluent sulphate concentration, a large drop in the effluent pH and a large increase in the percentage sulphur species recovery that could be accounted for. On days 5 and 6 the percentage sulphur species recovery was greater than 100% (114% and 105%) respectively.

During the following 2 days (days 7 and 8) the sulphide loading rate was increased, this resulted in a slight decrease in the effluent sulphate concentration, a steady increase in the effluent pH and a decrease in the percentage sulphur recovery which could be accounted for. During the period from day 7 onward no large amounts of sulphide (i.e. $>2 mg.L^{-1}$) detected in the reactor effluent even though the sulphide loading rate was continually increase between days 7 and 13 of the investigation.

The increase in the pH of the reactor effluent and the visual observations that a white precipitate was accumulating within the reactor suggested that sulphur production was occurring within the reactor, but this sulphur was not being detected in large quantities in the reactor effluent. In order to investigate the fate of the suspected sulphur produced within the reactor, depth profile studies utilising the sampling ports incorporated into the reactor design were carried out.

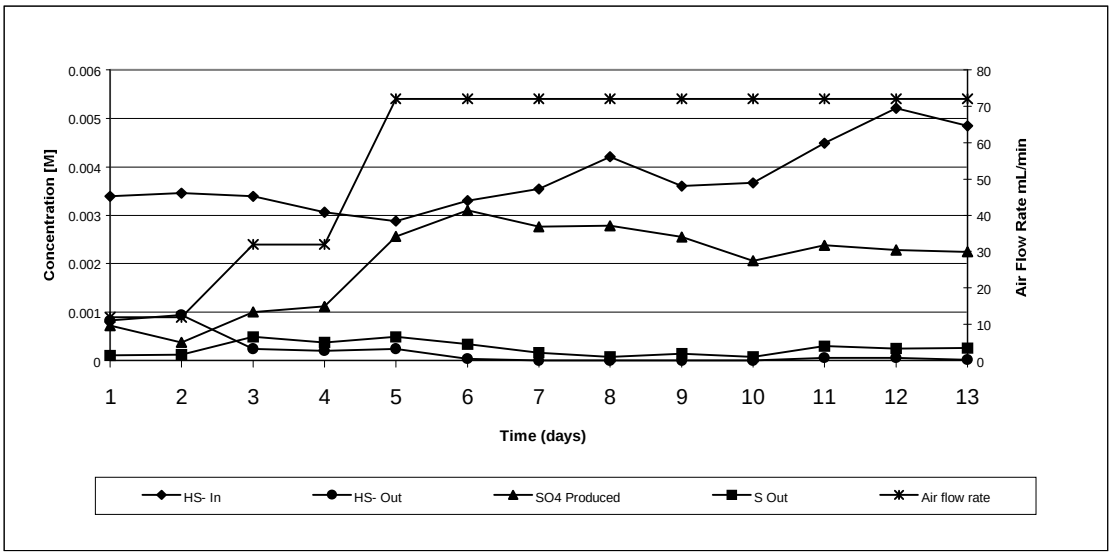


Figure 5.3: Sulphur species concentrations as determined during the investigation after a sulphide oxidising population had been established over a 45 day period. SO_4^{2-} produced = $[SO_4^{2-}]_{effluent} - [SO_4^{2-}]_{influent}$

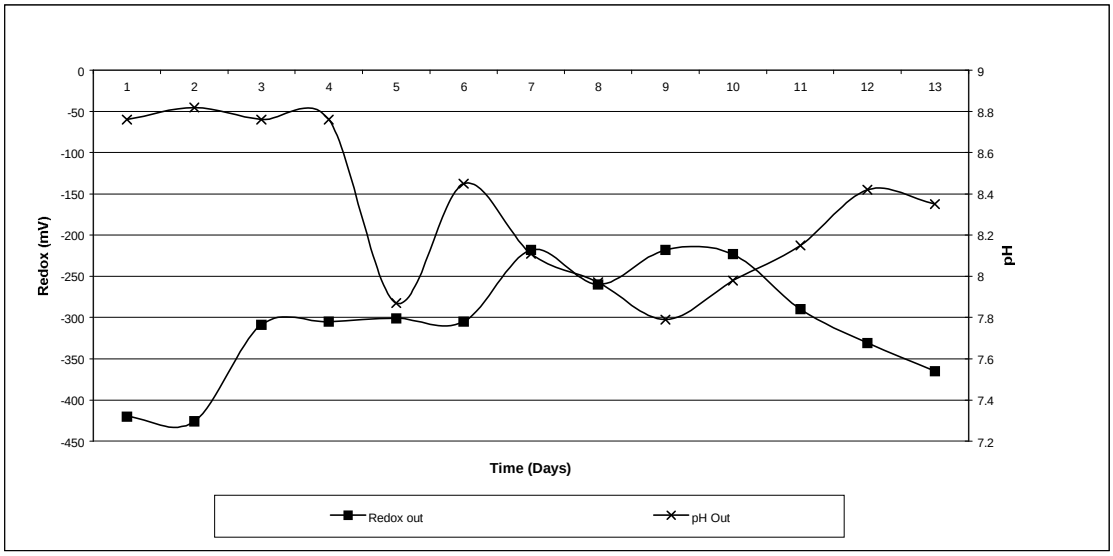


Figure 5.4: Effluent pH and Redox measurements obtained during the investigation

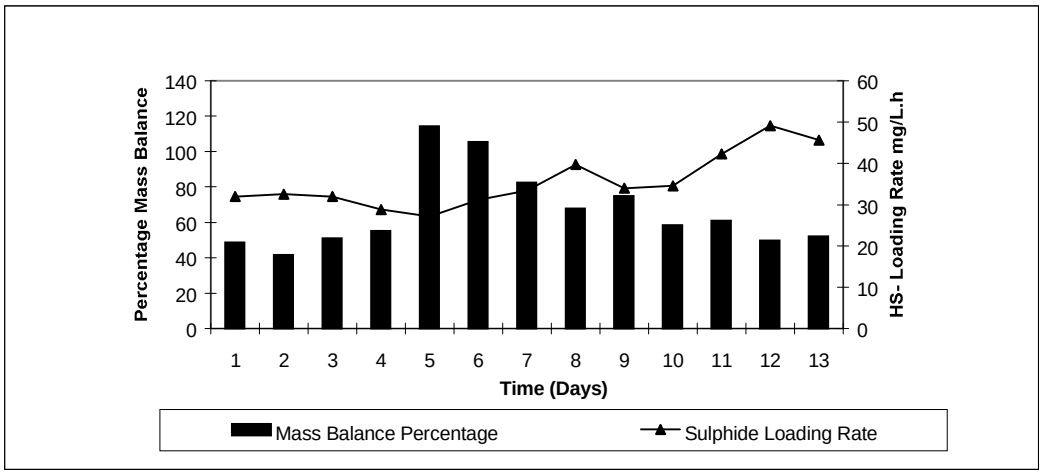


Figure 5.5: Percentage sulphur species recovery accounted for at each sulphide loading rate.

5.3.2 Depth profile studies

Figures 5.6, 5.7, 5.8, 5.9, 5.10 represent depth profiles for sulphide, sulphate, sulphur, pH and the measured redox on days 1, 3, 6, 9 and 12 respectively. The following general observations apply to these depth profiles:

1) The largest changes in sulphide concentration, sulphate concentration and pH tend to occur within the top 13cm of the reactor. Changes were also noted between the last sampling port (at 13 cm) and the effluent.
2) No decrease in the sulphate concentration or increase in the sulphide concentration was noted within the reactor for any of the depth profiles suggesting that sulphate reduction was not detectable as a significant process within the reactor.
3) The trends for elemental sulphur concentrations within the reactor are not clear although it is important to note that on days 9 and 12 (high air flow rates) the elemental sulphur concentration showed a decrease between the first sampling port and those further down the reactor, possibly indicating that sulphur that was produced in the upper reaches of the reactor was being oxidised as it travelled down the reactor.

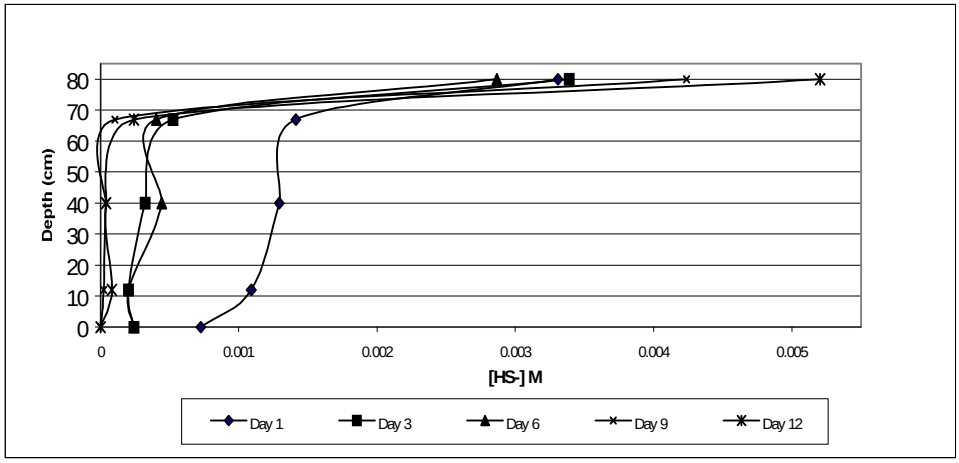


Figure 5.6 Sulphide concentration vs. depth on days 1, 3, 6, 9, 12 of the investigation

