BIOOXIDATION OF SULPHIDE UNDER DENITRIFYING CONDITIONS IN AN IMMOBILIZED CELL BIOREACTOR

A Thesis Submitted to the College of Graduate Studies and Research in Partial Fulfillment of the Requirements for the Degree of Master of Science in the Department of Chemical Engineering University of Saskatchewan Saskatoon

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ABSTRACT

Hydrogen sulphide (H₂S) is a serious problem for many industries, including oil production and processing, pulp and paper, and wastewater treatment. In addition, H₂S is usually present in natural gas and biogas. It is necessary to control the generation and release of H₂S into the environment because H₂S is corrosive, toxic, and has an unpleasant odour. In addition, the removal of H₂S from natural gas and biogas is essential for preventing the emission of SO₂ upon combustion of these gases. Physicochemical processes have been developed for the removal of H₂S. These processes employ techniques such as chemical or physical absorption, thermal and catalytic conversion, and liquid phase oxidation. In comparison, biological processes for the removal of sulphide typically operate at ambient temperature and pressure, with the feasibility for the treatment of smaller streams, and the absence of expensive catalysts. The objective of the present work was to study the biooxidation of sulphide under denitrifying conditions in batch system and a continuous immobilized cell bioreactor using a mixed microbial culture enriched from the produced water of a Canadian oil reservoir.

In the batch experiments conducted at various initial sulphide concentrations, an increase in the sulphide oxidation and nitrate reduction rates was observed as the initial sulphide concentration was increased in the range 1.7 to 5.5 mM. An extended lag phase of approximately 10 days was observed when sulphide concentrations around or higher than 14 mM were used. This, when considered with the fact that the microbial culture was not able to oxidize sulphide at an initial concentration of 20 mM, indicates the inhibitory effects of sulphide at high concentrations.

The effect of the initial sulphide to nitrate concentrations ratio (ranging from 0.3 to 4.0) was also studied. As the initial sulphide to nitrate ratio decreased, the sulphide oxidation rates increased. The increasing trend was observed for initial nitrate concentrations in the range of 1.3 to 7.3 mM, corresponding to ratios of 4.08 to 0.83. The increase in nitrate reduction rates was more pronounced than that of the sulphide oxidation rates. However at nitrate concentrations higher than 7.3 mM (ratios lower than 0.83) the nitrate
reduction rate remained constant. The percentage of sulphide that was oxidized to sulphate increased from 2.4% to 100% as the initial sulphide to nitrate ratio decreased from 4.08 to 0.42. This indicated that at ratios lower than 0.42, nitrate would be in excess and at ratios exceeding 4.08, nitrate would be limiting.

In the continuous bioreactor systems, at sulphide loading rates ranging from 0.26 to 30.30 mM/h, sulphide conversion remained in the range of 97.6% to 99.7%. A linear increase in the volumetric oxidation rate of sulphide was observed as the sulphide loading rate was increased with the maximum rate being 30.30 mM/h (98.5% conversion). Application of immobilized cells led to a significant increase in oxidation rate of sulphide when compared with the rates obtained in a bioreactor with freely suspended cells. At nitrate loading rates ranging from 0.19 to 24.44 mM/h, the nitrate conversion ranged from 97.2% to 100% and a linear increase in volumetric reduction rate was observed as the nitrate loading rate was increased, with the maximum rate being 24.44 mM/h (99.7% conversion).

A second bioreactor experiment was conducted to investigate the effects of sulphide to nitrate concentrations ratio on the performance of the system. Sulphide conversion was complete at sulphide to nitrate ratios of 1.1 and 1.3, but decreased to 90.5% at the ratio of 3.1 and 65.0% at the ratio of 5.0, indicating nitrate was limiting for sulphide to nitrate ratios of 3.1 and 5.0. The increase in the sulphide to nitrate ratio (and the resulting limitation of nitrate) caused a decrease in the volumetric reaction rate of sulphide.

Nitrate conversion was complete at sulphide to nitrate ratios of 1.3, 3.1, and 5.0; however, at a ratio of 1.1, the conversion of nitrate dropped to 59.6%, indicating that nitrate was in excess, and sulphide was limiting. The volumetric reaction rate of nitrate decreased as the sulphide to nitrate ratio increased for ratios of 1.3, 3.1, and 5.0; this was due to the decrease in the nitrate loading rate. For sulphide to nitrate ratios of 1.1 and 1.3, 7.2% and 19.6% of the sulphide was converted to sulphate, respectively. At ratios of 3.1 and 5.0, no sulphate was generated. For ratios between 1.3 and 5.0, an increase in the ratio caused a decrease in the generation of sulphate.
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DEDICATION

This thesis is dedicated to my family and friends for their support throughout this process. To my mother, Charlene Nijhawan, and my father, Eric Tang, for creating a life for me in which this was possible. Thank you to my brothers and sisters, Jeffrey, Janelle, Steven, Samara, and Rachel, for helping me maintain balance in my life. Finally, to my friends, particularly those who pursued graduate studies with me, for understanding without needing to understand. Thank you.
# TABLE OF CONTENTS

**PERMISSION TO USE**................................................................. i

**ABSTRACT** ............................................................................. ii

**ACKNOWLEDGEMENTS** ......................................................... iv

**DEDICATION** ........................................................................... v

**TABLE OF CONTENTS** ............................................................ vi

**LIST OF TABLES** ..................................................................... x

**LIST OF FIGURES** ................................................................... xi

1. **INTRODUCTION** ................................................................. 1

2. **LITERATURE REVIEW** .......................................................... 4

   2.1 Applications of Sulphide Biooxidation ........................................ 4

      2.1.1 Removal of H₂S from Gaseous Streams ................................. 4

      2.1.2 Control of Souring in Oil Reservoirs ..................................... 4

      2.1.3 Treatment of H₂S-containing Wastewater and Produced Water .. 5

      2.1.4 Generation of Electricity .................................................... 6

   2.2 Physicochemical Methods of Sulphide Removal .......................... 6

      2.2.1 Alkanolamine Process ...................................................... 6

      2.2.2 Claus Process ................................................................. 8

      2.2.3 LO-CAT® Process .......................................................... 9

   2.3 Biological Removal of Sulphide ............................................... 11

      2.3.1 Biological Sulphur Cycle Reactions ...................................... 11

      2.3.2 Indirect Oxidation of Sulphide .......................................... 13

      2.3.3 Direct Biooxidation of Sulphide ......................................... 14

         2.3.3.1 Phototrophic Oxidation .............................................. 14

         2.3.3.2 Chemolithotrophic Oxidation .................................... 15

   2.4 Kinetics of Sulphide Biooxidation .......................................... 17

      2.4.1 Indirect Biooxidation Kinetics ........................................... 17

      2.4.2 Direct Biooxidation Kinetics ............................................ 17
2.4.2.1 Phototrophic Biooxidation Kinetics ............................................. 17
2.4.2.2 Chemolithotrophic Biooxidation Kinetics ...................................... 20
2.4.3 Bioreaction End Products ............................................................... 25
  2.4.3.1 Phototrophic Bioreaction End Products ...................................... 25
  2.4.3.2 Chemolithotrophic Bioreaction End Products ................................ 27

3. RESEARCH OBJECTIVES ......................................................................... 30

4. MATERIALS AND METHODS .............................................................. 31
  4.1 Microbial Culture and Medium ........................................................... 31
    4.1.1 Medium ...................................................................................... 31
    4.1.2 Culture Conditions ...................................................................... 31
    4.1.3 Bioreactor Medium ..................................................................... 32
  4.2 Batch Experiments .............................................................................. 32
    4.2.1 Experimental Conditions ............................................................. 32
    4.2.2 Effect of Initial Sulphide Concentration ...................................... 33
    4.2.3 Effect of Initial Sulphide to Nitrate Ratio .................................... 33
  4.3 Specifications of the Up-Flow Packed Bed Bioreactors and
    Experimental Systems ........................................................................... 34
  4.4 Experimental Procedures .................................................................. 36
    4.4.1 Batch Operation of Bioreactor .................................................... 36
    4.4.2 Continuous Operation of Bioreactor ........................................... 37
    4.4.3 Effects of Volumetric Loading Rate of Sulphide ......................... 37
    4.4.4 Effects of Initial Sulphide to Nitrate Ratio .................................. 38
  4.5 Analytical Methods .......................................................................... 39
    4.5.1 Measurement of Sulphide ........................................................... 39
    4.5.2 Measurement of Nitrate, Nitrite, Sulphate, and Thiosulphate ........ 39
    4.5.3 Measurement of Sulphur ............................................................. 40
    4.5.4 Measurement of Protein ............................................................. 40
    4.5.5 X-Ray Diffraction ....................................................................... 41
  4.6 Statistical Methods ........................................................................... 41
    4.6.1 Sulphide Analysis ....................................................................... 41
    4.6.2 Ion Chromatography .................................................................. 42
5. **RESULTS AND DISCUSSION** ................................................................. 43

5.1 Batch Experiments ................................................................................. 43

5.1.1 Effect of Initial Sulphide Concentration ........................................... 43

5.1.2 Effect of Sulphide to Nitrate Ratio .................................................. 47

5.2 Continuous Bioreactor Experiments ..................................................... 56

5.2.1 Effect of Volumetric Loading Rate of Sulphide ................................. 56

5.2.2 Total Protein Concentration in the Liquid Phase .............................. 59

5.2.3 Analysis of Solid Samples Taken from the First Bioreactor ............... 60

5.2.3.1 X-Ray Diffraction and Sulphur Analysis ...................................... 60

5.2.3.2 Quantity of Attached Biomass (mg Protein/mg Sand) ..................... 62

5.2.4 Effect of Sulphide to Nitrate Ratio .................................................... 64

5.2.5 Comparison to Previous Work ....................................................... 68

6. **CONCLUSIONS AND RECOMMENDATIONS** .............................. 70

6.1 Conclusion ............................................................................................ 70

6.1.1 Batch Experiments ........................................................................... 70

6.1.2 Continuous Experiments .................................................................. 71

6.2 Recommendations for Future Work .................................................... 72

7. **REFERENCES** ................................................................................... 74

8. **APPENDICES** ..................................................................................... 79

A. Sample Calculations ................................................................................ 79

A.1 Sulphide Oxidation Rate and Nitrate Reduction Rate ............................ 79

A.2 Volumetric Loading Rate ..................................................................... 80

A.3 Volumetric Reaction Rate .................................................................... 81

A.4 Percent Conversion .............................................................................. 82

B. Experimental Data from Batch Experiments ........................................... 83

B.1 Effect of Initial Sulphide Concentration ............................................. 83

B.2 Effect of Initial Sulphide to Nitrate Ratio .......................................... 84

B.3 Control Experiments ......................................................................... 96

C. Calibration Curves for Analytical Methods .......................................... 99

C.1 Calibration Curve for Sulphide Measurement .................................... 99
C.2 Calibration Curve for Total Protein Measurement ............................................................ 100
LIST OF TABLES

Table 2.1. Stoichiometry for complete oxidation of various electron donors with sulphate reduction

Table 2.2 Environmental conditions for optimum growth of several phototrophic sulphur oxidizing species

Table 2.3 Environmental conditions for optimum growth of several chemolithotrophic sulphur oxidizing species

Table 2.4 Summary of phototrophic sulphide biooxidation kinetics and the corresponding conditions as reported in various works

Table 2.5 Chemolithotrophic sulphide biooxidation kinetics and the corresponding conditions as reported in various works

Table 4.1 Experimental conditions in the first bioreactor

Table 4.2 Experimental conditions in the second experimental run

Table 4.3 Standard deviation values for ion chromatography measurements

Table 5.1 Summary data for batch experiments conducted at various initial sulphide concentrations

Table 5.2 Summary data for batch experiments for various sulphide to nitrate ratios

Table 5.3 Sulphur deposition in the first bioreactor

Table 5.4 Protein content of the sand matrix in the first bioreactor

Table 5.5 Comparison of sulphide oxidation rate to previous works
LIST OF FIGURES

Figure 2.1. Simplified flow diagram of the alkanolamine process ......................6
Figure 2.2. Claus process diagram .......................................................................9
Figure 2.3. Conventional LO-CAT® process diagram .......................................11
Figure 2.4. Biological sulphur cycle ....................................................................12
Figure 2.5. H₂S utilization rates versus light energy for S⁰-optimized trials .......25
Figure 2.6. Modified van Niel curve adapted from Henshaw and Zhu (2001) ....26
Figure 4.1. Schematic diagram of the experimental setup (top) and photograph of experimental setup (bottom) ............................................................35
Figure 5.1. Sulphide, sulphate, nitrate, and thiosulphate concentrations profile during the oxidation of sulphide at initial sulphide concentrations of 1.9 mM (at left) and 1.7 mM (at right) .......................................................................................44
Figure 5.2. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide concentration of 5.4 mM ........................................................................................................46
Figure 5.3. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide concentration of 14.4 mM ........................................................................................................47
Figure 5.4. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratio of 4.08 ........................................................................................................49
Figure 5.5. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratio of 2.30 ........................................................................................................50
Figure 5.6. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratios of 1.14 (at left) and 1.12 (at right) .................................................................51
Figure 5.7. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratio of 0.77. ........................................................................................................52
Figure 5.8. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratio of 0.56.

Figure 5.9. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratio of 0.46.

Figure 5.10. Sulphide and nitrate removal rates as functions of the initial sulphide to nitrate ratio.

Figure 5.11. The effect of the initial sulphide to nitrate ratio on the percentage of sulphide converted to sulphate.

Figure 5.12. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at initial sulphide to nitrate ratio of 1.20.

Figure 5.13. Transient data for the first bioreactor.

Figure 5.14. The effect of the sulphide loading rate on the performance of the first bioreactor.

Figure 5.15. The effect of the nitrate loading rate on the performance of the first bioreactor.

Figure 5.16. Protein concentration in the liquid phase for steady states at each sulphide loading rate for the first bioreactor.

Figure 5.17. First bioreactor after completion of experimental run.