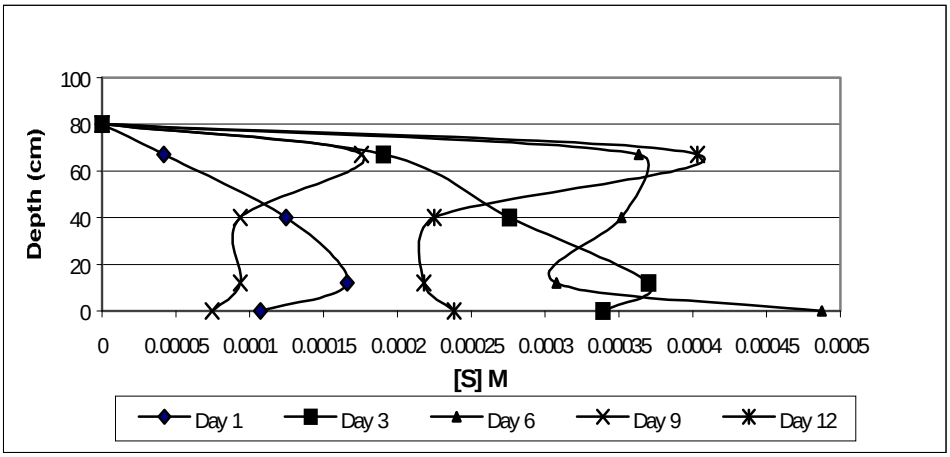


Figure 5.7 Sulphur concentration vs. depth on days 1, 3, 6, 9, 12 of the investigation

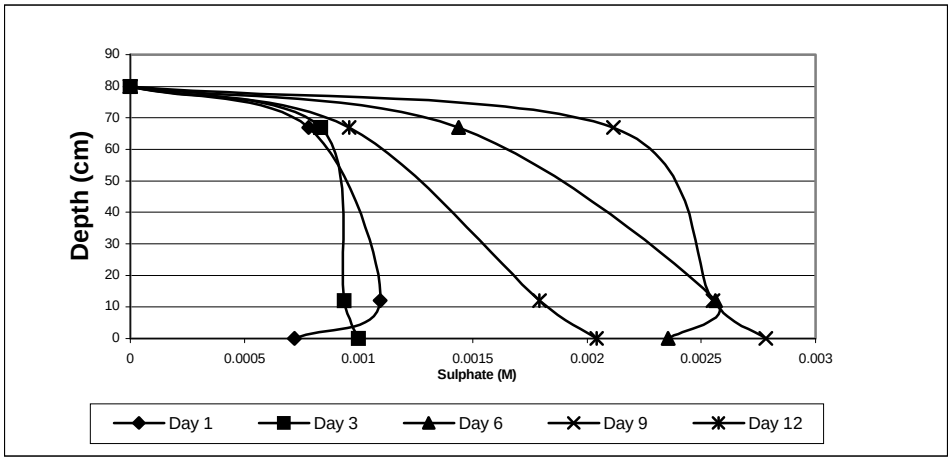


Figure 5.8 Sulphate concentrations vs. depth on days 1, 3, 6, 9, 12 of the investigation

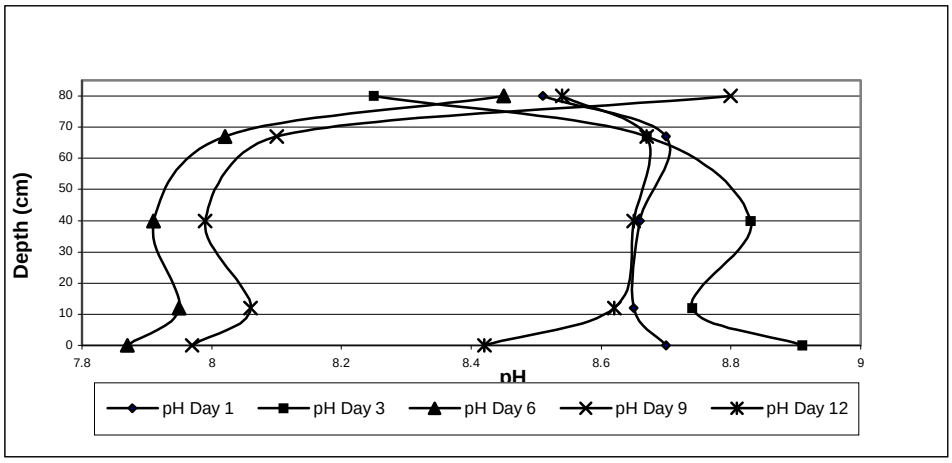


Figure 5.9 pH vs. depth on days 1, 3, 6, 9, 12 of the investigation

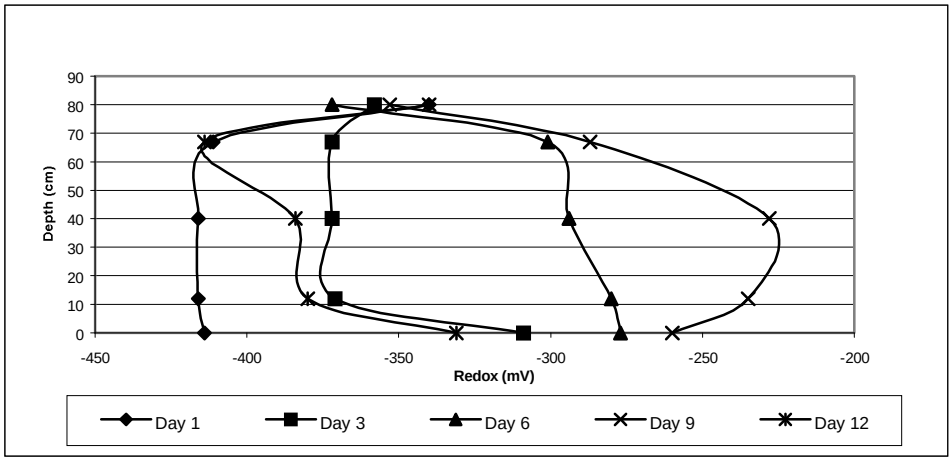


Figure 5.10 Measured redox vs. depth on days 1, 3, 6, 9, 12 of the investigation

5.3.3 General observations

The growth of grey-white filamentous biofilm was noted on the reactor walls (see Figures 5.11 and 5.12). Deposition of the white material occurred throughout the reactor and the grey-white biofilm growth seemed concentrated near the bottom of the reactor.

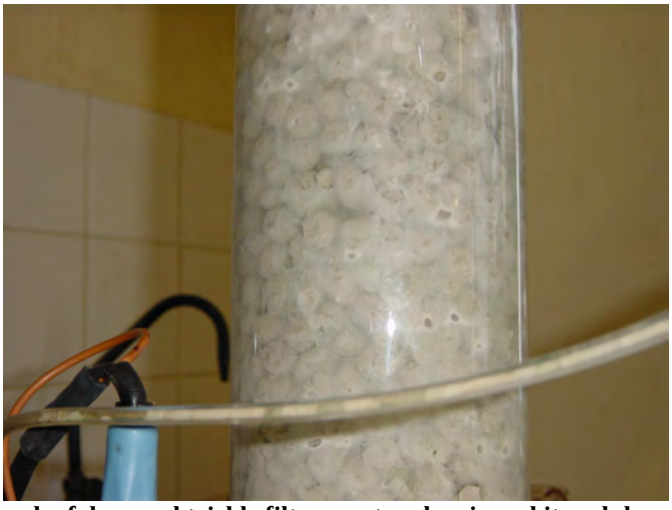


Figure 5.11: Photograph of drowned trickle filter reactor showing white sulphur accumulation on the reactor walls

Figure 5.12 Photograph of drowned trickle filter reactor showing white sulphur accumulation and filamentous bacterial growth on the reactor wall

5.3.4 Oxidation of accumulated elemental sulphur

In order to further establish whether quantities of elemental sulphur were being retained within the reactor the sulphide feed was stopped and replaced with 1:1 primary settled sewage supernatant:distilled water pH 8.0 feed. Following cessation of the sulphide feed sulphate concentration and effluent pH were monitored. Effluent pH and sulphate concentrations were monitored for 12 hours after cutting the sulphide feed. Results of this experiment are presented in Figure 5.13. The sulphate concentration in the reactor effluent was found to steadily increase during the 12-hour period after stopping the

sulphide feed. The pH of the reactor effluent was found to steadily decrease throughout the 12-hour experiment indicating that accumulated sulphur was being oxidised within the reactor.

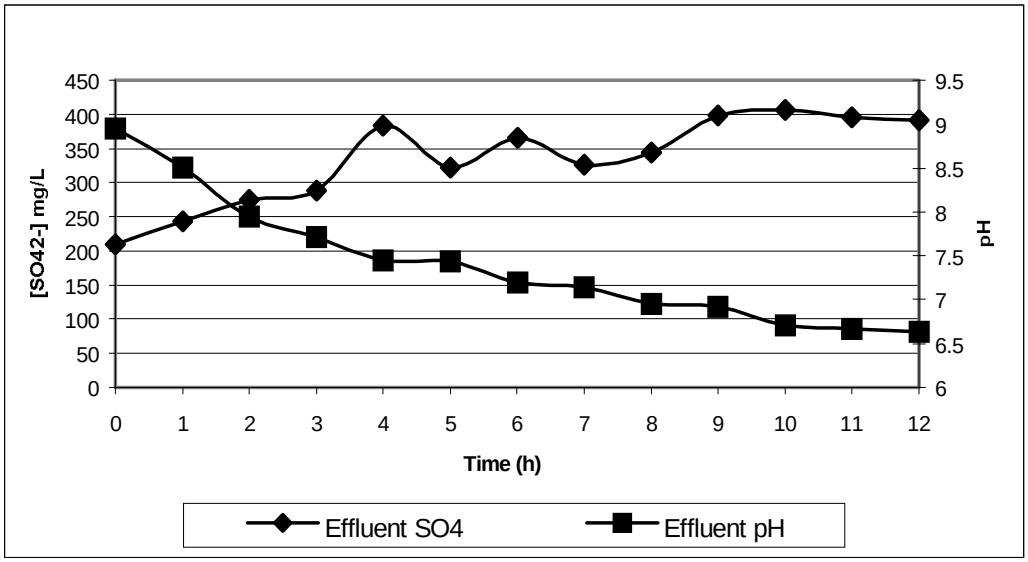


Figure 5.13: Sulphate concentration and measured pH in effluent after stopping the sulphide feed to the reactor

5.3.5 Light Microscopy

Light microscopy studies carried out on effluent from the reactor revealed the presence of large numbers of filamentous bacteria. Photographs of these bacteria are shown in Figures 5.14 and 5.15.

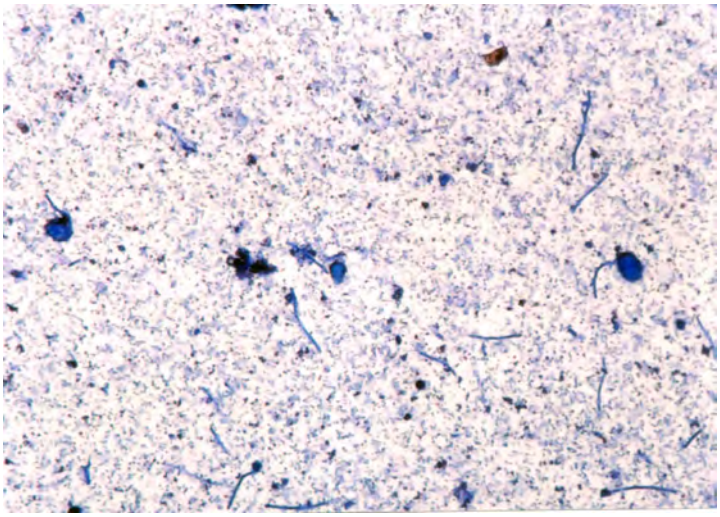


Figure 5.14 Micrograph of reactor effluent (400X) showing abundance of filamentous bacteria

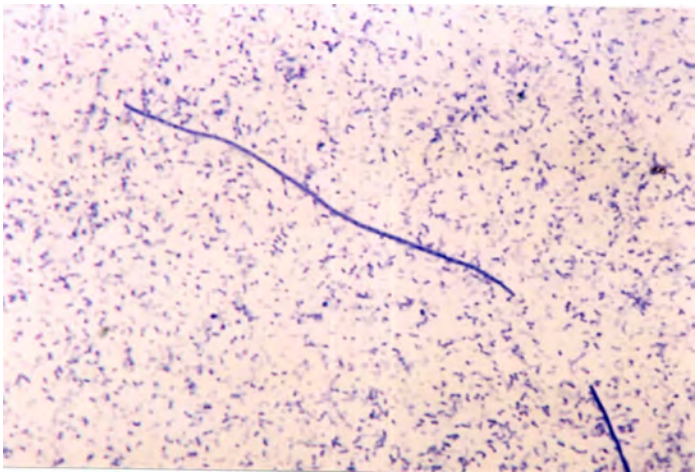


Figure 5.15 Micrograph of reactor effluent (1000X) showing a filamentous bacterium. Close inspection shows intracellular unstained inclusions which could be intracellular elemental sulphur - characteristic of filamentous *Thiothrix* -like bacteria

5.3.6 Transmission electron microscopy

Transmission electron microscopy studies that were conducted on embedded section of bacteria from the reactor revealed a diverse population of bacteria. Photographs of the bacteria observed during TEM investigations are shown in Figures 5.16 and 5.17.white intracellular inclusions were noted in a number of the bacteria. These inclusions were thought to consist of elemental sulphur.

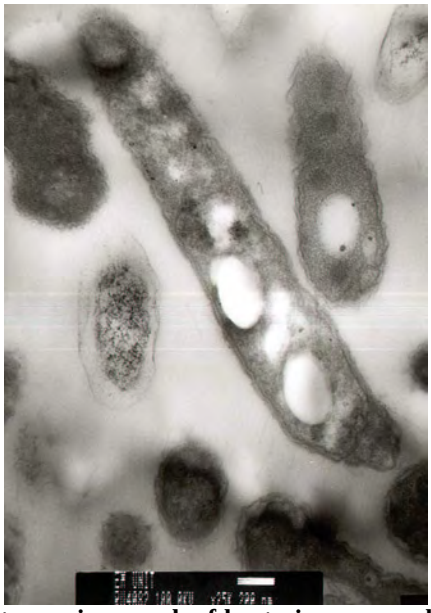


Figure 5.16: Transmission electron micrograph of bacteria recovered from reactor effluent showing white intracellular inclusions, which could be sulphur.

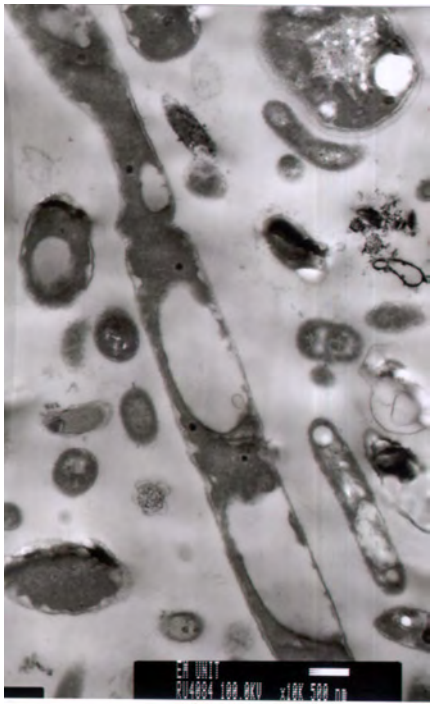


Figure 5.17 Transmission electron micrograph of reactor effluent showing filamentous bacterium with intracellular void structures.

5.3.7 TEM-EDX

The nature of these intracellular inclusions was investigated using TEM-EDX analysis on specially prepared TEM sections containing embedded bacteria. The white inclusions could not be positively identified as elemental sulphur

5.4 Discussion

Following establishment of steady state operating conditions sulphide was present in the in the reactor effluent during the first two days of the 15 day monitoring investigation. This suggests that at these sulphide loading and air flow rates, insufficient air was being supplied to the biomass to completely oxidise all the sulphide, or that reduction of oxidised sulphur intermediates was taking place within the reactor. Depth profiles of sulphide in the reactor (Figure 5.6) do show a general decrease in the sulphide concentration down the length of the reactor indicating that insufficient oxygen was being supplied rather than sulphate reducing bacterial activity ocurring. The effluent pH during these first two days was elevated. This is consistent with the explanation that under these oxygen limited conditions elemental sulphur was being produced resulting in the elevated pH according to equation (5). White deposits were noted on the reactor walls (Figure 5.11) and grey-white biofilm was noted to have established themslves in the lower

regions of the bioreactor (Figure 5.12) suggesting that elemental sulphur was being retained within the column and that a portion of this was due to intracellular accumulation of sulphur by filamentous sulphide oxidising bacteria. Confirmation of this observation was obtained by assessing the pH and sulphate concentration of the reactor effluent after cutting the sulphide feed to the reactor.

Cutting the sulphide feed to the reactor resulted in an increase in the effluent sulphate concentration and decrease in the effluent pH (Figure 5.13). The steady increase in the sulphate and decrease in pH were probably as a result of the oxidation of accumulated elemental sulphur within the reactor according to the equation (11). Sulphur reclamation from a reactor such as this, run under these conditions would be dependent on recovering the attached biofilm and the associated sulphur and would be dependent on sloughing of the accumulated biofilm. It is likely that intracellular accumulation of sulphur was occurring due to the presence of filamentous sulphide oxidising bacteria similar to *Thiothrix* and *Beggiatoa*. The growth of filamentous sulphur oxidising bacteria has been reported to be dependent on the sulphide-loading rate of the reactor. Sulphide loading rates of greater than 105 $mg.L^{-1}.h^{-1}$ ($3.28mmol.L^{-1}.h^{-1}$) were found to inhibit *Thiothrix* growth in a fixed film up flow reactor (Buisman *et al.*, 1990b). The down-flow trickle reactor used in this study was never run at an overall sulphide-loading rate of higher than 49 $mg.L^{-1}.h^{-1}$ ($1.4mmol.L^{-1}.h^{-1}$). This supports the observation that a large filamentous sulphide oxidising population had developed and was involved in sulphur production within the reactor.

White inclusions that were noted in bacteria during TEM studies could not be positively identified as sulphur during TEM-EDX studies. Knickerbocker *et al.*, (2000) noted that sulphur particles were vaporised under a high energy (60 kV) electron beam and this may have occurred during this investigation.

Depth profile analyses obtained for the column during the investigation showed that the largest oxidative changes occurred within the top 13cm of the reactor as well as in areas near the effluent port of the reactor. This may be explained by the best oxygen transfer occurring where the surface tension of the water was broken most effectively. This occurred at the top of the reactor where air bubbles broke the surface and at the point where air was introduced into the reactor. At the top of the reactor the escaping air

bubbles caused good mixing of air and aqueous environments. At the bottom of the reactor (between the last sampling port and the effluent) the liquid had to pass the air stone supplying air to the reactor. Bubbling the effluent gas stream though a zinc acetate trap and testing for the presence of sulphide discounted the loss of sulphide due to a sparging effect. No sulphide was detected in the gaseous effluent stream.

Depth profiles that were obtained for sulphide and sulphate indicated that sulphate reduction was probably not taking place within in the reactor since no corresponding decrease in sulphate and increase in sulphide concentration could be discerned for the reactor depth profiles (Figures 5.6 and 5.7). This however doesn't preclude the existence of oxidation-reduction cycles being present or cycling of the less oxidised or reduced sulphur species occurring within the reactor as proposed in Figure 1.5.