Figure 5.18. Comparison of the volumetric oxidation rate of sulphide in the first and the second bioreactors.

Figure 5.19. Comparison of the volumetric reduction rate of nitrate in the first and the second bioreactors.

Figure 5.20. Transient data from ports 1 and 3 during sulphide to nitrate ratio experiments.

Figure 5.21. The effect of the initial sulphide to nitrate ratio.

Figure 5.22. Effect of the sulphide to nitrate ratio on the volumetric oxidation rate and percent conversion of sulphide.

Figure 5.23. Effect of sulphide to nitrate ratio on volumetric reduction rate and percent conversion of nitrate.
Figure A.1.1. Complete sulphide and nitrate data for batch experiment with initial sulphide concentration of 14.4 mM ..............................................................79

Figure A.1.2. Sulphide and nitrate data selected for rate calculations. Linear trendlines and equations are shown for both sulphide and nitrate .......................80

Figure B.1.1. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide concentration of 5.5 mM .................................................................................................83

Figure B.1.2. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide concentration of 15.8 mM .................................................................................................84

Figure B.2.1. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 4.08. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small ................................................85

Figure B.2.2. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 4.08. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small ................................................85

Figure B.2.3. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 2.30. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small ................................................86

Figure B.2.4. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 2.30. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small ................................................86

Figure B.2.5. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 2.17. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small ................................................87
Figure B.2.6. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 2.17. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small........................................................................... 87

Figure B.2.7. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 1.14. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small........................................................................... 88

Figure B.2.8. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 1.14. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small........................................................................... 88

Figure B.2.9. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 1.12. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small........................................................................... 89

Figure B.2.10. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 1.12. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small........................................................................... 89

Figure B.2.11. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 0.83. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small........................................................................... 90

Figure B.2.12. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.83. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small........................................................................... 90

Figure B.2.13. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate
ratio of 0.77. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.................................................................91

Figure B.2.14. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.77. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.................................................................91

Figure B.2.15. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 0.59. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.................................................................92

Figure B.2.16. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.59. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.................................................................92

Figure B.2.17. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 0.56. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.................................................................93

Figure B.2.18. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.56. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.................................................................93

Figure B.2.19. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 0.46. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.................................................................94

Figure B.2.20. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.46. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.................................................................94
Figure B.2.21. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 0.42. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small...............................................................95

Figure B.2.22. Complete set of data (290 h) for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.42. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small...........................................................................95

Figure B.3.1. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at an initial sulphide concentration of 6.2 mM, and an initial sulphide to nitrate ratio of 0.59.................................................................96

Figure B.3.2. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at an initial sulphide to nitrate ratio of 4.16. ..........97

Figure B.3.3. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at an initial sulphide to nitrate ratio of 2.20. ..........97

Figure B.3.4. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at an initial sulphide to nitrate ratio of 0.77. ..........98

Figure B.3.5. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at an initial sulphide to nitrate ratio of 0.39. ..........98

Figure C.1.1. Calibration curve for sulphide measurement. Error bars indicate one standard deviation. Some error bars are not visible as the associated error is small.................................................................99

Figure C.2.1. Calibration curve for protein measurement. Error bars indicate one standard deviation. Some error bars are not visible as the associated error is small. .................................................................100
1. INTRODUCTION

Hydrogen sulphide (H$_2$S) is a problem in many industries, including oil production and processing, pulp and paper, and wastewater treatment. Given that H$_2$S is corrosive, toxic, and has an unpleasant odour, it is necessary to control its generation and release into the environment. In addition, H$_2$S is usually present in natural gas and biogas. The removal of H$_2$S from natural gas and biogas is essential for preventing the emission of SO$_2$ upon combustion of these gases. Physicochemical processes have been developed for the removal of H$_2$S; these processes employ techniques including chemical or physical absorption, thermal or catalytic conversion, and liquid phase oxidation. Three common processes are the alkanolamine process, the Claus process, and the LO-CAT$^\text{®}$ process. In comparison, biological processes for the removal of sulphide which rely on biooxidation of sulphide by microorganisms offer the advantage of operation at ambient temperature and pressure, feasibility for the treatment of smaller streams, and the absence of expensive catalysts.

Biological sulphide oxidation can occur indirectly or directly. The indirect biooxidation of sulphur involves a secondary oxidizing agent which oxidizes the sulphide and is regenerated by a microorganism. The direct biooxidation of sulphide can occur phototrophically or chemolithotrophically. Phototrophic biooxidation of sulphide is generally anaerobic, while chemolithotrophic biooxidation of sulphide can be aerobic or anaerobic. In anaerobic chemolithotrophic biooxidation of sulphide, an electron acceptor such as nitrate is required. Biooxidation of sulphide has been studied extensively. However, information regarding the kinetics of sulphide biooxidation in biofilm systems is rather limited. Furthermore, the majority of earlier works have utilized pure cultures of sulphide oxidizing bacteria. The efficiency of this process with a mixed microbial culture, especially a culture originated from an oil reservoir, has not been studied extensively. In addition, many of these cultures have a low tolerance for sulphide, which limits their application.
The objective of this work was to study the biooxidation of sulphide in conjunction with the reduction of nitrate using a microbial culture enriched from the produced water of a Canadian oilfield reservoir. The focus of this work is the biooxidation of sulphide. Simultaneous reduction of nitrate is carried out because the microbial culture used in this work is anaerobic and utilizes nitrate or nitrite as an electron acceptor in sulphide oxidation. The combination of sulphide oxidation and nitrate reduction could treat waste streams which contain sulphide and nitrate. In addition, conducting this study under denitrifying conditions (anaerobic) would provide insight regarding the in situ removal of sulphide in oil reservoirs which is carried out through the addition of nitrate to injection water.

The biooxidation of sulphide was studied in a batch system as well as in continuous immobilized cell bioreactors. In the batch system the effects of sulphide initial concentration and sulphide to nitrate concentration ratio on the kinetics and stoichiometry of the system were investigated. The experiments in immobilized cell bioreactors aimed to evaluate the possibility for improving the reaction rate and to identify the effects of sulphide volumetric loading rate and the ratio of sulphide to nitrate on the extent of reaction rate and the composition of the end products.

The thesis presented here consists of seven chapters. Chapter 1 provides general information regarding the research topic, objectives and scopes of the research. Chapter 2 includes a detailed literature review of the applications of sulphide biooxidation, the physicochemical processes developed for the removal of sulphide, a brief description of biological sulphur cycle and a detailed review and comparison of previous works on biological removal of sulphide with both phototrophic and chemolithotrophic organisms. Chapter 3 briefly discusses the knowledge gap and an outline of the objectives of this research. The materials and methods utilized in this research have been described in Chapter 4.

Chapter 5 contains the results of this work. This includes the experimental data as well as the discussion of the results from the batch experiments. The results and discussion of the experimental data from the continuous experiments are also provided in Chapter 5. Conclusions from this work and
recommendations for future work are included in Chapter 6. Chapter 7 provides the list of references. Sample calculations and additional experimental data which were not included in the results section are included in the appendix.
2. LITERATURE REVIEW

2.1 Applications of Sulphide Biooxidation

The applications of sulphide biooxidation are widespread. Such applications include: removal of H$_2$S from gaseous streams; control of souring in oil reservoirs; treatment of H$_2$S containing wastewater and produced water; control of emission of H$_2$S from livestock operations and generation of electricity during the treatment of sulphide containing streams.

2.1.1 Removal of H$_2$S from Gaseous Streams

Many processes produce gaseous streams which contain H$_2$S, including natural gas and biogas. It is necessary to remove H$_2$S from gaseous streams to prevent corrosion during transmission and distribution, as well as to prevent sulphur dioxide emission upon combustion of the gases (Mousavi et al., 2006). In the pulp and paper industry, effluent gas from processing equipment in the Kraft processes such as rotary kilns, evaporators and washers contains H$_2$S (Iliuta and Larachi 2003). Natural gas, whether produced from a condensate field or gas associated with an oil reservoir, frequently contains hydrogen sulphide (Maddox 1974). Biogas produced as a value added product from anaerobic digestion of agricultural wastes such as swine manure also contains H$_2$S (Hansen et al., 1998).

2.1.2 Control of Sourcing in Oil Reservoirs

In many oil fields, oil is produced by enhanced recovery methods, in which an external fluid, usually water in onshore and seawater in offshore reservoirs, is pumped into the reservoir through injection wells to increase the pressure in the
reservoir, thus forcing the residual oil out. Sourcing – an increase in the concentration of hydrogen sulphide – which can be caused chemically or biologically, is one of the major problems observed in oil reservoirs subjected to water flooding. Chemical souring occurs through thermochemical sulphate reduction or when metal sulphides such as pyrite in the rock dissolve. It is widely accepted that biological souring is the most significant mechanism of H$_2$S production in reservoirs subjected to enhanced recovery methods (Ollivier and Magot 2005). Biological souring is caused by sulphate reducing bacteria (SRB) which reduce the SO$_4^{2-}$, either available in the formation water or introduced by the injected water, to hydrogen sulphide using a variety of organic compounds available in the reservoir as carbon and/or energy sources (Antonio et al., 2000). Addition of nitrate and or sulphide-oxidizing bacteria to the injected water has been shown to effectively decrease the level of sulphide in oil reservoirs (Nemati et al., 2001).

2.1.3 Treatment of H$_2$S-containing Wastewater and Produced Water

Sulphide containing wastewaters are generated in various industries such as petrochemical plants, tanneries, and viscose rayon manufacturing. These wastewaters can also be produced as a result of anaerobic treatment of sulphate-containing wastewaters, such as those generated by the pulp and paper industry (Janssen et al., 1999). The formation of sulphide is also a common problem during anaerobic treatment of wastewater (van der Zee 2006). Agricultural activities, such as swine production, also produce wastewater streams containing sulphate that can be converted to sulphide by SRB (Hansen et al., 1998).

In addition to contaminating the oil and gas, souring in oil reservoirs subjected to water flooding results in contamination of the injected water with sulphide. The water which is recovered with oil and gas (commonly referred to as produced water) needs treatment prior to further use. Biooxidation of sulphide
has been suggested as an effective means to treat these sour waters (Nemati et al., 2001).

2.1.4 Generation of Electricity

Until recently, microbial fuel cells have been targeted primarily at converting carbon-based substrates to electricity. Given the prevalence of sulphur compounds in organic wastes and wastewaters, the use of a microbial fuel cell would turn a waste stream into a valuable energy source. A recent study by Rabaey et al. (2006) explored using microbial fuel cells to convert dissolved sulphide to elemental sulphur. The microbial fuel cells were capable of removing sulphate via sulphide and converting the produced sulphide to elemental sulphur, which demonstrated that digester effluents can be polished by a microbial fuel cell for both residual carbon and sulphur compounds (Rabaey et al., 2006).

2.2 Physicochemical Methods of Sulphide Removal

Currently, several well established physicochemical methods exist for the removal of H₂S from gaseous streams. These processes employ techniques including chemical or physical absorption, thermal or catalytic conversion, and liquid phase oxidation. Three common physicochemical processes are described below; the Alkanolamine process, the Claus process, and the LO-CAT® process.

2.2.1 Alkanolamine Process

Alkanolamines have been used as chemical absorbents for acidic gases since the 1930’s. Amines frequently used in commercial gas purification are monoethanolamine (MEA), diethanolamine (DEA), and methyldiethanolamine (MDEA). The selection of the amine is determined by the pressure, temperature and composition of the contaminated gas, as well as the purity requirements of the treated gas (Kohl and Nielsen 1997).
The alkanolamine process flow diagram is shown in Figure 2.1. Sour gas enters the bottom of the absorber (1) and flows countercurrently against the amine solution. Purified gas leaves the absorber. Lean amine solution is pumped from a heat exchanger (5) to the top of the absorber, flowing down across the trays and absorbing the acid components. The rich amine solution leaves the bottom of the absorber to a heat exchanger (2) where it is heated by lean solution prior to entering the stripper column (3). Heat is supplied from the amine reboiler (4) at the bottom of the stripper column and the acid gases are separated from the rich amine solution. The acid gas is cooled in a heat exchanger (6) to condense the excess steam prior to entering the reflux accumulator (7), and the condensate is pumped back to the column.

![Figure 2.1. Simplified flow diagram of the alkanolamine process (Adapted from Jensen and Webb, 1995).](image)

The acid gas generated in the alkanolamine process is typically treated in a sulphur recovery process, such as the Claus Process.
2.2.2 Claus Process

The Claus process is used to recover sulphur from gas streams containing hydrogen sulphide. It employs thermal and catalytic conversion of H₂S to elemental sulphur. The reactions of the Claus process are as follows (Monnery 2000):

\[ \text{H}_2\text{S} + \frac{3}{2}\text{O}_2 \rightarrow \text{SO}_2 + \text{H}_2\text{O} \]  \hspace{1cm} (2.1)

\[ 2\text{H}_2\text{S} + \text{SO}_2 \rightarrow \frac{3}{2}\text{S}_2 + 2\text{H}_2\text{O} \]  \hspace{1cm} (2.2)

The process (shown in Figure 2.2) begins by feeding the acid gas stream and air into a reactor furnace typically operating at a temperature in the range 982 to 1371 °C where a substantial amount of elemental sulphur is formed. The air is provided at a rate sufficient enough to convert one-third of the H₂S to SO₂. The formed sulphur is condensed by cooling the gases in a waste heat boiler followed by a sulphur condenser. The reaction gases leaving the sulphur condenser are reheated prior to passing through a catalytic converter where the remaining H₂S and SO₂ react to form sulphur. The gases leaving the converter are cooled and sulphur is condensed. The process of reheating, catalytically reacting, and sulphur condensing can be repeated one, two, or even three times (Kohl and Nielsen 1997). The typical catalyst used in the Claus process is non-promoted spherical activated alumina (Larraz 2002). For processes using two catalytic stages, H₂S conversion efficiency ranges from 94 to 95%; with three catalytic stages, H₂S conversion efficiency ranges from 96 to 97% (Kohl and Nielsen 1997).
2.2.3 LO-CAT® Process

The LO-CAT® process removes H$_2$S in a liquid redox process using an aqueous solution of ferric iron. The ferric iron, which is held in solution by organic chelating agents, oxidizes the H$_2$S ions absorbed into the solution. Elemental sulphur is generated, and the ferric iron is reduced to the ferrous iron. The ferrous iron is regenerated with air in an oxidizer. The LO-CAT® process operates at ambient temperature, requires no heating or cooling of the solution,
and has a high H₂S removal efficiency, of up to 99.99% (Kohl and Nielsen 1997). The operating pressures of the LO-CAT® process are below 1500 psi (10.3 MPa) (Speight 1993).