The sulphur depth profiles suggest that two trends predominated within this reactor: firstly sulphur was oxidised as it travelled down the reactor and secondly sulphur was retained within the biofilm and did not appear in the effluent due to a low rate of sloughing in the reactor. Sloughing may have become more predominant as the biofilm became older and may have been observed had the reactor been run over a longer period of time.

5.5 Conclusions

During these laboratory scale investigations into the use of a Submerged Trickle-Filter Reactor for the microbial oxidation of sulphide to sulphur under high organics conditions the following has been shown:

1) Sulphur accumulation within the reactor, possibly due to intracellular sulphur accumulation by filamentous sulphide oxidising bacteria such as *Thiothrix* ;

2) The reactor had a very limited functional area. At higher airflow rates (Day 9 and Day 12), all the sulphide was found to be removed within the first 13cm of the reactor.

3) Changes in airflow rates had profound effects on the products of sulphide oxidation with the presence of the organics seeming to have little ability to buffer these changes.

Employing the following measures could possibly have mitigated these disadvantages:

a) In order to prevent the development of filamentous sulphur accumulating sulphide-oxidising population, a higher sulphide-loading rate could have been applied. Previous reports suggest that loading rates greater than 105mg.L^{-1}.h^{-1} (3.28mmol.L^{-1}.h^{-1}) (Buisman *et al.*, 1990b) inhibit the growth of these organisms.
b) The reactor could have been shortened, decreasing the percentage of the reactor that was subjected to lower sulphide-loading rates therefore decreasing the areas in which a filamentous sulphide oxidising population may have developed.

Based on observations and these proposed mitigating measures made during the investigations, a reactor that will produce elemental sulphur as a major product of sulphide oxidation under heterotrophic conditions will need to be run under the following configuration and general conditions:

1) Higher sulphide loading rates – this could discourage the growth of heterotrophic sulphide oxidising bacteria;

2) Due to the high sulphide loading rates the oxygen supply rates would have to be correspondingly high;

3) Produced elemental sulphur should be removed from the oxygen rich zone quickly to prevent further oxidation;

The development of a heterotrophic sulphide oxidising population is likely to develop in at least some part of the reactor acting as a “sulphur sink” and therefore provision should be made for the easy removal of such a population

Chapter 6

Sulphide Oxidation in a Silicone Tubular Reactor

6.1 Introduction

Biological sulphide oxidation occurs predominantly at liquid-air interfaces where bacteria are able to effectively compete with the abiotic chemical oxidation of sulphide (Jorgensen, 1982). Sulphur containing biofilms have been observed to develop on the surface of sulphide rich waters associated with tanneries (Figure 1.3), hot springs (Figure 1.2) and anaerobic sulphate reducing reactors such as the RSBR (Figure 1.4). Investigations on the biofilms that develop on the surface of the RSBR revealed that the bacterial population consisted of a variety of predominantly heterotrophic bacteria. The development of crystalline sulphur (Figure 1.5) as opposed to the liquid amorphous sulphur associated with biological sulphide oxidation occurred within these biofilms (Gilfillan, 2000). The investigations reported on in chapters 4 and 5 of this thesis revealed that although evidence suggested that sulphur production was occurring within the reactors it was occurring within very specific areas of the reactor and any sulphur that was produced was either retained in or associated with the biomass. Sulphur that was produced was prone to further oxidation to sulphate within the reactor.

The development of a white film has been observed in this laboratory to develop when a non-sterile sulphide containing solution is pumped through silicone tubing and this film is thought to consist at least in part of biologically produced sulphur. Silicone is permeable to a wide number of chemical compounds and has been reported to be highly permeable to 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 silicone membranes 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 can also been used as a means of separating sulphate reducing bacteria from toxic metals solutions in the treatment of metals containing wastewater (Chuichulcherm *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 Reactor. Due to the chemical characteristics of silicone, the conditions that favour the formation of natural sulphur biofilms could be reliably reproduced in this novel reactor configuration. The studies conducted aimed to confirm that these sulphur biofilms could be reliably produced within the silicone tubing and gain a general understanding of the microbiology and chemistry of these biofilms.

6.2 Materials and Methods

6.2.1. Reactor Configuration

The reactors in this investigation consisted of lengths of silicone tubing (13,2m in length, 5mm (ID) x 8mm (OD)). This gave a total reactor volume of 272mL, a HRT of 47 min at a flow rate of 5.8mL.min^{-1}. The surface area of the reactor was calculated to be 2902cm^2. A sulphide/sewage mix was fed to the reactor as previously described. The reactor was attached to a 2.5m plastic mesh column by means of cable ties. The reactor was fed from the top downward. A photograph of the laboratory configuration of the silicone tube reactor is shown in Figure 6.1.

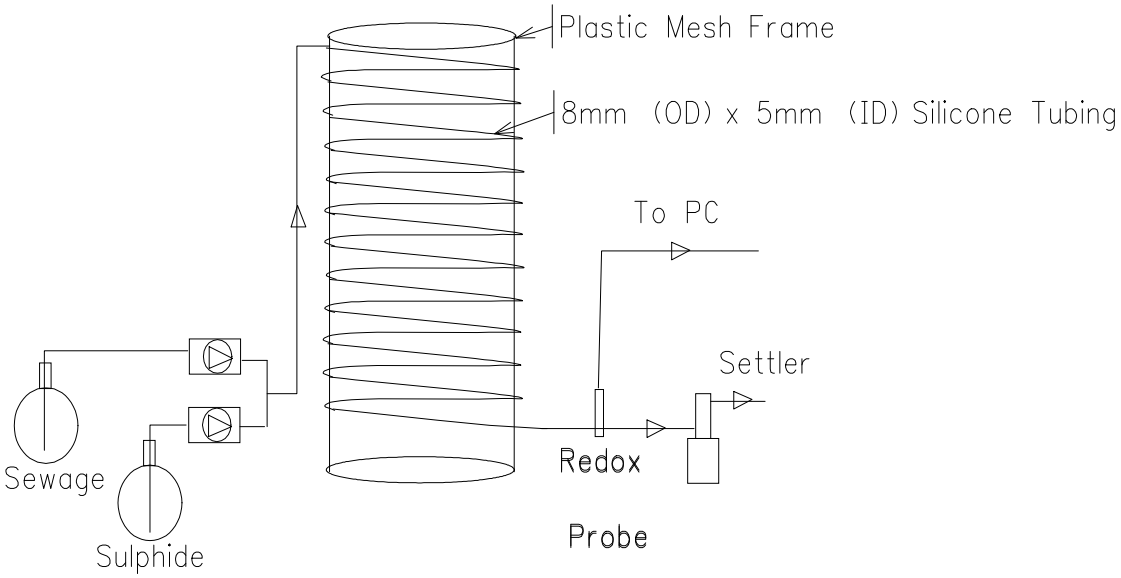


Figure 6.1 Diagrammatic representation of Silicone Tubular Reactor set-up

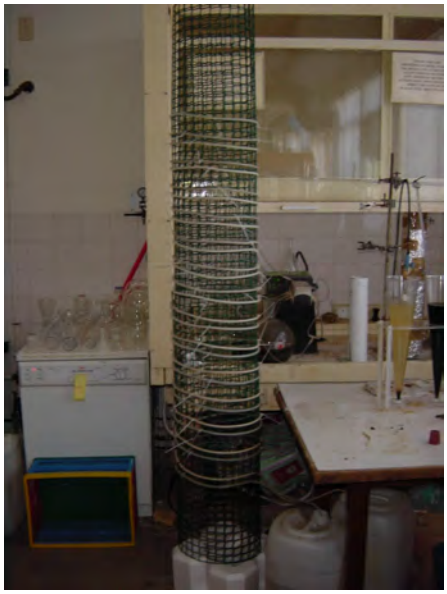


Figure 6.2 Photograph of the Silicone Tubular Reactor laboratory set-up. Silicone tubing was supported on a plastic mesh column

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.

6.2.2 Reactor Operation

6.2.2.1 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 the investigation.

6.2.2.2 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 as previously described in chapter 2.

6.2.2.3 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 5.8mL^{-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 .

6.2.2.4 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 (125mL.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 these upset conditions.

6.2.3 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 - 2.2m 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 2m intervals along the reactor. These pieces were prepared for SEM as previously described

6.2.4 Light Microscopy

Reactor effluent containing fragments of sloughed biofilm were viewed under phase contrast conditions at 400 X on a light microscope. Effluent samples containing biofilm were heat-fixed on glass microscope slides, stained with methylene blue and observed on a phase contrast microscope.

6.2.5 Analytical Methods

Sulphide, sulphate and elemental sulphur concentrations, pH and redox levels were determined as described previously. TEM samples prepared as described previously.

6.3 Results

6.3.1 Reactor Start –Up

Figure 6.3 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 6.4. 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 4mM (81-133mg.L^{-1}), pH 8.5 and a flow rate of 2mL.min^{-1}. During this stage the effluent contained 0.7-1.7mM (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 0.8mM (6 mg. L^{-1}). During this time the effluent sulphur concentration increased to a maximum of 1mM (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 4.4mL.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 0.7mM (20mg.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 0.5mM (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 1.07×10^{-3} M.h^{-1}.

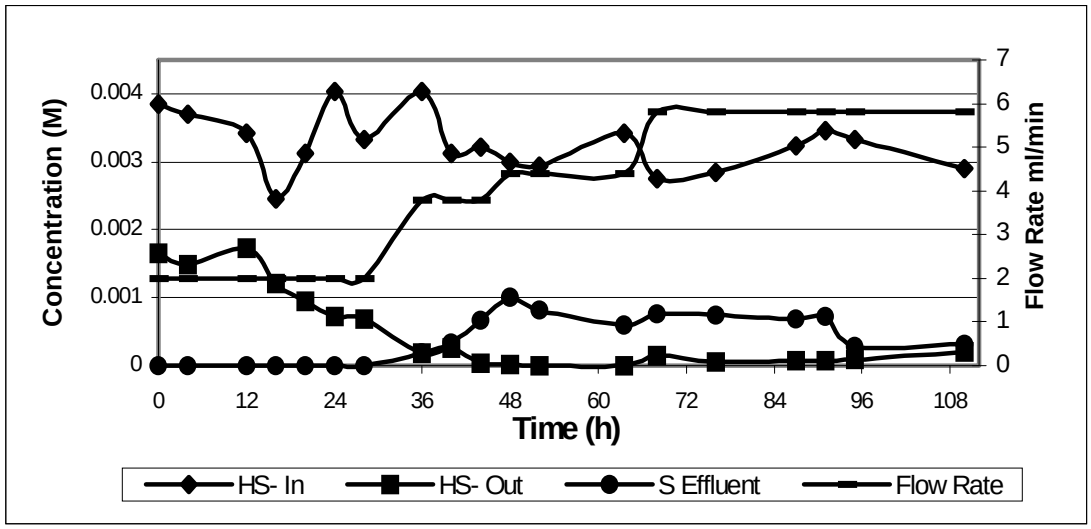


Figure 6.3 Sulphide influent and effluent concentrations, sulphur effluent concentration and liquid flow rate for a Silicone Tubular Reactor on start-up using a fresh length of tubing.

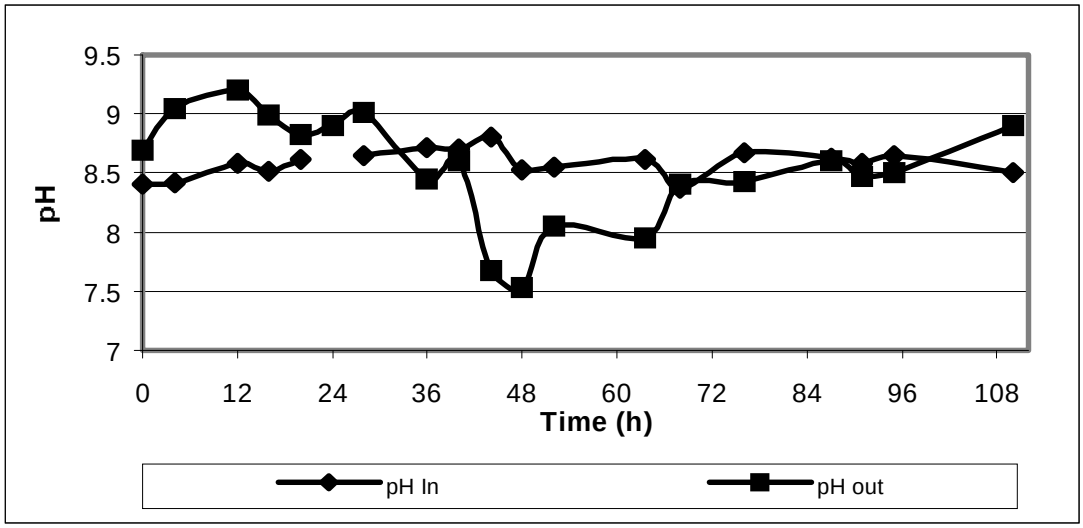


Figure 6.4 Influent and effluent pH measurements over during silicone reactor start-up fresh length of silicone tubing

A thick white biofilm was observed to have developed after 10 days of continuous reactor operation (Figure 6.5). 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 6.6).

Figure 6.5: Photograph of silicone tube reactor showing white biofilm development within the tubing (top tube) compared to a fresh length of silicone tubing.

Figure 6.6: Photograph of dried biofilm harvested from Silicone Tubular Reactor.

6.3.2 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 6.7. The influent and effluent pH for the same period are shown in Figure 6.8.

Re-starting the reactor after harvesting the previous biofilm at a flow rate of 5.8mL.min^{-1}at a sulphide concentration of 3mM (100mg.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 1.8mM HS^- (10mg.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 1.7mM (125 and 163mg.L^{-1}), effluent elemental sulphur ranged between 0.5 and 0.8mM (16 and 26mg.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.

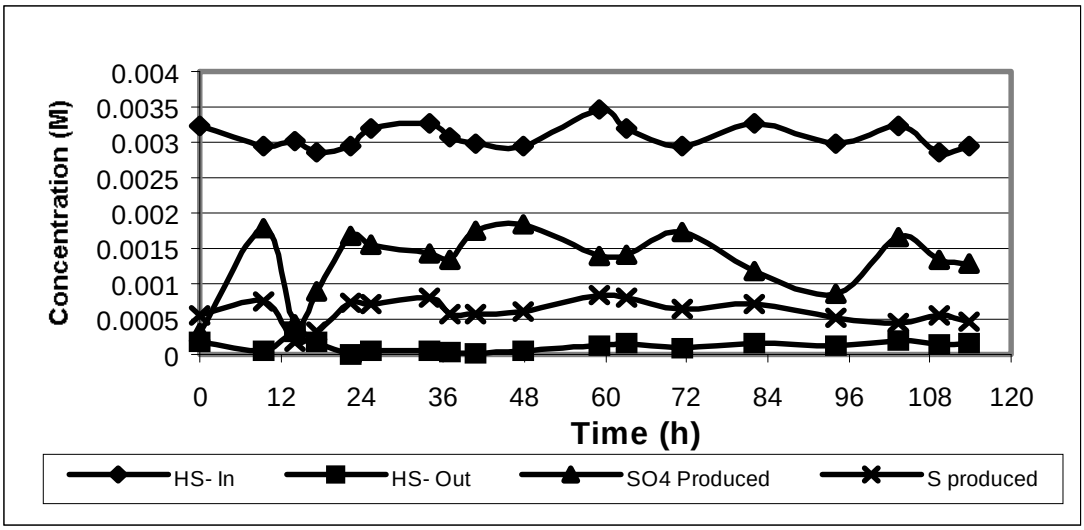


Figure 6.7 Influent and effluent sulphide concentrations produced sulphate and effluent sulphur concentrations for the Silicone Tubular Reactor started up a directly after removal of a previous biofilm.

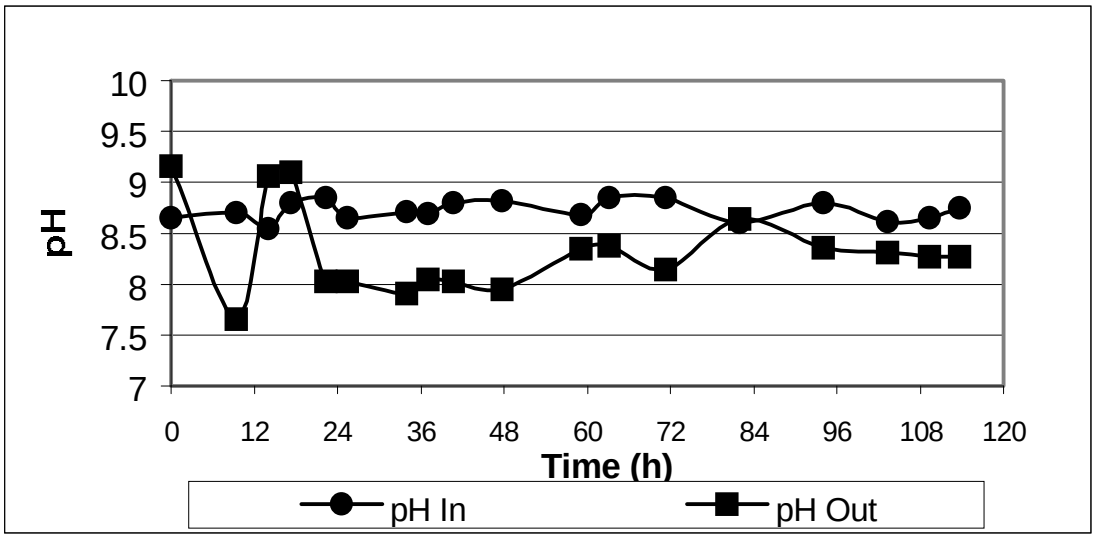


Figure 6.8 Influent and effluent pH measurements during operation of the Silicone Tubular Reactor started up directly after removal of a previous biofilm.

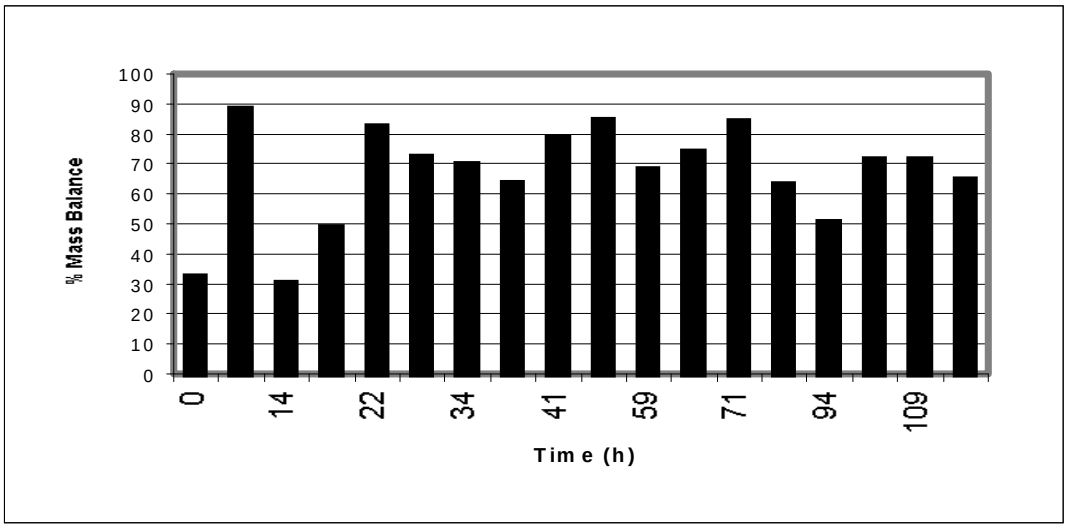


Figure 6.9 Percentage sulphur species recovery obtained during operation of Silicone Tubular Reactor started up directly after removal of a previous biofilm. Percentage S species recovery = $[HS^-]_{if}/[SO_4^{2-}]_{elf}$ $[S]_{Fe}$ + $[HS^-]_{Fe}$) x 100

6.3.3 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 6.10 and 6.11 respectively. The amount of particulate matter collected from the reactor at a flow rate of 5.6mL.min^{-1} during the first six days of the experiment ranged between 9 and 46mg (average 26 +/- 14.7mg) and the elemental sulphur present ranged between 1 and 6.5mg (average 4.3 +/-1.9mg) (Figure 6.9). The total sulphide load per day (assuming a constant sulphide concentration of 3mM HS^-, 100mg.L^{-1}) at 5.6 mL.min^{-1} was 806 mg.day^{-1}.