The LO-CAT® process consists of two primary reactions. The first (2.3) is the oxidation of bisulphide to sulphur, which takes place in the absorber. The second reaction (2.4) is the oxidation of the reduced iron by dissolved oxygen in the oxidizer.

\[
2\text{Fe}^{3+} + \text{HS}^{-} \rightarrow 2\text{Fe}^{2+} + \text{S} + \text{H}^+ \quad (2.3)
\]

\[
2\text{Fe}^{2+} + \frac{1}{2}\text{O}_2 + \text{H}_2\text{O} \rightarrow 2\text{Fe}^{3+} + 2\text{OH}^- \quad (2.4)
\]

A conventional LO-CAT® process flow diagram is shown in Figure 2.3. The ferric iron solution and sour gas are combined in a Venturi pre-contactor before entering the liquid filled absorber. Sweet gas and the ferrous solution come out of the absorber. The ferrous solution is fed to the oxidizer where sulphur is removed and the iron is oxidized back to the ferric state (Kohl and Nielsen 1997).
2.3 Biological Removal of Sulphide

2.3.1 Biological Sulphur Cycle Reactions

The microbial reduction of sulphate to sulphide and the oxidation of sulphide back to sulphate are considered as the main reactions involved in biological sulphur cycle. Sulphate reduction may occur in two ways; dissimilatory reduction and assimilatory reduction. In dissimilatory reduction, sulphate (or sulphur) is reduced to inorganic sulfide by obligatory anaerobic bacteria, such as *Desulfovibrio* and *Desulfotomaculum*. The electrons needed for sulphate reduction can be generated by the oxidation of a variety of organic compounds serving as carbon and energy source, as well as by hydrogen. The ATP
produced as a result of oxidation of the carbon source is used in the reduction of sulphate to sulphide (Postgate 1984). In assimilatory reduction, the sulphur is assimilated into sulphur-containing amino acids and other metabolites (Huxtable 1986). Figure 2.4 summarizes the biological sulfur cycle.

\[ 4H_2 + SO_4^{2-} = S^{2-} + 4H_2O \] (2.5)

The overall dissimilatory reduction of sulphate to sulphide is given by the following reaction (Huxtable 1986):

The overall reactions for the complete oxidation of various electron donors used in conjunction with the reduction of sulphate are summarized in Table 2.1.

Figure 2.4. Biological sulphur cycle (Adapted from Janssen et al., 1999)
Table 2.1. Stoichiometry for complete oxidation of various electron donors with sulphate reduction. Adapted from Amend et al., 2004.

<table>
<thead>
<tr>
<th>Electron Donor</th>
<th>Stoichiometry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>$4\text{CH}_3\text{COCOO}^- + 4\text{H}_2\text{O} + 5 \text{SO}_4^{2-} \rightarrow 12\text{HCO}_3^- + 5\text{HS}^- + 3\text{H}^+$</td>
</tr>
<tr>
<td>Lactate</td>
<td>$2\text{CH}_3\text{CHOHCOO}^- + 3\text{SO}_4^{2-} \rightarrow 6\text{HCO}_3^- + 3\text{HS}^- + \text{H}^+$</td>
</tr>
<tr>
<td>Butyrate</td>
<td>$\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2.5\text{SO}_4^{2-} \rightarrow 4\text{HCO}_3^- + 2.5\text{HS}^- + 0.5\text{H}^+$</td>
</tr>
<tr>
<td>Acetate</td>
<td>$\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} \rightarrow 2\text{HCO}_3^- + \text{HS}^-$</td>
</tr>
</tbody>
</table>

Madigan et al. (2003) identified the oxidation reactions of sulphide to sulphur (2.6) and sulphur to sulphate (2.7) as:

$$\text{HS}^- + \text{H}^+ + \frac{1}{2}\text{O}_2 \rightarrow \text{S}^0 + \text{H}_2\text{O}$$  \hspace{1cm} (2.6)

$$\text{S}^0 + \frac{3}{2}\text{O}_2 + \text{H}_2\text{O} \rightarrow \text{SO}_4^{2-} + 2\text{H}^+$$  \hspace{1cm} (2.7)

The complete oxidation of sulphide to sulphate can be represented by following reaction:

$$\text{H}_2\text{S} + 2\text{O}_2 \rightarrow \text{SO}_4^{2-} + 2\text{H}^+$$  \hspace{1cm} (2.8)

The electrons produced in reaction 2.8 are transferred to the dissolved oxygen, the final electron acceptor. For aerobic oxidation, $\text{O}_2$ is reduced to $\text{H}_2\text{O}$ by the following reaction:

$$2\text{H}^+ + \frac{1}{2}\text{O}_2 \rightarrow \text{H}_2\text{O}$$  \hspace{1cm} (2.9)

Under anaerobic conditions in which sulphide oxidation is coupled to nitrate reduction, $\text{NO}_3^-$ is reduced to $\text{NO}_2$ by the following reaction (Madigan et al., 2003):

$$2\text{H}^+ + \text{NO}_3^- \rightarrow \text{NO}_2^- + \text{H}_2\text{O}$$  \hspace{1cm} (2.10)

This reduction reaction can proceed until $\text{NO}_3^-$ and/or $\text{NO}_2^-$ is reduced to $\text{N}_2$.

### 2.3.2 Indirect Oxidation of Sulphide

The following reactions, proposed by Pagella and de Faveri (2000), describe the two step process for indirect biological removal of sulphide in which
ferric iron oxidizes the sulphide to elemental sulphur and iron oxidizing bacteria such as *Acidithiobacillus ferrooxidans* oxidizes ferrous ion to ferric ion:

\[
H_2S + 2Fe^{3+} \rightarrow S^0 + 2Fe^{2+} + 2H^+ \quad (2.11)
\]

\[
Fe^{2+} \rightarrow Fe^{3+} + e^- \quad (2.12)
\]

*Acidithiobacillus ferrooxidans* is a chemoautotrophic aerobic bacterium that uses CO\(_2\) as its carbon source. It can be employed to oxidize sulphide indirectly, through an iron bioprocess (Giro et al., 2006). Other bacterial species capable of biooxidation of iron (reaction 2.12) include *Leptospirillum ferrooxidans* (Madigan et al., 2003), and *Sulpholobus acidocaldarius* (Brock et al., 1976).

### 2.3.3 Direct Biooxidation of Sulphide

The direct biological oxidation of sulphide does not involve an oxidizing agent such as ferric iron. Rather, the sulphide is directly oxidized through the cellular metabolism of the bacterial species. Direct oxidation of sulphide is carried out by both phototrophs and chemolithotrophs.

#### 2.3.3.1 Phototrophic Oxidation

Phototrophic biooxidation of sulphide does not typically occur aerobically. Phototrophic sulphur biooxidation is anaerobic, and carried out by green sulfur bacteria, such as *Chlorobium*, and purple sulphur bacteria, such as *Allochromatium* (Madigan et al., 2003). These bacteria utilize H\(_2\)S as an electron donor for CO\(_2\) reduction in photosynthesis, thus oxidizing the H\(_2\)S to S\(^0\) by the following reaction which is often referred to as the van Niel reaction (Janssen et al., 1999, Madigan et al., 2003, Blum 1937):

\[
2H_2S + CO_2 \xrightarrow{\text{light}} 2S^0 + H_2O + \text{Carbohydrate} \quad (2.13)
\]

The environmental growth conditions for some photosynthetic anaerobic sulphur oxidizing bacteria are summarized in Table 2.2.
Table 2.2. Environmental conditions for optimum growth of several phototrophic sulphur oxidizing species as identified in Bergey’s Manual of Systematic Bacteriology (Garrity 2001, 2005a, 2005b, 2005c).

<table>
<thead>
<tr>
<th>Species</th>
<th>pH1</th>
<th>Temperature1 (°C)</th>
<th>Carbon Source(s)2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorobium limicola</td>
<td>6.8 (6.5-7.0)</td>
<td>25-35</td>
<td>Bicarbonate</td>
</tr>
<tr>
<td>Chlorobium tepidum</td>
<td>6.8-7.0</td>
<td>47-48 (32-52)</td>
<td>Bicarbonate</td>
</tr>
<tr>
<td>Rhodospirillum rubrum</td>
<td>6.8-7.0</td>
<td>30-35</td>
<td>Acetate, lactate, malate, arginine, butyrate, propionate</td>
</tr>
<tr>
<td>Rhodopseudomonas palustris</td>
<td>6.9</td>
<td>30-37</td>
<td>Lactate, malate, benzoate, acetate</td>
</tr>
</tbody>
</table>

1 Optimum value is given, where available, the possible range is indicated in parentheses.
2 Except where bicarbonate is the source, only a selection of several sources are listed.

2.3.3.2 Chemolithotrophic Oxidation

In aerobic biological oxidation of H₂S and other reduced sulphur compounds, the reduced sulphur compound serves as the electron donor, and the electron acceptor is oxygen. Aerobic sulphur-oxidizing prokaryotes have been identified by Friedrich et al. (2001) as belonging to genera such as Acidithiobacillus, Bacillus, Beggiatoa, Pseudomonas, Thiobacillus, Xanthobacter, and others. Generally, aerobic sulfur-oxidation occurs through the metabolic activity of chemolithotrophic species, such as Thiobacillus denitrificans, which is an obligate lithoautotroph, and a facultative anaerobe (Friedrich et al., 2005). Other Thiobacillus strains such as Thiobacillus thioparus and Thiobacillus thiooxidans are also capable of oxidizing sulphide (Jensen and Webb 1995). Janssen et al. (1999) identified the following reaction for aerobic sulfide oxidation to sulphur (2.14) or sulphate (2.15):

\[
2\text{HS}^- + \text{O}_2 \rightarrow 2\text{S}^0 + 2\text{OH}^- \quad (2.14)
\]
\[
2\text{HS}^- + 4\text{O}_2 \rightarrow 2\text{SO}_4^{2-} + 2\text{H}^+ \quad (2.15)
\]

In chemolithotrophic anaerobic biooxidation of sulphide, the sulphide serves as the electron donor, while nitrate or nitrite serves as the electron acceptor. The oxidation of sulphide can result in the formation of elemental sulphur or sulphate. The reduction of nitrate can result in the formation of nitrite,
which can also be reduced, resulting in the formation of other reduced nitrogen compounds, such as \( \text{N}_2 \) or \( \text{NH}_3 \) (Amend et al., 2004). Several anaerobic chemolithotrophic sulphide oxidizing bacteria have been identified in literature and used for biooxidation of sulphide. Such species include \textit{Thiobacillus denitrificans}, \textit{Thiomicrospira denitrificans} (Viaopoulou et al., 2005), \textit{Thiomicrospira} sp. CVO (Nemati et al., 2001), and a \textit{Beggiatoa} enrichment culture (Kamp et al., 2006).

According to Davidova et al. (2001) the following reactions summarize the oxidation of sulphur with nitrate (2.16) or nitrite (2.17) reduction:

\[
\begin{align*}
5\text{HS}^- + 2\text{NO}_3^- + 7\text{H}^+ & \rightarrow 5\text{S}_0 + \text{N}_2 + 6\text{H}_2\text{O} \quad (2.16) \\
3\text{HS}^- + 2\text{NO}_2^- + 5\text{H}^+ & \rightarrow 3\text{S}_0 + \text{N}_2 + 4\text{H}_2\text{O} \quad (2.17)
\end{align*}
\]

Table 2.3 summarizes the environmental conditions for optimum growth of several chemolithotrophic sulphur oxidizing species.

Table 2.3. Environmental conditions for optimum growth of several chemolithotrophic sulphur oxidizing species. Data is from Bergey's Manual of Systematic Bacteriology (Garrity 2001, 2005a, 2005b, 2005c) except where indicated.

<table>
<thead>
<tr>
<th>Species</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>Carbon source(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acidithiobacillus thiooxidans</td>
<td>2.0-3.0 (0.5-5.5)</td>
<td>28-30 (10-37)</td>
<td>( \text{CO}_2 )</td>
</tr>
<tr>
<td>Acidithiobacillus ferroxidans*</td>
<td>2.5 (1.3-4.5)</td>
<td>30-35 (10-37)</td>
<td>( \text{CO}_2 )</td>
</tr>
<tr>
<td>Leptospirillum ferroxidans*1</td>
<td>1.5</td>
<td>43</td>
<td>( \text{CO}_2 )</td>
</tr>
<tr>
<td>\textit{Thiobacillus thioparus}</td>
<td>6.6-7.2 (4.5-7.8)</td>
<td>28</td>
<td>( \text{CO}_2 )</td>
</tr>
<tr>
<td>\textit{Thiobacillus denitrificans}</td>
<td>6.8-7.4</td>
<td>28-32</td>
<td>( \text{CO}_2 )</td>
</tr>
<tr>
<td>\textit{Thiomicrospira denitrificans}</td>
<td>7.0</td>
<td>22</td>
<td>( \text{CO}_2 )</td>
</tr>
<tr>
<td>\textit{Thiomicrospira} sp. CVO²</td>
<td>5.5-8.5</td>
<td>5-35</td>
<td>( \text{CO}_2, \text{Acetate} )</td>
</tr>
</tbody>
</table>

* Sulphur oxidation occurs indirectly

¹ from Okibe et al., 2003
² from Gevertz et al., 2000
2.4 Kinetics of Sulphide Biooxidation

2.4.1 Indirect Biooxidation Kinetics

The kinetics of oxidation of ferrous iron by *Acidithiobacillus ferrooxidans* has been studied extensively, for free cells as well as immobilized cells (Nemati et al., 1998). Son and Lee (2005) initially studied indirect H$_2$S oxidation by *Acidithiobacillus ferrooxidans* in a single stage reactor at H$_2$S concentrations ranging from 20 ppm to 510 ppm, but found that at higher concentrations, the H$_2$S had an inhibitory effect on the iron-oxidizing capabilities of the bacteria. Subsequently, they developed a hybrid reactor in which the oxidation of sulphide by ferric iron took place in a liquid catalyst reactor, and the biological regeneration of ferric iron occurred in a packed bed reactor. The iron oxidation medium regenerated by *Acidithiobacillus ferrooxidans* was able to achieve a 99.99% H$_2$S removal rate at a concentration of 2000 ppm, with a flow rate of 1.22 L/min. Giro et al. (2005) used a process consisting of a packed-bed bioreactor with PVC strands as a carrier matrix for *A. ferrooxidans* with an absorber column for H$_2$S oxidation by ferric iron. With a gas flow rate of 120 L/h, a removal efficiency of approximately 100% was achieved with an inlet gas concentration of 20 000 ppm H$_2$S in 500 minutes. The reactor was operated at a temperature of 30°C, and the pH of the growth medium was adjusted to 1.7.