The amount of sulphur collected at 5.6mL.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 125mL.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 3912mg of particulate material was collected of which 1137mg was determined to be elemental sulphur. On the following four days an average of 53+/-24.8mg of which 10.6+/-7.25mg was determined to be elemental sulphur. On the last day 550mg of particulate matter was collected of which 110mg of elemental sulphur was determined to be elemental sulphur.

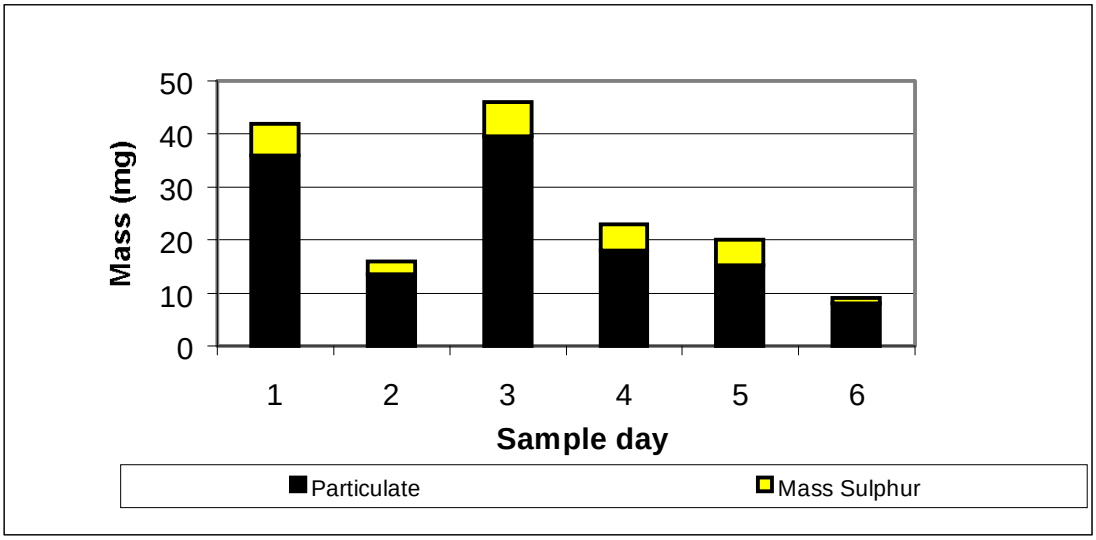


Figure 6.10 Daily mass of particulates collected from effluent and the proportion of the mass that was made up by sulphur with the reactor being run at 5.6mL.min^{-1}.

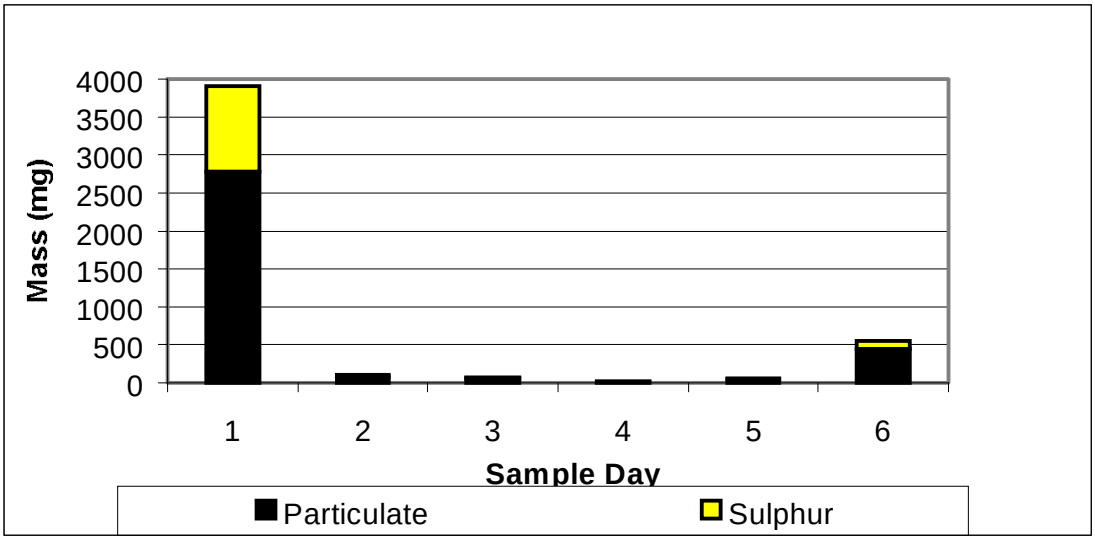


Figure 6.11 Daily mass of particulates collected from effluent and the proportion of the mass that was made up by sulphur with the reactor being run with purging ever 3 hydraulic retention times

6.3.4 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 6.12 and 6.13. Figure 6.12 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 –415mV quickly as the flow rate and sulphide loading are increased (at 5.35 hours) but returned to the previous level of -382mV after 0.592 h (35.52 minutes) (see Figure 6.13). The increased sulphide load did not affect the oxidising capacity of the biomass once the load had washed out of the reactor.

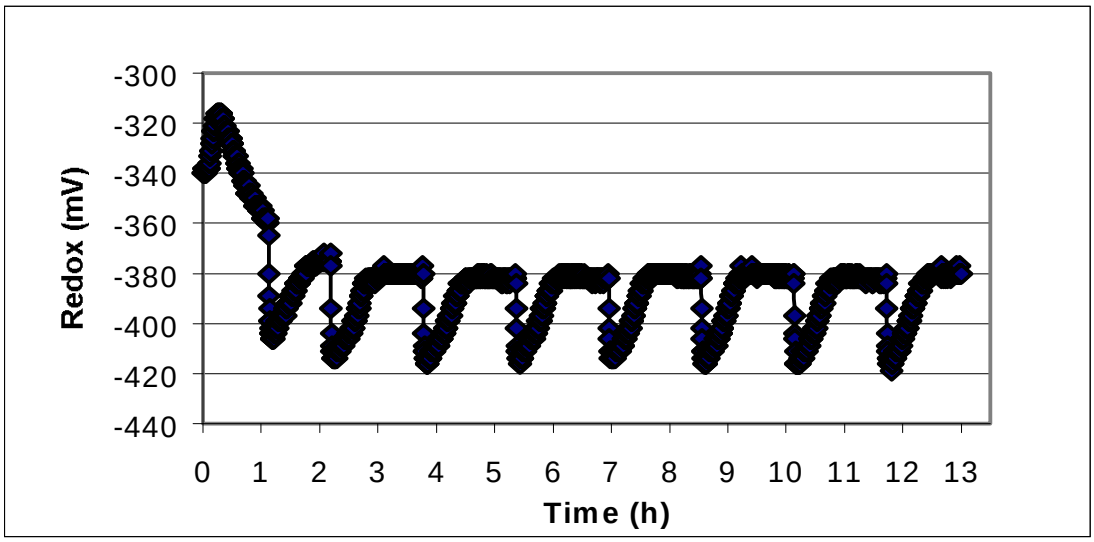


Figure 6.12 Measured redox potential over a 13 hour period with Silicone Tubular Reactor being run with purging every 3 hydraulic retention times

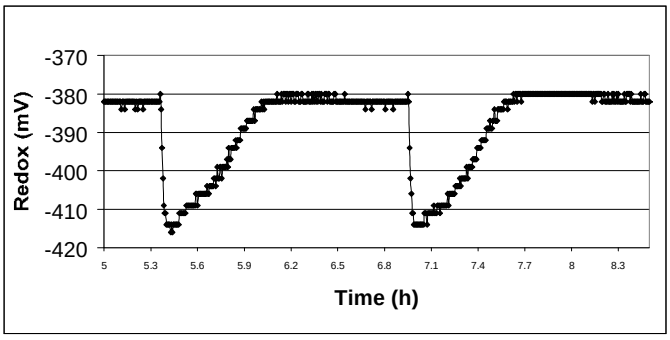


Figure 6.13 Measured redox potential between 5 and 8.3 hours of the same experiment shown in Figure 6.12, highlighting the time taken for the reactor to return to previous condition after purging.

6.3.5 EM and light studies on population present in silicone reactor

Scanning electron micrographs of the attached bacterial population present in the Silcone Tubular Reactor are shown in Figures 6.14 to 6.20. The following was noted:

1) A diverse bacterial biofilm had developed on the tube wall. Examples of cocci, bacilli and filamentous organisms were noted;

2) The biofilm contained large amounts of a polymeric substance, probably exopolysaccharide (Figure 6.14);

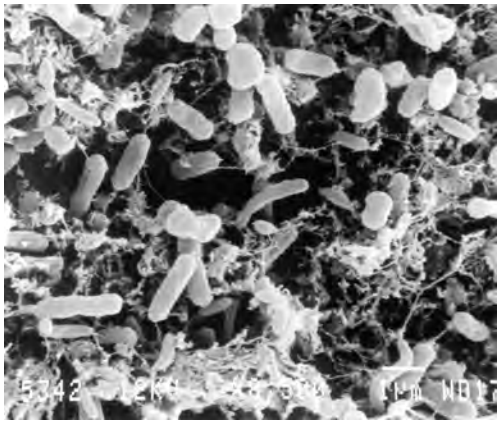


Figure 6.14 Scanning electron micrograph of attached bacterial population from uppermost section of the reactor. Evidence of polymeric exopolysaccharide production and a diverse bacterial population are shown.

3) Microbiologically produced elemental sulphur was observed only in sections from the first four meters of the reactor (Figure 6.15 and Figure 6.16). The population present in this area was varied with a variety of bacteria exhibiting extracellular sulphur globules;

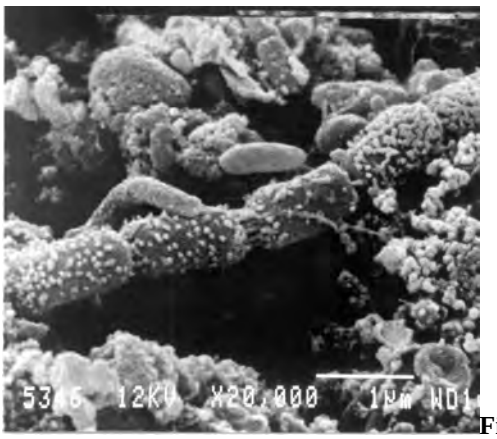


Fig.6.15

Fig 6.16

Figures 6.15 and 6.16 Scanning electron micrographs of attached bacterial population from the second portion of the reactor that was sampled. Evidence of bacterial sulphur production is shown with both filamentous and coccal bacteria producing elemental sulphur

4) Areas of apparently single microbial morphology were noted (Figure 6.17);

Figure 6.17 Scanning electron micrograph of attached bacterial population from the third section of the reactor that was sampled. A relatively dense, but uniform bacterial population seems to have developed

5) Large crystals that were suspected to be elemental sulphur were observed as part of the biofilm (Figures 6.18 and 6.19);

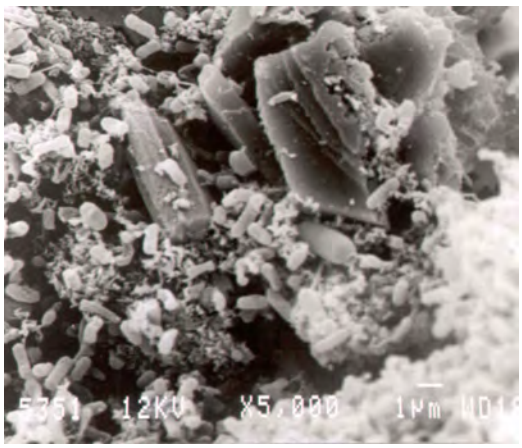


Figure 6.18 Scanning electron micrograph of bacterial population from the fourth section of the reactor that was sampled. Apart from the bacterial population, which developed large crystalline structures, suspected to be sulphur were observed.

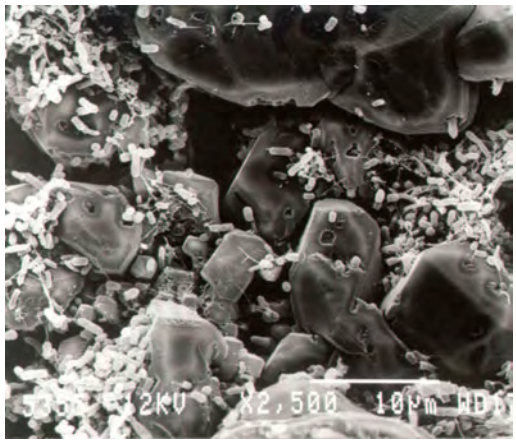


Figure 6.19 Scanning electron micrograph of the bacterial population from the fifth section of the reactor that was sampled. Crystalline structures suspected to be elemental sulphur are observed. Bacterial interaction with this crystalline sulphur is also observed

6) Possible evidence of the bacterial colonisation of elemental sulphur was noted. Pitting of the large crystalline structures was noted (Figure 6.20);

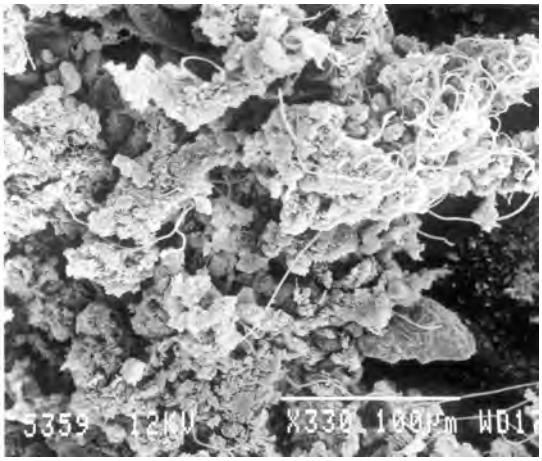


Figure 6.20 Scanning electron micrograph of the attached bacterial population from the sixth section of the reactor that was sampled. Evidence for the development of a filamentous bacterial population was observed.

7) 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 6.21). This is where the sulphide loading rate was lowest.

6.3.6 Phase contrast light microscopy

Reactor effluent was viewed under phase contrast light microscopy at 400 X magnification. Photographs of sloughed pieces of biofilm are shown in Figures 6.21 and 6.22. Elemental sulphur appears as bright specks under phase contrast conditions. Elemental sulphur was observed to be present in a number of the biofilm fragments present in the reactor effluent. Bright white areas, consistent with the presence of elemental sulphur were observed. The sulphur seemed to be present within discrete areas of the biofilm fragments.

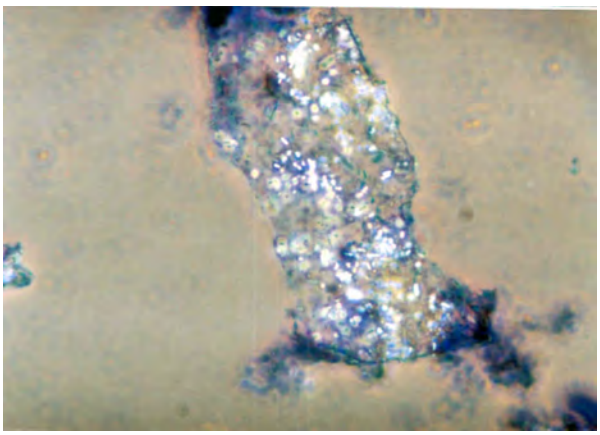


Figure 6.21 Biofilm fragment observed under phase contrast at 400X. The bright white areas represent elemental sulphur. The elemental sulphur appears to be associated with bacterial growth.

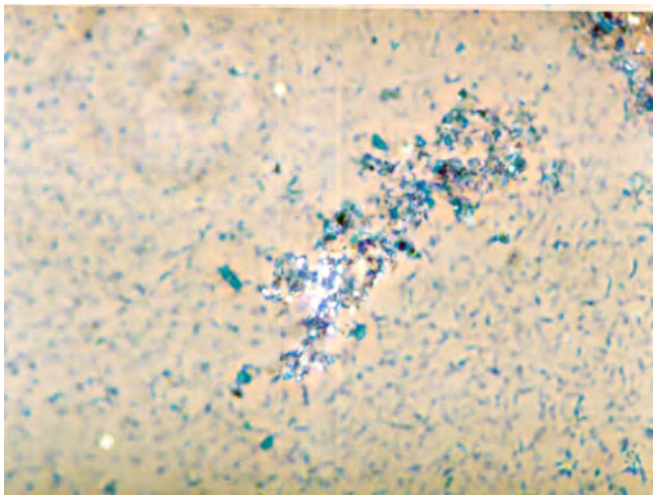


Figure 6.22 Biofilm fragment observed under phase contrast at 400X magnification. Elemental sulphur production is noted in discrete sections of the biofilm.

6.4 Discussion

6.4.1 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 is probably due to a lack of an attached biofilm. The rate of sulphide oxidation during the first 12 hours was 4.32×10^{-4} $M.h^{-1}$ ($8.1mg.L^{-1}.h^{-1}$) which compares well with the predicted initial chemical oxidation rate of a 100 mg. $L^{-1}.h^{-1}$ HS^- at an oxygen concentration of $3mg.L^{-1}$. 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.

The chemical 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 critcal role in determining the oxygen transfer capability to the developed biomass.

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

6.4.2 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 12.

It is probable that the reactor was initially operating under non-steady state coditions 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.

Sulphide loading rate for reactor of this type where oxygen 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.

6.4.3 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**.**

6.4.4 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 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.

6.5 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 reactor 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 6.14 and 6.15). 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.

The trickle filter and drowned trickle filter that were investigated prior to the silicone tube reactor both had the disadvantage of inability to control the oxygen supply to the sulphide oxidising zones of the reactors, resulting in very little sulphur being produced as a product of sulphide oxidation. Furthermore the development of filamentous sulphur accumulating organisms was shown to occur which could not be easily removed once established within the reactor. Due to the oxygen permeability and flexibility of silicone, an environment seems to be created in which sulphur can be produced biologically and

the possibility exists where filamentous populations can easily be removed from the tubing periodically, possibly enhancing the sulphur recovery process.

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 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.

Chapter 7

General Discussion and Conclusion

Biological oxidation of sulphide under high organic concentrations has been reported on in natural environments with chemical parameters and the bacterial populations present in these natural systems being well documented. Little work has been carried out on the potential biotechnological application of bacterial sulphide oxidation under high organic concentrations, although the biotechnological applications and development of such technology would be diverse. The development of a reliable technology for sulphur recovery under heterotrophic conditions would increase the choice of possible carbon sources and especially waste carbon sources for biotechnological sulphate reduction. A process that can produce large amounts of elemental sulphur from sulphide under heterotrophic conditions would contribute significantly to the development of an integrated biological process to treat the large volumes of AMD which are predicted to occur in South Africa.