2.4.2 Direct Biooxidation Kinetics

2.4.2.1 Phototrophic Biooxidation Kinetics

Phototrophic biooxidation of sulphide has been studied extensively. It has been demonstrated that simultaneous control of molar flow rates of incoming gases and bioreactor photon flux is important in optimizing the van Niel reaction (Jensen and Webb 1994). Henshaw and Zhu (2001) studied biooxidation of sulphide with *Chlorobium limicola* in a fixed-film continuous flow photo-reactor, obtaining 100% conversion at a sulphide loading rate of 286 mg/L-h, with a
sulphide concentration of 143±12 mg/L. The end product of the sulphide oxidation was elemental sulphur.

A green sulphur bacteria (GSB) enrichment culture was studied by Hurse and Keller (2004) in a substratum-irradiated photosynthetic biofilm reactor. A maximum concentration of 11.5 mg/L of sulphide was applied, with a flow rate between 1.11 mL/min and 1.18 mL/min, and a temperature between 26.5 and 28.5°C. The maximum areal net sulphide removal rate was found to be 2.08 g/m²·d. The end products of the sulphide oxidation by this GSB enrichment were elemental sulphur and sulphate.

Kobayashi et al (1983) studied hydrogen sulphide removal from anaerobic waste treatment effluent by an enrichment culture of green and purple sulphur bacteria in a packed column as well as in a submerged tube system. The column was maintained with a 24-h retention time. With a S²⁻ loading rate of 107 mg/day, 95% removal was achieved. In the photosynthetic tube, with a retention time of 0.66 h and a sulphide concentration of 23.9 mg/L (loading rate of 36.2 mg/L·h⁻¹), 98% of the sulphide was removed (removal rate of 35.5 mg/L·h⁻¹). Both sulphate and elemental sulphur were observed in the end products.

Kim and Chang (1991) compared H₂S removal rates using Chlorobium thiosulphatophilum in: an immobilized-cell reactor and sulphur-settling free-cell reactors. Both fed-batch (2 and 4 L) and continuous (4 L, with cell recycle) modes of operation were studied in the free-cell reactor. The immobilized-cell reactor achieved a removal rate of 0.259 µmol H₂S min⁻¹ (mg of protein)⁻¹L⁻¹, which was significantly higher than the removal rate of 0.106 µmol H₂S min⁻¹ (mg of protein)⁻¹L⁻¹ in the free-cell reactor of the same volume (2 L). The removal rate for a 4 L free-cell reactor with cell recycle was 0.205 µmol min⁻¹(mg of protein)⁻¹L⁻¹. It was also determined that the light-energy requirement of the immobilized cell reactor was 600 W/m² for an H₂S removal rate of 2 mM/L·h, while the free cell reactor required 850 W/m² for the same removal rate. The pH and temperature of the reactor medium were maintained at 6.8-6.9 and 30 °C, respectively.
Henshaw et al. (1997) studied sulphide conversion to elemental sulphur by *Chlorobium limicola* in a CSTR with freely suspended cells. The system was able to achieve a sulphide removal rate of 3.2 mg/L-h, with 100% conversion to elemental sulphur (within the cumulative error of the measurements). The reactor was operated at a pH of 6.8-7.2 and temperature of 30 °C.

Syed and Henshaw (2005) investigated the performance of a tubular fixed-film photobioreactor with light emitting diodes (LEDs) and infrared light bulbs as the light source. A modified van Niel curve was generated for the LEDs and infrared bulb, and it was concluded that with the same light intensity, the experiments with LEDs achieved higher loading rates (1.3 to 1.7 fold) than those with infrared bulbs. The highest sulphide loading rate with complete sulphide removal was 338 mg/L-h. The bioreactors were operated at a pH in the range of 6.8 to 7.0, and a temperature in the range 27-29°C. The end products in this study were not discussed; however, given that the sulphide loading rates were achieved in accordance with a modified van Niel curve, it is likely the end product was elemental sulphur.

Borkenstein and Fischer (2006) investigated sulphide removal by the mutant *Allochromatium vinosum* strain 21D. This strain contained an inactivated *dsrB* gene, and is unable to oxidize intracellular sulphur to sulphate, making it ideal for sulphide removal with elemental sulphur recycling. The sulphide removal process was semi-continuous, and consisted of three successive fed-batch sections. Each section was initiated with photoorganoheterotrophic growth using malate and acetate to generate higher cell yields. After each sulphide addition, the culture grew photolithoheterotrophically with malate/acetate and sulphide. The highest sulphide removal rate was 49.3 µM/h. Given that the sulphur was stored intracellularly, Borkenstein and Fischer recommend the industrial or agricultural use of a “biomass sulphur” product, in which the sulphur would not have to be separated from the bacterial cells. The pH and temperature of the reactor in this case were 6.9 and 30 °C, respectively.

Table 2.4 contains a summary of the research works conducted with respect to phototrophic sulphide biooxidation kinetics. The species, reactor
configuration, pH, temperature, and electron acceptor are listed for each work, where available. In addition, the maximum sulphide loading rate and end products of the reaction are shown.

Table 2.4. Summary of phototrophic sulphide biooxidation kinetics and the corresponding conditions as reported in various works.

<table>
<thead>
<tr>
<th>Species</th>
<th>Reactor Configuration</th>
<th>pH</th>
<th>T (°C)</th>
<th>Electron Acceptor</th>
<th>Maximum Sulphide Oxidation Rate</th>
<th>End Product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chlorobium limicola</td>
<td>Fixed-film continuous flow photobioreactor</td>
<td>6.8-7.0</td>
<td>27-29</td>
<td>CO₂</td>
<td>1451 mg/L-h</td>
<td>Sulphur</td>
<td>Syed and Henshaw, 2003</td>
</tr>
<tr>
<td>Chlorobium limicola</td>
<td>Fixed-film continuous flow photoreactor</td>
<td>6.8-7.2</td>
<td>27</td>
<td>CO₂</td>
<td>286 mg/L-h</td>
<td>Sulphur</td>
<td>Henshaw and Zhu, 2001</td>
</tr>
<tr>
<td>Chlorobium limicola</td>
<td>Free cell reactor with cell recycle</td>
<td>6.8-6.9</td>
<td>30</td>
<td>CO₂</td>
<td>0.205 µmol/min-mg protein-L</td>
<td>Sulphur</td>
<td>Kim and Chang 1991</td>
</tr>
<tr>
<td>Chlorobium limicola</td>
<td>Fixed-film continuous flow photobioreactor</td>
<td>6.8-7.0</td>
<td>27-29</td>
<td>CO₂</td>
<td>338 mg/L-h</td>
<td>Sulphur</td>
<td>Syed and Henshaw 2005</td>
</tr>
<tr>
<td>Chlorobium limicola</td>
<td>Suspended cells CSTR</td>
<td>6.8-7.2</td>
<td>30</td>
<td>CO₂</td>
<td>3.2 mg/L-h</td>
<td>Sulphur</td>
<td>Henshaw et al. 1997</td>
</tr>
<tr>
<td>Enrichment culture of green and purple sulphur bacteria</td>
<td>Packed column</td>
<td>7.0</td>
<td>-</td>
<td>CO₂</td>
<td>101.65 mg/day</td>
<td>Sulphur and Sulphate</td>
<td>Kobayashi et al 1983</td>
</tr>
<tr>
<td>Enrichment culture of green and purple sulphur bacteria</td>
<td>Photosynthetic tube</td>
<td>7.0</td>
<td>-</td>
<td>CO₂</td>
<td>35.5 mg/L-h</td>
<td>Sulphur and Sulphate</td>
<td>Kobayashi et al 1983</td>
</tr>
<tr>
<td>GSB enrichment culture</td>
<td>Substratum irradiated Photosynthetic biofilm reactor</td>
<td>-</td>
<td></td>
<td>CO₂</td>
<td>2.08g/m²d</td>
<td>Sulphur and Sulphate</td>
<td>Hurse and Keller 2004</td>
</tr>
<tr>
<td>Allochromatium vinosum strain 21D</td>
<td>Fed-batch stirred tank reactor</td>
<td>6.9</td>
<td>30</td>
<td>CO₂</td>
<td>49µM/h</td>
<td>Biomass sulphur</td>
<td>Borkenstein and Fischer 2006</td>
</tr>
</tbody>
</table>

2.4.2.2 Chemolithotrophic Biooxidation Kinetics

The kinetics of chemolithotrophic biooxidation of sulphide have been studied for a number of organisms, including *Thiobacillus denitrificans*, an enrichment culture dominated by *Thiomicrospira* sp. CVO, and *Thiomicrospira* sp. CVO.
Sublette and Sylvester (1987) studied batch oxidation of sulphide by *Thiobacillus denitrificans* in a bench fermentor. At loading rates of 4-5 mmol H$_2$S/h g biomass, with an agitation rate of 300 rpm, H$_2$S was undetectable in the outlet gas. No elemental sulphur was detected in the reactor, and sulphate accumulated in the medium as H$_2$S was removed from the feed gas. The residence time of a bubble of feed gas was estimated as 1-2 s. The effect of temperature on the growth of *T. denitrificans* was tested at temperatures ranging from 15 to 45°C. The maximum optimal density was obtained at a temperature of 30°C. The pH for these experiments was 7.0.

Lee and Sublette (1993) studied aerobic oxidation of sulphide by *Thiobacillus denitrificans* in an upflow bubble column. The cells were immobilized by co-culture with floc-forming heterotrophs. This method of immobilization was originally proposed by Ongcharit et al. (1990) because it allows for the microorganism to be recovered from the effluent stream and recycled back to the reactor. The system was able to achieve complete sulphide removal without upset at loading rates of 12.7 to 15.4 mmol/h. Lee and Sublette determined that the molar sulphide feed rate was more important in determining the capacity of the reactor for sulphide oxidation than the hydraulic retention time or the influent sulphide concentration. The product of sulphide oxidation in this case was sulphate (Lee and Sublette 1993). Krishnakumar et al. (2005) proposed the use of a novel reverse fluidized loop reactor (RFLR) for aerobic sulphide oxidation by *T. denitrificans*. The reactor consists of an outer tube enclosing a draft tube. The aeration regime inside the reactor created loop flow between the tubes, fluidizing the biofilm carrier particles. When the pH was controlled at 8, the maximum sulphide loading rate of 19 kg/m$^3$/d resulted in complete removal of sulphide (100%).

The use of a novel biocatalyst system utilizing an anaerobic enrichment culture from the Coleville oil field in Saskatchewan, Canada was proposed by McComas et al. (2001) for oxidation of sulphide. The culture was dominated by *Thiomicrospira* sp. CVO, but was also known to contain another novel species, *Arcobacter* sp. FWKO B. Freely suspended cells were cultured in a bench-scale
reactor which was maintained at a pH of 7.4, and a temperature of 32°C. The maximum sulphide loading was 5.8 mmol H₂S/g-biomass hr, which was observed to be comparable to *T. denitrificans* under similar conditions. However, the enrichment culture was shown to be more tolerant of extremes in pH and elevated temperatures, as well as salinity when compared with *T. denitrificans*.

In order to accommodate sulphide-containing effluents at higher temperatures (such as those from the pulp and paper industry), Datta et al. (2007) studied biotrickling filtration of hydrogen sulphide at temperatures of 40, 50, 60, and 70°C. The microbial community in this study was obtained from three hot pools around Lake Rotorua in New Zealand. DNA extraction was performed and it was found that the community contained several species including *Oceanobacillus, Virgibacillus, Bacillus, Orchobuctrum, Rhizobium*, and *Desulfitobacterium*. Glucose and monosodium glutamate were added to the reactors as a carbon source and compatible solute, respectively. The biotrickling filters were operated aerobically, and the pH in the reactor was in the range of 4-5. The maximum removal capacity of the biotrickling filters approached 40 g of H₂S/m³h, at temperatures up to 70°C. The end products of sulphide oxidation were not identified in this study.