In an attempt to develop a biotechnological sulphide oxidising process the chemical parameters of abiotic and biotic sulphide oxidation were studied in classical flask experiments. Results suggested that the presence of organics and a heterotrophic bacterial population were able to decrease oxygen concentrations thereby favouring biological sulphide oxidation and the subsequent formation of elemental sulphur as an oxidation porduct. Results of these studies also suggested that the overall oxygen supply rate was a key parameter in determining the major product of microbial sulphide oxidation under heterotrophic conditions.

Armed with this knowledge a Fixed-Film Trickle Filter system was assessed for its feasibility as a reactor configuration for the biotechnological production of sulphur from sulphide. The Fixed-Film Trickle Filter system proved to be less than ideal as a reactor configuration for this purpose with the supply of the correct amount of oxygen to the correct bacterial population being difficult to achieve in practice.

In an attempt to deliver a more predictable and uniform oxygen profile to the sulphide oxidising population a Submerged Trickle-Filter Reactor with counter-current liquid/gas flows was investigated. This reactor had the disadvantage of accumulating large amounts

of sulphur within the reactor, making significant sulphur recovery difficult. This sulphur accumulation was ascribed to the development of filamentous sulphide oxidising population, consisting of *Thiothrix* and *Beggiatoa* species. The functional area of the Submerged Trickle-Filter Reactor was found to be small in comparison with the total reactor volume with most of the sulphide oxidation occurring at the top of the reactor at the oxygen/sulphide interface. Any extracellular sulphur that was produced in this upper zone was found to be oxidised at lower levels in the reactor.

Based on observations of the development of white films in silicone tubing in which non-sterile sulphidic solutions were being pumped, and that sulphide oxidation reactions, mediated by bacteria were prone to take place at interfaces, a simple length of silicone was assessed as a possible reactor configuration. Results of investigations on this Silicone Tubular Reactor showed that an attached biofilm developed within the reactor. Electron microscopy studies revealed that the prevailing microbial population changes down the length of the reactor as the chemical environment changed.

Once a bacterial biofilm was established on the tube wall, sulphur was detected as a component of the effluent and sulphur made up about 20% by mass of the biofilm. Electron microscopy studies revealed the presence of a bacterial population that was producing extracellular sulphur deposits. The presence of these extracellular deposits was taken as evidence of either obligate or facultative autotrophic growth. Autotrophic growth was only observed near the top of the reactor where sulphide concentrations were highest and this type of metabolism would offer a selective advantage to the bacterial population present in this environment. Sulphur crystal accumulations were noted further down the reactor and were different from the sulphur globules associated with bacterial autotrophic metabolism. The possibility exists that bacterial sulphur production near the top of the reactor results in crystallisation of sulphur in lower sections of the reactor. The use of silicone membranes presents a possible novel manner in which to produce sulphur under heterotrophic conditions although further work will be required to develop this technology to a process level. Of prime importance will be the determination of optimal sulphide loading rates under various biomass conditions and the development of a reliable means of harvesting accumulated sulphur within the reactor.

7.1 Conceptual Model

The investigations carried out here and a survey of the literature suggests that biological sulphur production is highly dependent on supplying the correct oxygen to sulphide consumption ratio. Based on these observations the following conceptual model is proposed:

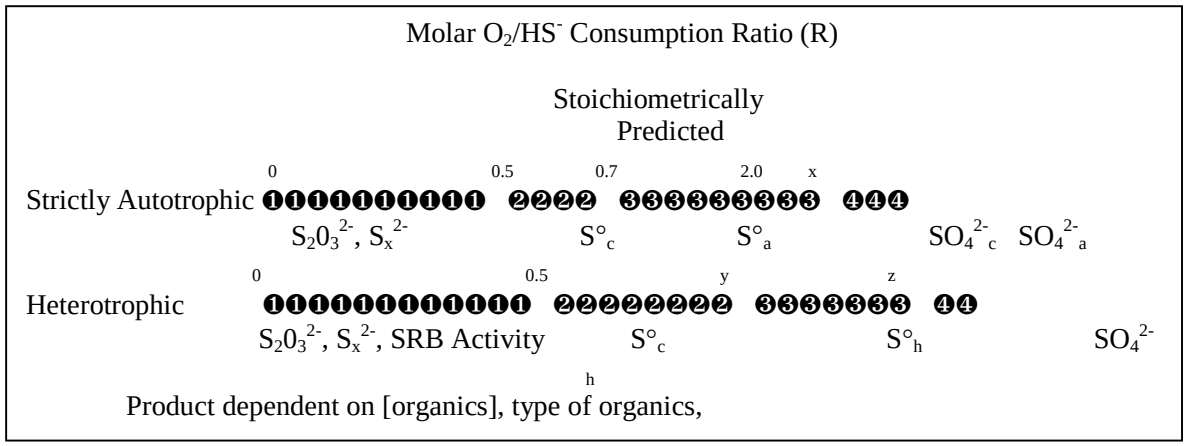


Figure 7.1: Graphical representation of the proposed relationship between the molar oxygen/sulphide consumption ratio and the predominant product of sulphide oxidation under autotrophic and heterotrophic conditions respectively. $S°_c$ – Stoichiometrically predicted $0_2/HS^-$ consumption ratio at which elemental sulphur is the main product. $S°_a$ – Optimal $0_2/HS^-$ consumption ratio for elemental sulphur production under autotrophic conditions. $SO_4^{2-}{}_c$- Stoichiometrically predicted $0_2/HS^-$ consumption ratio above which SO_4^{2-} is main product. $SO_4^{2-}{}_a$- $02/HS^-$ consumption ratio under autotrophic conditions above which SO_4^{2-} is the predominant product. $S°_h$ - Optimal $0_2/HS^-$ consumption ratio for elemental sulphur production under heterotrophic conditions. $SO_4^{2-}{}_h$- $0_2/HS^-$ consumption ratio under autotrophic conditions above which SO_4^{2-} is the predominant product

Table 7.1 Predicted scenarios in a biological sulphide oxidising environment for different O_2: HS^- consumption rates.

Scenarios	Effect on reactor
R << y	High HS^- breakthrough (Biomass overloaded and reduction of oxidised product occurring) (Region 1)
R< y	S_n^{2-}, $S_2O_3^{2-}$ and HS^- breakthrough (Region 2)
R = y	S° production predominates. Intracellular vs. extracellular production determined by absolute $[HS^-]$ micro-organisms exposed to.
y<R<z	S° and SO_4^{2-} both produced (Region 3)
R>z	$S0_4^{2-}$ predominant product.

Development of an efficient biological process for the production of elemental sulphur from sulphide is dependent on determining the ratio value y and then designing the reactor such that that ratio is maintained in a large a portion as possible. In a silicone tube type system this could be accomplished by employing tubing of different wall thicknesses

down the length of the reactor or by changing the tube diameter to change the flow rate down the reactor length.

7.2 The silicone reactor as a model for the study of floating sulphur biofilms

In natural environments biological sulphur production and in fact the prevalence of sulphide oxidising bacteria have been reported to occur at aqueous sulphide/air interfaces where bacteria can efficiently compete with chemical oxidation. An example of this phenomenon is the development of floating sulphur biofilms on the surface of sulphide-rich waters such as sulphate reducing bioreactors and tannery waste stabilisation ponds (Gilfillan 2000). These floating sulphide oxidising biofilms have attracted attention in the Rhodes University Environmental Biotechnology Group due to their potential as biotechnological sulphur processes and as models for the development of fundamental knowledge on the underlying microbial ecology.

A number of difficulties may be encountered in studying these floating sulphur biofilms. Floating sulphur biofilms tend to be very thin (no more that 2-3mm thick) biofilms rendering them very fragile. Collection of representative samples of these biofilms is difficult. The development of the Silicone Tubular Reactor may be regarded as an attached version of these floating biofilms and presents a model environment for studying these biofilms and represent a vertical section of these floating biofilms along its length. Work currently being carried out in the Molecular Microbial Ecology Unit of the Environmental Biotechnology Group at Rhodes University will contribute to this hypothesis and will be reported by Bowker (2001)

7.3 Proposed Future Investigations

Based on the work conducted during this study the following future work could be carried out to assess the feasibility of utilizing a silicone based membrane system as a potential reactor configuration for the biotechnological production of elemental sulphur under heterotrophic conditions:

1) The effect of overall organic concentration on:
    - Overall sulphide oxidation rate

- The type of populations that form in the reator
- Maximum sulphur production
- Settling characteristics of the sulphur produced

2) Effect of specific organic molecules eg. VFA and lignocellulosic breakdown products on particularly overall sulphide oxidation rate and the settling properties of the sulphur produced

3) Reactor configurations other than a Silicone Tubular Reactor system. Other configurations could include some form of sheet membrane sytem where sulphide rich water flows between sheets of silicone membrane.

4) Assessment of the kinetics of sulphur containing biofilm develoment on the silicone surface.

5) Investigations into effective ways in which to harvest the sulphur rich biofilm.

7.4 Conclusion

The work carried out in fulfillment of this thesis suggests that elemental sulphur may be produced as a major product of biological sulphide oxidation under heterotrophic conditions. Work carried out here and the flask experiments in particular, suggest that the presence of organics is able to poise redox conditions such that elemental sulphur is the predominant product of biological sulphide oxidation. It seems feasible to propose that this buffering of redox conditions occurs as a result of background heterotrophic metabolism during which oxygen is consumed, creating optimal redox conditions for oxidation of sulphide to sulphur.

Chapter 8

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