Annachhatre and Suktrakoolvait (2001) studied sulphide oxidation by biomass from an activated sludge reactor treating distillery water in a fluidized bed reactor with the goal of producing sulphur sludge with suitable settling characteristics. The oxidation of sulphide was studied at various dissolved oxygen (DO) concentrations within the reactor. At DO concentrations greater than 0.1 mg/L, sulphate was the main product of sulphide oxidation. By increasing the sulphide loading rate, the sulphate production was decreased, and elemental sulphur production increased. At DO concentrations less than 0.1 mg/L, sulphur was the main end product. An elemental sulphur production of 76% was obtained with a sulphide loading rate of 1.6 kgS/m³d. Sulphide removal rates greater than 90% were achieved at sulphide loading rates of 0.13-1.6 kgS/m³d. The pH in the reactor was maintained at 7.8, and the temperature was in the range 25-30 °C.
Huang et al. (1996) studied biofiltration of H$_2$S by autotrophic bacteria, *Thiobacillus* sp. CH11, and heterotrophic bacteria, *Pseudomonas putida* CH11, isolated from swine waste water. The H$_2$S concentration applied to these biofilters was 60 ppm. The biofilters achieved >95% removal of H$_2$S at flow rates ranging from 18 to 93 L/h. The retention time corresponding to a flow rate of 93 L/h is 28s. At each flow rate, the removal efficiency of the heterotrophic biofilter was lower than that of the autotrophic reactor. To examine the effect of H$_2$S on the removal capacity of the reactors, a flow rate of 150 L/h was selected, and concentrations varied in the range 5 to 200 ppm. It was found that a critical concentration of 100 ppm corresponded to the highest removal capacity of 25 gS/m$^3$h in the heterotrophic biofilter. At a concentration of 150 ppm, the heterotrophic biofilter showed sudden decrease in removal efficiency. The autotrophic biofilter was able to achieve greater removal rates as the inlet concentration of H$_2$S increased to 200 ppm. The study also found that the autotrophic biofilter showed a high affinity for H$_2$S but failed to reliably remove low concentrations of H$_2$S over a long period, while the heterotrophic biofilter showed the opposite tendency. The temperature for the continuous system was controlled in the range of 28-30 °C.

Table 2.5 contains a summary of research conducted with respect to chemolithotrophic sulphide biooxidation kinetics. The species, reactor configuration, pH, temperature, electron acceptor, and maximum sulphide loading rate are listed for each work, where available. In addition, the end products of the reactions are included where available.
Table 2.5. Chemolithotrophic sulphide biooxidation kinetics and the corresponding conditions as reported in various works.

<table>
<thead>
<tr>
<th>Species</th>
<th>Reactor Configuration</th>
<th>pH</th>
<th>T (°C)</th>
<th>Electron Acceptor</th>
<th>Maximum Sulphide Oxidation Rate</th>
<th>End Product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass from an activated sludge reactor</td>
<td>Fluidized bed reactor</td>
<td>7.8</td>
<td>25-30</td>
<td>O₂</td>
<td>1.44 kg-S/m³d</td>
<td>Sulphur and Sulphate</td>
<td>Annachhatre and Suktrakoolvait 2001</td>
</tr>
<tr>
<td>Microbial community in hot pools around Lake Rotorua in New Zealand</td>
<td>Biotrickling filter</td>
<td>4.5</td>
<td>70°C</td>
<td>O₂</td>
<td>40 g/m³h</td>
<td>-</td>
<td>Datta et al. 2007</td>
</tr>
<tr>
<td>Acidithiobacillus thiooxidans immobilized on activated carbon</td>
<td>Horizontal biotrickling filter</td>
<td>4.5</td>
<td>25-30</td>
<td>O₂</td>
<td>113 gH₂S/m³h</td>
<td>-</td>
<td>Duan et al. 2005</td>
</tr>
<tr>
<td>Thiomicrospira sp. CVO</td>
<td>Continuous flow reactor</td>
<td>7.0</td>
<td>-</td>
<td>NO₃</td>
<td>3.2 mM/h</td>
<td>No Sulphate formed</td>
<td>Gadekar et al., 2006</td>
</tr>
<tr>
<td>Thiobacillus sp. CH11</td>
<td>Packed column</td>
<td>-</td>
<td>28-30</td>
<td>O₂</td>
<td>7328 mg/L-h</td>
<td>-</td>
<td>Huang et al. 1996</td>
</tr>
<tr>
<td>Pseudomonas putida CH11</td>
<td>Packed column</td>
<td>-</td>
<td>28-30</td>
<td>O₂</td>
<td>25 g-S/m³h</td>
<td>-</td>
<td>Huang et al. 1996</td>
</tr>
<tr>
<td>Thiobacillus denitrificans</td>
<td>Reverse fluidized loop reactor</td>
<td>9.5</td>
<td>-</td>
<td>O₂</td>
<td>19 kg/m³d</td>
<td>Sulphur and Sulphate</td>
<td>Krishnakumar et al., 2005</td>
</tr>
<tr>
<td>Thiobacillus denitrificans</td>
<td>Upflow bubble column</td>
<td>-</td>
<td>30</td>
<td>O₂</td>
<td>12.7-15.4 mmol/h</td>
<td>Sulphate</td>
<td>Lee and Sublette 1993</td>
</tr>
<tr>
<td>Acidithiobacillus thiooxidans</td>
<td>Packed-bed reactor</td>
<td>-</td>
<td>-</td>
<td>O₂</td>
<td>670g/m³h</td>
<td>Sulphate</td>
<td>Lee et al., 2006</td>
</tr>
<tr>
<td>Thiomicrospira sp. CVO enrichment</td>
<td>Fed-batch bench scale fermentor</td>
<td>7.4</td>
<td>32</td>
<td>NO₃</td>
<td>5.8 mmol/g-biomass-protein</td>
<td>Sulphur and Sulphate</td>
<td>McComas et al., 2001</td>
</tr>
<tr>
<td>Thiomonas sp.</td>
<td>Batch biofilter column</td>
<td>-</td>
<td>-</td>
<td>O₂</td>
<td>0.01 mg-H₂S/min-g biocarbon</td>
<td>-</td>
<td>Ng et al. 2004</td>
</tr>
<tr>
<td>Thiobacillus denitrificans</td>
<td>CSTR with total biomass recycle</td>
<td>-</td>
<td>-</td>
<td>O₂</td>
<td>3.2 mmol/L-h</td>
<td>Sulphate</td>
<td>Oncharit et al., 1990</td>
</tr>
<tr>
<td>Thiobacillus denitrificans</td>
<td>Batch fermentor</td>
<td>7.0</td>
<td>30</td>
<td>NO₃</td>
<td>5 mmol-H₂S/g-biomass</td>
<td>Sulphate</td>
<td>Sublette and Sylvester 1987</td>
</tr>
<tr>
<td>Thiobacillus denitrificans immobilized on activated carbon</td>
<td>Packed column</td>
<td>6.8-7.4</td>
<td>30-35</td>
<td>O₂</td>
<td>667 mg-H₂S/L-d</td>
<td>Sulphur</td>
<td>Ma et al., 2006</td>
</tr>
</tbody>
</table>
2.4.3 Bioreaction End Products

2.4.3.1 Phototrophic Bioreaction End Products

Using a continuously stirred tank reactor described as an anaerobic gas-to-liquid phase fed-batch reactor, Cork et al. (1985) investigated the effects of H$_2$S flow rate and light energy on the composition of oxidized sulphur compounds formed by the photoautotroph *Chlorobium limicola* forma *thiosulfatophilum*. Cork et al. determined a relationship between the H$_2$S oxidation rates and the light energy for trials that were optimized for S$^0$ production, shown in Figure 2.5.

![Graph showing H$_2$S utilization rates versus light energy for S$^0$-optimized trials](image)

**Figure 2.5.** H$_2$S utilization rates versus light energy for S$^0$-optimized trials (Adapted from Cork et al., 1985).

The area above the curve (Region I) represents oxidative metabolism of sulphur by equation 2.18:

$$2nH_2S + nCO_2 \rightarrow 2nS + nCH_2O + nH_2O \quad (2.18)$$
The area under the curve (Region II) represents inhibition of specific growth rate and growth yield caused by high soluble sulphide concentrations. According to Cork et al., photons may be described as a limiting substrate, given that the \( \text{H}_2\text{S} \) utilization rate increases with a corresponding increase in light energy level. The formation of oxidized sulphur species occurs at lower \( \text{H}_2\text{S} \) flow rates and light energy levels according to reaction 18 (Region I).

Henshaw and Zhu (2001) investigated \( \text{H}_2\text{S} \) oxidation to elemental sulphur in a fixed-film continuous flow photo-reactor. The results of their experiments were reported in a modified van Niel curve, which is the sulphide loading rate versus the radiant flux per volume times the bacteriochlorophyll concentration. The modified van Niel curve from the study conducted by Henshaw and Zhu is shown in Figure 2.6. The area above the curve represents over-loading of sulphide, resulting in sulphide in the effluent of the reactor. The area below the curve represents under-loading of sulphide, where the light input results in further oxidation of elemental sulphur to sulphate.

![Modified van Niel curve](image)

**Figure 2.6.** Modified van Niel curve adapted from Henshaw and Zhu (2001). Reproduced using the equation of the line is \( y=356(1-e^{-0.00298x}) \).
2.4.3.2 Chemolithotrophic Bioreaction End Products

McComas et al. (2001) studied the stoichiometry of the sulphide oxidation using nitrate as the electron acceptor. Assuming that nitrate is reduced to N\textsubscript{2}, 0.4 mol of nitrate would be required to oxidize one mol of H\textsubscript{2}S to S\textsuperscript{0}, and 1.6 mol of nitrate would be required for oxidation of one mol of sulphide to sulphate. In batch studies, elemental sulphur appeared to be the main product of sulphide oxidation in a culture of Thiomicrospira sp. CVO and Arcobacter sp. FWKO B. According to Gevertz et al. (2000), CVO does oxidize sulphide to sulphate when sulphide concentrations are low and nitrate is not limiting, but FWKO B oxidizes sulphide to elemental sulphur only.

Gadekar et al. (2006) reported the reaction kinetics and stoichiometry of anaerobic sulphide oxidation by *Thiomicrospira* sp. CVO (herein referred to as CVO) in batch and continuous systems. Utilizing nitrate as electron acceptor, CVO was able to oxidize sulphide at concentrations as high as 19 mM. Two phases of sulphide oxidation were observed: the first resulting in the formation of sulphur followed by a second phase of sulphate formation. In the continuous reactor, complete removal of sulphide was observed at loading rates reaching 1.6 mM/h. The sulphide to nitrate ratio had a significant effect on the composition of the end products of sulphide oxidation. At a ratio of 0.28, 93% of the reaction products was sulphate; at a ratio of 1.6, only 9.3% of the products was sulphate.

Alcantara et al. (2004) utilized a microbial consortium primarily made up of *Thiobacillus* to oxidize sulphide in a recirculation reactor system. In this system, the sulphide oxidation and the aeration of the liquid phase were spatially separated, allowing for control of the oxygen concentration and the creation of turbulence inside the reactor. The effect of oxygen on the sulphide oxidation was investigated for a range of oxygen to sulphide (O\textsubscript{2}/S\textsuperscript{2-}) ratios. It was found that an oxygen to sulphide ratio of 0.5 - 1.5 would result in partial oxidation of sulphide to elemental sulphur, and a ratio of 1.5-2 would result in complete oxidation to sulphate. It was also concluded that ratios below 0.5 would result in low sulphide oxidation.
Annachhatre and Suktrakoolvait (2001) studied sulphide oxidation by biomass from an activated sludge reactor treating distillery water in a fluidized bed reactor with the goal of producing a sulphur sludge with suitable settling characteristics. The oxidation of sulphide was studied at various dissolved oxygen (DO) concentrations within the reactor. At DO concentrations greater than 0.1 mg/L, sulphate was the main product of sulphide oxidation. By increasing the sulphide loading rate, the sulphate production was decreased, and elemental sulphur production increased. At DO concentrations less than 0.1 mg/L, sulphur was the main end product.

Wang et al. (2005) studied simultaneous desulfurization and denitrification (SDD) by *Thiobacillus denitrificans*. The objective of this study was to maximize the production of elemental sulphur from sulphide. Utilizing nitrate as the electron acceptor, the influences of sulphide concentrations and the ratio of S$^{2-}$/NO$_3^-$ on the efficiency of the simultaneous desulfurization and denitrification were investigated in both continuous flow and batch tests. The stoichiometric equations for desulphurization and denitrification by *Thiobacillus denitrificans* were given as:

$$12H^+ + 2NO_3^- + 5S^{2-} \rightarrow N_2 + 5S + 6H_2O \quad (2.19)$$

$$5S^0 + 6NO^-_3 + 8H_2O \rightarrow 5H_2SO_4 + 6OH^- + 3N_2 \quad (2.20)$$

From equation 2.19, the stoichiometric ratio of S$^{2-}$/NO$_3^-$ was 5:2. In order to ensure complete oxidation of the sulphide, a ratio of 5:3 was used in the investigation of the effects of initial sulphide concentration. Initial sulphide concentrations of 100, 200, 300, 400, and 500 mg/L were studied in batch tests. It was found that at initial sulphide concentration of 100 mg/L, 99% of the sulphide was removed. At a concentration of 300 mg/L, a sulphide conversion of 67.9% was observed. At concentrations of 400 mg/L and 500 mg/L, the sulphide removal dropped to 22.9 and 17.2%, respectively.
Krishnakumar et al. (2005) proposed the use of a novel aerobic bioreactor for sulphide oxidation by *T. denitrificans*; referred to as a reverse fluidized loop reactor (RFLR). The study cited that a molar sulphide to oxygen ratio for sulphur production (rather than sulphate) would be 0.6-1.0. Given the difficulties in maintaining a narrow sulphide to oxygen ratio, the authors opted to maintain an optimum redox potential (ORP) instead. The ORP range of -300 mV to -200 mV was maintained to maximize sulphur production.

van der Zee et al. (2007) investigated sulphide oxidation in batch anaerobic bioreactors. Three oxygen levels were examined, corresponding to initial molar O$_2$/sulphide ratios of 0.53, 1.1, and 3.5. When oxygen was introduced into the batch vials sulphide disappeared rapidly, and elemental sulphur and thiosulphate were formed. Substantial sulphate formation was only observed after the second injection of oxygen in the batch tests with the O$_2$/sulphide ratio of 3.5. In this study, large fractions of the initial sulphide concentration were not retrieved as elemental sulphur, thiosulphate or sulphate, but rather presumed to be polysulphide. Comparing the rate constants at different O$_2$/sulphide ratios, it was established that a higher ratio resulted in a higher rate constant for sulphide oxidation.
3. **RESEARCH OBJECTIVES**

A review of the available literature reveals that the biooxidation of sulphide has been studied extensively. However, information regarding the kinetics of sulphide biooxidation in a biofilm system is rather limited. In addition, the majority of the earlier works have utilized pure cultures of sulphide oxidizing bacteria and the efficiency of this process with a mixed microbial culture, particularly a culture originated from an oil reservoir, has not been studied extensively. In addition, many of these cultures have a low tolerance for sulphide, which limits their application.

The objective of the present work was to study the biooxidation of sulphide under denitrifying conditions in batch system, and in continuous immobilized cell bioreactor, using a mixed microbial culture enriched from the produced water of a Canadian oil reservoir. Given that the microbial culture was obtained from an oilfield, it may be possible to apply the result of this research in control of souring in an oil reservoir as well.

In the batch experiments, the effect of sulphide concentration on the activity of the microbial culture and biooxidation of sulphide under denitrifying conditions was examined. In addition, the effect of the initial sulphide to nitrate ratio on the composition of the end-products was investigated.

The experiments in the continuous immobilized cell bioreactors primarily aimed to evaluate the possibility for improving the reaction rate. In addition the effects of the loading rates of sulphide and nitrate on the performance of the bioreactor with respect to sulphide oxidation and nitrate reduction were investigated. As in the batch experiments, the effect of the ratio of sulphide to nitrate concentrations on the performance of the bioreactor and the composition of bioreaction end products was studied.
4. **MATERIALS AND METHODS**

4.1. **Microbial Culture and Medium**

A mixed culture of sulphide oxidizing, nitrate reducing bacteria, enriched from the produced water of the Coleville oil field in Saskatchewan, Canada was used in this work.

4.1.1. **Medium**

The medium used for maintenance and growth of the microbial culture was the Coleville Synthetic Brine (CSB) as described earlier (Gadekar et al., 2006). CSB contained per litre: 7.0 g NaCl, 0.68 g MgSO$_4$•7H$_2$O, 0.24 g CaCl$_2$•2H$_2$O, 0.02 g NH$_4$Cl, 0.027 KH$_2$PO$_4$, 0.68 NaC$_2$H$_3$O$_2$•3H$_2$O, 1.0 g KNO$_3$, 1.9 g NaHCO$_3$, and 0.5 mL trace element solution. The trace element solution contained per litre: 0.5 mL concentrated H$_2$SO$_4$, 2.28 g MnSO$_4$•H$_2$O, 0.5 g ZnSO$_4$•7H$_2$O, 0.5 g H$_3$BO$_3$, 0.025 g Na$_2$MoO$_4$•2H$_2$O, 0.045 g CoCl$_2$•6H$_2$O, and 0.58 g FeCl$_3$. The medium was buffered using 0.05 M (6.06 g/L) Tris Base (C$_4$H$_11$NO$_3$). The pH of the medium was adjusted to the range of 6.8-7.2 using either 2 or 4 M HCl.

4.1.2. **Culture Conditions**

Subculturing and maintenance of the enrichment culture was carried out in 125 mL serum bottles. The bottles were charged with 100 mL of prepared CSB medium. The medium was then purged with nitrogen gas for 3 minutes and then sealed with rubber septum and aluminum caps. The bottles were autoclaved for 30 minutes at 121 °C to ensure sterilization. The sterilized serum bottles were used for the maintenance of the enrichment culture at room temperature (22°C). Prior to inoculation, 0.55 mL of 1 M Na$_2$S stock solution was added to achieve a sulphide concentration of 5 mM, and the pH was readjusted to the range of 6.8-
7.2 using 2 M HCl. The bottles were inoculated with the enrichment culture at 10% v/v. The initial enrichment culture was obtained from the produced water of the Coleville oilfield in Saskatchewan, Canada, by adding the produced water to the CSB medium (25% v/v) containing 5 mM sulphide and allowing the establishment of the microbial consortium at room temperature. The established microbial culture was then used as inoculum in subsequent subculturing as described above. Subculturing was carried out on a bi-weekly basis to maintain an active enrichment culture.

4.1.3. Bioreactor Medium

The CSB medium previously described was also used in the continuous bioreactors. The medium was prepared in a glass flask or large bottles (10 L), and autoclaved for 30 minutes at 121 °C. Once cooled to room temperature, the medium was purged with filter sterilized nitrogen gas for 30 minutes per litre of medium. The sulphide was added to the medium until the desired sulphide concentration (15-18 mM) was achieved using a sterilized 1 M Na$_2$S stock solution, and the pH was readjusted to within the range of 6.8 - 7.2 using 2 M (batch experiments) or 4 M (continuous experiments) stock solution of HCl. The medium was then transferred from the bottle or flask to the sterile collapsible medium bag connected to the bioreactor by introducing pressurized sterilized nitrogen gas into the bottle or flask. The collapsible medium bag was a 5 L bag, except at high loading rates, when 10 L and 20 L bags were used.

4.2. Batch Experiments

4.2.1. Experimental Conditions

The batch experiments were conducted in 125 mL serum bottles. The bottles were charged with 100 mL of CSB medium and purged with N$_2$ gas for three minutes. A sterilized stock solution of 1 M Na$_2$S was used to adjust the sulphide concentration to the designated level, and the pH was readjusted to the range of 6.8 - 7.2 using sterilized 2 M HCl prior to inoculation. In each of the
batch tests, the bottles were inoculated with a 3-day-old enrichment culture (10% v/v).

### 4.2.2. Effect of Initial Sulphide Concentration

The effect of initial sulphide concentration on the activity of the microbial culture and biooxidation of sulphide was studied in 125 mL serum bottles containing 100 mL of CSB medium with 10 mM nitrate and 2.1, 5.5, 16.3, or 20.6 mM of sulphide. A 3-day-old enrichment culture was used as the inoculum (10% v/v). Experiments were conducted at room temperature (22°C). Samples were taken from the serum bottles during the course of the experiments and the sulphide concentration was determined immediately after sampling. The samples were then centrifuged at 14000 rpm for 5 minutes and preserved in a freezer (-75°C) for further analysis. Thiosulphate, sulphate, nitrate, and nitrite concentrations in these samples were determined at the end of the experimental runs, using ion chromatography. All the experimental runs were carried out in duplicates. A control run was conducted with 5 mM sulphide, in the absence of inoculum.

### 4.2.3. Effect of Initial Sulphide to Nitrate ratio

The effect of the initial sulphide to nitrate concentrations ratio was determined by conducting a series of batch experiments at sulphide to nitrate initial concentration ratios of 0.33, 0.50, 0.67, 1.0, 2.0, and 4.0. The initial sulphide concentration was around 5 mM for each of the tests, while the nitrate concentration was adjusted to 1.25 mM, 2.5 mM, 5.0 mM, 7.5 mM, 10 mM, and 15 mM to achieve ratios of 4.0, 2.0, 1.0, 0.67, 0.50, and 0.33, respectively. The experiments were carried out in 125 mL serum bottles containing 100 mL of CSB medium. A three-day-old enrichment culture was used as the inoculum (10% v/v). All other conditions and monitoring approaches were similar to those described in section 4.2.2. All the experimental runs were carried out in duplicates. Control runs were conducted under similar conditions in the absence of inoculum.
4.3. Specifications of the Up-Flow Packed Bed Bioreactors and Experimental Systems

Two identical experimental systems were used in this study. Each experimental set-up consisted of an up-flow packed-bed bioreactor made of a glass column with a diameter of 4 cm, and a height of 36 cm. Three sampling ports were devised at 12.5 cm intervals along the length of the column. A polymeric mesh pad was used at the bottom of the reactor to support the carrier matrix used for the establishment of a biofilm. The carrier matrix utilized in this experiment was quartz sand with a mesh size of -50 to +70 and an average diameter of 225 µm. The surface area of the sand, as measured by Krishnan (2006) was 0.321 m²/g. The three sampling ports were sealed using rubber septum. Polyvinyl chloride (PVC) tubing and a multispeed peristaltic pump were used to transfer the medium from a collapsible medium bag (5 L, 10 L, or 20 L, Cole-Parmer Canada, Montreal, Canada) to the bioreactor, and from the bioreactor to the effluent container. A schematic diagram and photograph of the experimental system is shown in Figure 4.1.
Figure 4.1. Schematic diagram of the experimental setup (top) and photograph of experimental setup (bottom).
4.4. Experimental Procedures

4.4.1. Batch Operation of Bioreactor

CSB medium containing 16.12 ± 0.43 mM sulphide and 13.50 ± 0.35 mM nitrate (values were calculated as average ± one standard deviation) was pumped into the bottom of the bioreactor through PVC tubing using a multispeed peristaltic pump. Medium was pumped through the bioreactor until approximately two pore volumes passed through the system, ensuring that the void spaces were filled with medium. Flow of the medium was then stopped and the bioreactor was inoculated by injecting 20 mL of 3 day old enrichment culture into each sampling port (total of 60 mL), beginning at the bottom port to prevent outflow of inoculum from the top of the bioreactor. During the batch operation the sulphide concentration was monitored in all three ports. Batch operation was sustained until complete conversion of sulphide was achieved throughout the bioreactor.

4.4.2. Continuous Operation of Bioreactor

Following the complete oxidation of sulphide in all parts of the bioreactor, the bioreactor was switched to continuous mode by pumping medium into the reactor at a low flow rate (0.5 mL/h) for approximately 48 hours to allow the passive immobilization of the bacteria and the formation of the biofilm. The flow rate was then increased in steps, after the bioreactor reached the steady state with respect to sulphide oxidation at a given flow rate. Steady state was defined as the establishment of complete conversion of sulphide in all three sampling ports, or when the sulphide concentration was constant over a period of at least two to three residence times. The flow rates applied to the reactor ranged from 0.5 mL/h to 71 mL/h. The experiments were carried out at room temperature (22 °C), and the feed pH was maintained within the range of 6.8-7.2.

One sample was taken from each port on a regular basis and the sulphide concentration was determined immediately and prior to freezing the remaining of
the samples at -75 °C for further analysis. The sulphate, nitrate, nitrite, and thiosulphate concentrations in the preserved samples were determined using an ion chromatograph. Ion chromatography analysis was only carried out for the samples taken after establishment of steady-state conditions. At each steady state point samples were taken from all ports to determine the protein concentration in the liquid phase. The steady state data at each volumetric loading rate were used to determine the volumetric reaction rates for oxidation of sulphide and reduction of nitrate in the bioreactor.

Two independent experimental runs were conducted in this work. In the first run, the effect of the volumetric loading rate of sulphide was studied. The second run was conducted for two reasons: first to confirm the reproducibility of the results in the first bioreactor, and second to study the effect of the sulphide to nitrate concentrations ratio on the bioreactor performance. The batch operation and procedures used for the establishment of the biofilm were identical in both runs.

4.4.3. Effects of Volumetric Loading Rate of Sulphide

The effects of the volumetric loading rate of sulphide were determined by increasing the flow rate of the feed medium into the bioreactor while maintaining a constant sulphide concentration and sulphide to nitrate concentrations ratio in the feed. All other components of the CSB medium were maintained constant at the same level as described in section 4.1.1. For this experiment, the sulphide concentration in the medium was 16.12 ± 0.43 mM, and the nitrate concentration was 13.50 ± 0.35 mM. The applied flow rates were in the range 0.5-71 mL/h. Each flow rate was maintained until the achievement of steady state. Samples were taken from each port on a regular basis. Table 4.1 shows the flow rates applied in the primary bioreactor, along with the corresponding sulphide and nitrate loading rates.
### Table 4.1. Experimental conditions in the first bioreactor*

<table>
<thead>
<tr>
<th>Flow Rate (mL/h)</th>
<th>Sulphide Loading Rate (mM/h)</th>
<th>Nitrate Loading Rate (mM/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.26</td>
<td>0.19</td>
</tr>
<tr>
<td>3.20</td>
<td>1.37</td>
<td>1.25</td>
</tr>
<tr>
<td>4.25</td>
<td>2.00</td>
<td>1.70</td>
</tr>
<tr>
<td>5.60</td>
<td>3.07</td>
<td>2.22</td>
</tr>
<tr>
<td>6.50</td>
<td>3.22</td>
<td>2.52</td>
</tr>
<tr>
<td>11.1</td>
<td>4.51</td>
<td>4.62</td>
</tr>
<tr>
<td>20.2</td>
<td>8.62</td>
<td>8.43</td>
</tr>
<tr>
<td>38.0</td>
<td>17.94</td>
<td>13.21</td>
</tr>
<tr>
<td>71.0</td>
<td>30.30</td>
<td>24.44</td>
</tr>
</tbody>
</table>

*See section A.2 of Appendix A for sample calculation of loading rates.

### 4.4.4. Effects of Initial Sulphide to Nitrate Ratio

The initial period of operation of the second bioreactor in which sulphide and nitrate concentrations were maintained at the same level as the first run was used to assess the reproducibility of the results obtained in the first run. The flow rates and corresponding sulphide and nitrate loading rates for this period of operation are shown in Table 4.2.

### Table 4.2. Experimental conditions in the second bioreactor*

<table>
<thead>
<tr>
<th>Flow Rate (mL/h)</th>
<th>Sulphide Loading Rate (mM/h)</th>
<th>Nitrate Loading Rate (mM/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.08</td>
<td>0.55</td>
<td>0.30</td>
</tr>
<tr>
<td>2.89</td>
<td>1.52</td>
<td>0.83</td>
</tr>
<tr>
<td>4.75</td>
<td>2.22</td>
<td>1.41</td>
</tr>
<tr>
<td>12.82</td>
<td>5.26</td>
<td>3.53</td>
</tr>
</tbody>
</table>

*See section A.2 of Appendix A for sample calculation of loading rates.

The effects of the initial sulphide to nitrate concentrations ratio on the performance of the bioreactor and the composition of the end products were determined by varying the concentration of nitrate, while maintaining a constant concentration of sulphide in the feed of $15.55 \pm 0.82$ mM. The flow rate was maintained at 12 mL/h for all ratios. Sulphide to nitrate ratios of 1.0, 1.5, 3.0, and
were studied. The corresponding nitrate concentrations for these ratios were 15 mM, 10 mM, 5 mM, and 2.5 mM, respectively. All other components of the CSB medium were kept constant at the same level described in section 4.1.1.

4.5. Analytical Methods

4.5.1. Measurement of Sulphide

The concentration of sulphide was determined using a spectrophotometric method (Cord-Ruwisch 1985). A solution of copper sulphate was added to the sample containing sulphide. Copper sulphide precipitated, and the absorbance of the solution (at 480 nm) was proportional to the sulphide concentration. A calibration curve was generated by adding 0.1 mL of standard sodium sulphide solutions (0-10 mM) to 0.9 mL of 5.0 mM acidic copper sulphate solution. The acidic copper sulphate solution contained 0.8 g/L of copper sulphate and 4.1 mL/L of HCl (36.5-38%). The absorbance of the mixture was measured at 480 nm using a spectrophotometer (SHIMADZU UVmini-1240 spectrophotometer). The calibration curve can be found in section C.1. of Appendix C.

4.5.2. Measurement of Nitrate, Nitrite, Sulphate, and Thiosulphate

The concentrations of sulphate, thiosulphate, nitrate, and nitrite were determined using ion chromatography. A Dionex Ion chromatograph (ICS-2500) with a conductivity detector (CD25A) equipped with an IonPac CG5A guard column and an IonPac CS5A analytical column was used. The eluent was 1.0 mM KOH. The applied flow rate of the eluent was 1.5 mL/h. The software used to operate the ion chromatograph was Chromeleon version 6.60 (Dionex Corporation, Sunnyvale, CA, U.S.A.). The system was calibrated using standard solutions of nitrite, nitrate, thiosulphate, and sulphate with concentrations in the range of 10 to 20 ppm.
The ions analyzed in the ion chromatograph were measured one time per sample. The standard deviations in concentration of the ions measured by ion chromatography were determined using standard solutions of these ions. The standards were analyzed in the ion chromatograph five times, and the standard deviation for these measurements was calculated for each ion.

### 4.5.3. Measurement of Sulphur

The measurement of sulphur content in the bioreactor after completion of the experiments was conducted by the Saskatchewan Research Council (SRC) Geoanalytical Laboratories (Saskatoon, SK). Samples (0.2 g) taken from three different sections of bioreactor in the vicinity of ports 1, 2 and 3 were analyzed in a LECO SC144DR C/S analyzer (LECO Corporation, St. Joseph, Michigan, U.S.A.) to measure total sulphur content of these samples.

### 4.5.4. Measurement of Protein

Protein in the liquid phase of the reactor was monitored each time steady state was achieved at a designated loading rate in the bioreactor. The samples (0.5 mL) were taken from each port at steady state. The protein analysis was conducted using a Coomasie Plus Bradford Assay Kit (Pierce, Rockford Illinois, U.S.A.). In order to determine the concentration of protein a standard curve was generated by measuring the absorbance of bovine serum albumin standards at 595 nm using a SHIMADZU UVmini-1240 spectrophotometer. To measure the concentration of protein in samples from the bioreactor, the samples were sonicated for 2 minutes at 10 Watts using a BRANSON Model 450 Sonifier (Branson Ultrasonics, Dansbury, CT, U.S.A.). The sample was then mixed with the assay reagent, and the absorbance was measured at 595 nm. A calibration curve developed using standard solutions of protein was used to determine the protein concentration. The calibration curve can be found in section C.2. of Appendix C.

After completion of the experiment, solid samples were taken from the first bioreactor to determine the protein concentration within the reactor. These
samples were washed with reverse osmosis water. The wash water was then centrifuged at 10x10$^3$ rpm for 5 minutes to remove large solid particles. The protein concentration in the wash water was then determined using the same method as was used for determining the protein concentration for liquid phase samples from the reactor. This was then used to determine the biomass hold-up in the bioreactor.

4.5.5. X-Ray Diffraction

In order to determine the composition and the type of sulphur compounds formed in the bioreactor, X-ray Diffraction (XRD) analysis was carried out by the Saskatchewan Research Council Geoanalytical Laboratories (Saskatoon, SK). XRD is conducted by illuminating a powdered sample with x-rays of a fixed wavelength. The intensity of the diffracted radiation is recorded and the angle of reflection is used to determine the atomic structure of the sample. After the experimental run was concluded in the first bioreactor, solid samples were taken from the bioreactor at the vicinity of each sampling port. The samples were analyzed in a Rigaku Miniflex, with Cu X-ray tube (Rigaku Americas Corporation, The Woodlands, Texas, U.S.A.).

4.6 Statistical Methods

4.6.1 Sulphide Analysis

In the batch experiments, sulphide was sampled periodically. The sample was taken once, and was then analyzed twice. The average of the two values was taken, and was reported with one standard deviation. In the continuous experiments, each port was sampled from once per sampling period. These samples were then analyzed twice for sulphide. As in the case of the batch experiments, the average of the two measurements was reported with one standard deviation, for each of the sampling ports. The standard deviations were calculated using Microsoft Excel®.
4.6.2 Ion Chromatography

In the batch experiments, samples were taken periodically. Each sample was taken one time, and was then analyzed in the ion chromatograph one time. In the continuous experiments, each port was sampled from once per sampling period. These samples were then analyzed once in the ion chromatograph. Standard deviation values for each ion were determined previously from standard samples which were analyzed five times with the ion chromatograph. The standard deviation values were calculated using Microsoft Excel®. The standard deviation values calculated from that analysis are reported in Table 4.3.

Table 4.3 Standard deviation values for ion chromatography measurements.

<table>
<thead>
<tr>
<th>Ion</th>
<th>Standard Deviation</th>
<th>(mg/L)</th>
<th>(mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate</td>
<td>0.017</td>
<td>2.7x10^{-4}</td>
<td></td>
</tr>
<tr>
<td>Nitrite</td>
<td>0.053</td>
<td>11.5x10^{-4}</td>
<td></td>
</tr>
<tr>
<td>Sulphate</td>
<td>0.009</td>
<td>0.94x10^{-4}</td>
<td></td>
</tr>
<tr>
<td>Thiosulphate</td>
<td>0.100</td>
<td>8.9x10^{-4}</td>
<td></td>
</tr>
</tbody>
</table>

5. RESULTS AND DISCUSSION

5.1. Batch Experiments

5.1.1. Effect of Initial Sulphide Concentration

The effect of the initial sulphide concentration on the sulphide biooxidation in batch system was studied at initial sulphide concentrations of approximately 2, 5, 15, and 20 mM, in duplicate. The initial nitrate concentration was 10 mM for all of these experiments. Sulphide measurements were conducted twice on each sample, and standard deviation for the measured concentration was calculated and included as error bars in the figures representing the experimental data. To show the reproducibility of the experimental data, profiles of sulphide, nitrate, sulphate and thiosulphate concentrations for experiments with initial sulphide concentrations of 1.9 mM and 1.7 mM are shown together in Figure 5.1. No nitrite was detected in either experiment. The comparison of the two data sets shows similar patterns: in both experiments, the majority of sulphide was oxidized within the first 6 hours, with corresponding sulphide oxidation rates for initial sulphide concentrations of 1.7 and 1.9 mM being 0.24 mM/h and 0.20 mM/h, respectively. Nitrate reduction rates in the presence of 1.7 and 1.9 mM sulphide were 0.29 mM/h and 0.22 mM/h, respectively. However, in both cases residual nitrate was detected in the cultures, suggesting that sulphide was the limiting substrate.
Figure 5.1 Sulphide, sulphate, nitrate, and thiosulphate concentrations profile during the oxidation of sulphide at initial sulphide concentrations of 1.9 mM (at left) and 1.7 mM (at right). Nitrite was not detected throughout these experiments. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
The sulphide, nitrate, nitrite, sulphate, and thiosulphate concentration profiles during oxidation of sulphide at initial concentrations of 5.4 mM, and 14.4 mM are shown in Figures 5.2, and 5.3, respectively. Experimental data for initial sulphide concentrations of 5.5 and 15.8 mM (repeated runs) are included in Section B.1 of Appendix B. With 5.4 mM sulphide, oxidation of sulphide occurred within the first 6 hours, with a corresponding rate of 0.74 mM/h. Nitrate reduction resulted in production of nitrite at a rate of 0.69 mM/h and was near complete over the first 6 hours. In the presence of 14.4 mM sulphide, sulphide oxidation was not significant during the first 238 hours which represented the lag phase in the bacterial activity. The sulphide oxidation and nitrate reduction rates were 0.68 mM/h and 0.53 mM/h, respectively. Nitrite was generated briefly during the period of sulphide oxidation and nitrate reduction; however, it was reduced immediately, and the nitrite concentration remained at a negligible level for the remainder of the experiment. Complete oxidation of sulphide in conjunction with complete reduction of nitrate and nitrite indicated that neither sulphide nor nitrate was limiting in this experiment. No microbial activity was observed in the experiment with an initial sulphide concentration of 20 mM (even after a period of 213 hours), indicating the strong inhibitory effect of sulphide at high concentrations. Table 5.1 presents the lag phase period and the rates of sulphide oxidation and nitrate reduction observed during these experiments. The measured initial sulphide concentrations are also included. The rates of sulphide oxidation and nitrate reduction were determined using the slope of the sulphide or nitrate concentration profiles during a period over which sulphide oxidation or nitrate reduction occurred (i.e. the part of the profiles representing the lag phase in microbial activity was not considered). A sample calculation can be found in Section A.1 of Appendix A.

For the initial sulphide concentrations of approximately 2 mM and 5 mM, no lag phase in bacterial activity was observed, while a lag phase of approximately 238 h (approximately 10 days) was seen with 15 mM sulphide. The experimental data indicated that the reduction of nitrate was somewhat coupled to the sulphide oxidation; that is, when the sulphide oxidation rate
increased or decreased, the nitrate reduction rate increased or decreased correspondingly.

A comparison of the rates presented in Table 5.1 indicate that increase in initial concentration of sulphide in the range 2 to 5 mM could lead to higher sulphide oxidation rate and thus a higher nitrate reduction rate during the exponential phase of bacterial activity. The rates do not increase between sulphide concentrations of 5.5 and 14.4 mM, and a decrease in the rates was observed at 15.8 mM. The extended lag phase observed with 14.4 and 15.8 mM sulphide in conjunction with the fact that bacteria was not able to oxidize 20 mM sulphide reveals the possible inhibitory effect of sulphide at high concentrations.

![Figure 5.2. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide concentration of 5.4 mM. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.](image)
Figure 5.3. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide concentration of 14.4 mM. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Table 5.1. Summary data for batch experiments conducted at various initial sulphide concentrations

<table>
<thead>
<tr>
<th>Initial Na₂S (mM)</th>
<th>Na₂S Oxidation Rate (mM/h)</th>
<th>NO₃ Reduction Rate (mM/h)</th>
<th>Lag Phase (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7</td>
<td>0.24</td>
<td>0.29</td>
<td></td>
</tr>
<tr>
<td>1.9</td>
<td>0.20</td>
<td>0.22</td>
<td>Not Observed</td>
</tr>
<tr>
<td>5.4</td>
<td>0.74</td>
<td>0.69</td>
<td></td>
</tr>
<tr>
<td>5.5</td>
<td>0.72</td>
<td>0.81</td>
<td></td>
</tr>
<tr>
<td>14.4</td>
<td>0.68</td>
<td>0.53</td>
<td>238</td>
</tr>
<tr>
<td>15.8</td>
<td>0.41</td>
<td>0.38</td>
<td>238</td>
</tr>
</tbody>
</table>

*Sample calculations for the rates are shown in section A.1 of Appendix A.

5.1.2. Effect of Sulphide to Nitrate Ratio

The effect of the initial sulphide to initial nitrate concentrations ratio was studied at initial sulphide to nitrate ratios of approximately 0.3 to 4.0. For each ratio, the experiment was conducted twice in order to show the reproducibility.
Control experiments in which no inoculation was added to the medium were also conducted for each ratio. The initial sulphide concentration was approximately 5 mM for each experiment, while the nitrate concentration was adjusted to achieve the designated sulphide to nitrate ratio. The experiments were carried out until the concentrations of sulphide, sulphate, nitrate, and nitrite stabilized; this period translated approximately to 290 hours. In order to best present the data during sulphide oxidation, only the first 52 hours of the experiments are shown here. After the initial 52 hours, sulphide oxidation was complete, and the nitrate level had stabilized. In some cases, nitrite reduction occurred after the initial 52 hours, along with additional generation of sulphate. Figures representing the concentration profiles for the entire experimental period (290 h) can be found in section B.2 of Appendix B. The experimental data for sulphide to nitrate ratios of 4.08, 2.30, 0.77, 0.56, and 0.46 are shown in Figures 5.4, 5.5, 5.7, 5.8, and 5.9, respectively. Figure 5.6 shows ratios of 1.14 and 1.12 together. As indicated in the materials and methods these experiments were conducted in duplicate. The experimental data for the ratios of 1.14 and 1.12 are both shown in order to demonstrate the reproducibility of the data for a similar sulphide to nitrate ratio. The experimental data for sulphide to nitrate ratios of 2.17, 0.83, 0.59, and 0.42 (representing the data generated in the replicates) are shown in Section B.2 of Appendix B.

For all of the tested sulphide to nitrate ratios, sulphide oxidation took place within the first 6 hours. For the ratio of 4.08, sulphide oxidation was not complete, while nitrate was completely reduced. Nitrite was generated and reduced during the period in which sulphide oxidation occurred. Only 2.4% of the initial sulphide was converted to sulphate in this experiment. These observations indicated that for these ratios, nitrate was the limiting reactant. An experimental error occurred during the nitrate adjustment of the duplicate experiment at this ratio (initial sulphide to nitrate ratio of 4.09); as a result, the data for this repeated experiment has been excluded.
Figure 5.4. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratio of 4.08. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Sulphide oxidation in all other tested ratios was complete. For the initial sulphide to nitrate ratio of 2.30, nitrate reduction was complete and nitrite was generated and reduced completely during the period of sulphide oxidation. Of the initial sulphide, 9.4% was converted to sulphate. In this case, neither sulphide nor nitrate was limiting. For the initial ratios of 1.12 and 1.14, nitrate reduction was complete and nitrite was generated during the period of sulphide oxidation, and reduced shortly after. For the ratio of 1.12, 59.2% of the initial sulphide was converted to sulphate while for the ratio of 1.14, 54.4% of the initial sulphide was converted to sulphate. This indicates that neither nitrate nor sulphide were limiting at these ratios. Nitrate reduction was also complete for the initial sulphide to nitrate ratio of 0.77 and 0.56. Nitrite was generated during nitrate reduction and was reduced gradually over the remainder of the experiment. For both ratios of 0.77 and 0.56, nitrite reduction was complete, although it did not occur within the first 52 hours of the experiment and as a
result is not shown. At a ratio of 0.77, 70.6% of the initial sulphide was converted to sulphate; for the ratio of 0.56, 93.8% of the initial sulphide was converted to sulphate. In the 0.46 sulphide to nitrate ratio experiment, nitrate reduction was not complete. Approximately 6% of the initial nitrate remained unconverted. Nitrite was generated during nitrate reduction and was completely reduced over the remainder of the experiment (not shown).

![Graph showing concentrations of sulphide, sulphate, nitrate, nitrite, and thiosulphate](image)

**Figure 5.5.** Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratio of 2.30. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure 5.6. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratios of 1.14 (at left) and 1.12 (at right). Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure 5.7. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratio of 0.77. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure 5.8. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratio of 0.56. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure 5.9. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide to nitrate ratio of 0.46. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

The calculated sulphide oxidation and nitrate reduction rates together with the ratios of initial sulphide to nitrate concentrations at which these rates were measured are shown in Table 5.2. (See section A.1 of Appendix A for a sample of calculations). For the purpose of discussion, the initial sulphide and nitrate concentrations for each experiment are also included.

The variation of nitrate reduction and sulphide oxidation rates as a function of the initial sulphide to nitrate ratio are shown in Figure 5.10. As the initial sulphide to nitrate ratio decreased, the sulphide oxidation rates appear to increase, although only slightly. The increase in the observed nitrate reduction rates is more obvious. This may be driven by the increase in the initial nitrate concentration and possible dependency of the reaction rate on concentration of nitrate. The increasing trend is apparent for initial nitrate concentrations within the range of 1.3 mM to 7.3 mM. At higher nitrate concentrations (7.3 mM to 14.6) a relatively constant rate is observed.
Table 5.2. Summary data for batch experiments for various sulphide to nitrate ratios*

<table>
<thead>
<tr>
<th>Initial Sulphide to Nitrate Ratio</th>
<th>Initial [Na₂S] concentration (mM)</th>
<th>Na₂S Oxidation Rate (mM/h)</th>
<th>Initial [NO₃] concentration (mM)</th>
<th>NO₃ Reduction Rate (mM/h)</th>
<th>Lag Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.08</td>
<td>5.3</td>
<td>0.68</td>
<td>1.3</td>
<td>0.39</td>
<td>Not Observed</td>
</tr>
<tr>
<td>2.30</td>
<td>5.5</td>
<td>0.84</td>
<td>2.4</td>
<td>0.65</td>
<td></td>
</tr>
<tr>
<td>2.17</td>
<td>5.4</td>
<td>0.86</td>
<td>2.5</td>
<td>0.68</td>
<td></td>
</tr>
<tr>
<td>1.14</td>
<td>5.6</td>
<td>0.89</td>
<td>4.9</td>
<td>0.98</td>
<td></td>
</tr>
<tr>
<td>1.12</td>
<td>5.3</td>
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<td>4.7</td>
<td>1.07</td>
<td></td>
</tr>
<tr>
<td>0.83</td>
<td>6.1</td>
<td>0.91</td>
<td>7.3</td>
<td>1.41</td>
<td></td>
</tr>
<tr>
<td>0.77</td>
<td>5.7</td>
<td>0.90</td>
<td>7.5</td>
<td>1.43</td>
<td></td>
</tr>
<tr>
<td>0.59</td>
<td>5.8</td>
<td>0.92</td>
<td>9.8</td>
<td>1.39</td>
<td></td>
</tr>
<tr>
<td>0.56</td>
<td>5.5</td>
<td>0.87</td>
<td>9.9</td>
<td>1.42</td>
<td></td>
</tr>
<tr>
<td>0.46</td>
<td>6.5</td>
<td>1.03</td>
<td>14.3</td>
<td>1.44</td>
<td></td>
</tr>
<tr>
<td>0.42</td>
<td>6.2</td>
<td>0.98</td>
<td>14.6</td>
<td>1.33</td>
<td></td>
</tr>
</tbody>
</table>

*Sample calculations for the rates are shown in section A.1 of Appendix A.

Figure 5.10. Sulphide and nitrate removal rates as functions of initial sulphide to nitrate ratio.
The effect of the initial sulphide to nitrate ratio on the composition of end products was also verified. It was found that the percentage of sulphide that was converted to sulphate increased as the initial sulphide to nitrate ratio decreased. At the sulphide to nitrate ratio of 0.42, all of the sulphide was converted to sulphate, suggesting that at ratios lower than 0.42, nitrate would be in excess, and sulphide would be the limiting reactant. At a sulphide to nitrate ratio of 4.08, only 2.4% of the sulphide added was oxidized to sulphate, indicating that at ratios in excess of 4.08, the nitrate would be the limiting reactant. The percentage of sulphide converted to sulphate as a function of the initial sulphide to nitrate ratio is shown in Figure 5.11.

![Figure 5.11](image)

**Figure 5.11.** The effect of the initial sulphide to nitrate ratio on the percentage of sulphide converted to sulphate.

Control experiments were conducted for sulphide to nitrate ratios of 4.16, 2.20, 1.20, 0.77, 0.59, and 0.31. The control experiments were carried out for 169 hours. In all of the control experiments, a small amount of sulphide oxidation took place initially which can be attributed to a spontaneous chemical oxidation of sulphide that occurred upon the addition of sulphide to the serum bottles. No significant nitrate reduction took place in the controls, and nitrite was not detected. This indicates that in the preceding...
experiments bacterial activity was responsible for sulphide oxidation, nitrate reduction, and subsequent generation and reduction of nitrite. The experimental data for the control experiment at a sulphide to nitrate ratio of 1.20 is shown in Figure 5.12. The remainder of the control data is shown in Section B.3 of Appendix B.

![Figure 5.12. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at an initial sulphide to nitrate ratio of 1.20. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.](image)

**5.2. Continuous Bioreactor Experiments**

**5.2.1. Effect of Volumetric Loading Rate of Sulphide**

The effect of the volumetric loading rate of sulphide on the performance of the first bioreactor was examined over a period of 5616 hours (234 days). During this period, sulphide loading rates ranging from 0.26 mM/h to 30.30 mM/h were applied and the performance of the bioreactor in terms of conversion and volumetric reaction rate for both sulphide oxidation and nitrate reduction was evaluated. Sample calculations for loading rate, reaction rate, and percent conversion can be found in Appendix A.
Transient residual sulphide concentration data for ports one and three (located at the bottom and top of the bioreactor) for the duration of the experiment are shown in Figure 5.13. Labeled sections on the graph represent the periods over which various loading rates were applied.

**Figure 5.13.** Transient data for the first bioreactor. Ports 1 and 3 are shown. Lettered sections indicate periods for each loading rates (A=0.26-0.59 mM/h, B=0.97 mM/h, C=1.37 mM/h, D=2.00 mM/h, E=3.07 mM/h, F=3.22 mM/h, G=4.51 mM/h, H=8.62 mM/h, I=17.94 mM/h, J=30.30 mM/h).

When an increase in the loading rate was applied, the residual sulphide concentration in the bioreactor increased temporarily. Over time, the bioreactor recovered from the perturbation and sulphide concentration decreased until steady state was reached. The increase in residual sulphide concentration in the bioreactor typically occurred in the bottom port but was not always observed in the middle and top ports. When residual sulphide was observed in all of the bioreactor ports, the sulphide concentration in the third port dropped first, followed by the second port, and lastly, the first port. It was also found that when sulphide concentrations greater than 18 mM were introduced into the bioreactor, due to inhibitory effect of sulphide a significant increase
in the residual sulphide concentration in the bioreactor was observed. The residual sulphide did not decrease until sulphide concentration in the feed (medium bag) was readjusted to the appropriate concentration (15-18 mM). This accounts for peaks (labeled by arrows) that are observed at times when the flow rate has not been increased. Steady state was achieved at all loading rates, except 3.07 mM/h (section E). During this stage, an unintentional high sulphide concentration of the feed caused an inhibitory effect, and it was necessary to decrease both the flow rate and the sulphide concentration in order to allow the bioreactor to recover. The percent conversion for sulphide at steady state remained within the range of 97.6% to 99.7% for all other loading rates. The steady state volumetric reaction rate and percent conversion of sulphide as a function of the volumetric loading rate of sulphide are shown in Figure 5.14. As can be seen, an increase in loading rate of sulphide initially decreased the conversion of sulphide. This decreasing trend however leveled off as loading rate was increased above 10 mM/h. A linear increase in volumetric oxidation rate of sulphide was observed as sulphide loading rate was increased with the slope of the line being 0.9841 ($R^2=1$).

![Figure 5.14](image-url)

**Figure 5.14.** The effect of the sulphide loading rate on the performance of the first bioreactor.
As the sulphide loading rate was increased, the nitrate loading rate was increased in proportion. In the first bioreactor, steady state nitrate loading rates ranged from 0.19 mM/h to 24.44 mM/h, and percent conversion ranged from 97.2% to 100%. The volumetric reaction rate and percent conversion of nitrate as functions of the volumetric loading rate of nitrate in the first bioreactor are shown in Figure 5.15. As can be seen, an increase in loading rate of nitrate initially increased the conversion of nitrate. This trend leveled off as the loading rate was increased above 1.5 mM/h. A linear increase in volumetric reduction rate of nitrate was observed as the nitrate loading rate was increased with the slope being 0.9981 (R²=1).

![Figure 5.15. The effect of the nitrate loading rate on the performance of the first bioreactor](image)

5.2.2. Total Protein Concentration in the Liquid Phase

The protein concentration (as an indication of biomass concentration) was determined for each sample taken from the liquid phase at each port in the first bioreactor at each loading rate of sulphide. The results are shown in Figure 5.16. At low sulphide loading rates, there appears to be a slight increase in the protein concentration as the volumetric loading rate increased. However, this trend appears to
level off for loading rates above 10 mM/h, indicating that the protein concentration in the liquid phase became insensitive to the increase in loading rate beyond 10 mM/h. The liquid samples taken from these ports showed similar protein concentrations at the same loading rates, indicating that the location in the reactor did not significantly influence the protein concentration in the liquid phase.

![Graph](image)

**Figure 5.16.** Protein concentration in the liquid phase for steady states at each sulphide loading rate for the first bioreactor. Error bars indicate one standard deviation.

5.2.3. Analysis of Solid Samples Taken from the First Bioreactor

5.2.3.1. X-Ray Diffraction and Sulphur Analysis

After completion of the experimental run, the first bioreactor was disassembled and solid samples taken from various parts were analyzed for sulphur deposition using sulphur analysis and X-Ray Diffraction (XRD), by the SRC Geoanalytical Laboratory. The sulphur analysis was completed by a sulphur analyzer working on the basis of a combination of combustion and infrared technology. Sulphur analysis was conducted for samples from five locations in the bioreactor while XRD was conducted for samples at three of these five locations. The analysis of the sample by XRD indicated that the samples taken from the bioreactor consists of only silica (the carrier used for cell
immobilization) and sulphur (i.e. no other sulphur compound was detected). The percentage of sulphur (wt%) in the samples taken from different locations, measured by two different methods, is shown in Table 5.3. The difference between the sulphur content reported by the two methods could be due to variability of the sulphur content of the samples used in these analytical methods. Figure 5.17 is a photograph demonstrating the sulphur deposition in the first bioreactor after the experiment was completed.

**Table 5.3. Sulphur Deposition in the first Bioreactor**

<table>
<thead>
<tr>
<th>Position from Bottom of Reactor (cm)</th>
<th>Sulphur Analysis [S] (wt%)</th>
<th>XRD Analysis [S] (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>0.27</td>
<td>0</td>
</tr>
<tr>
<td>21</td>
<td>0.91</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>4.38</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>9</td>
<td>11.8</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>25.0</td>
<td>26.3 ± 1.6</td>
</tr>
</tbody>
</table>

**Figure 5.17.** First bioreactor after completion of experimental run (left) and prior to experimental run (right).
5.2.3.2. **Quantity of Attached Biomass (mg Protein/g Sand)**

Samples from the first bioreactor were also analyzed for the quantity of protein. These samples were taken from the same five locations indicated in Table 5.4. The results are shown in Table 5.4. The protein content in the bottom 9 cm of the bioreactor was higher than that of the upper portion of the bioreactor. This indicates that bacterial growth was higher in the bottom portion of the bioreactor. Given that the reactor was up-flow (and therefore fed from the bottom), it is expected that the bacterial growth would be higher where the feed sulphide concentration was highest. As the medium flowed upward through the bioreactor, the concentration was reduced by the bacterial activity, thus reducing the amount of sulphide available for bacterial growth in the upper portion of the bioreactor.

**Table 5.4.** Protein content of the Sand Matrix in First Bioreactor.

<table>
<thead>
<tr>
<th>Position from Bottom of Bioreactor (cm)</th>
<th>Protein Content (mg protein/g sand)</th>
</tr>
</thead>
<tbody>
<tr>
<td>27</td>
<td>5.61±0.02</td>
</tr>
<tr>
<td>21</td>
<td>5.86±0.05</td>
</tr>
<tr>
<td>15</td>
<td>6.01±0.11</td>
</tr>
<tr>
<td>9</td>
<td>6.74±0.08</td>
</tr>
<tr>
<td>3</td>
<td>6.36±0.03</td>
</tr>
</tbody>
</table>

A second bioreactor experiment was conducted for the purpose of examining the effect of the sulphide to nitrate concentrations ratio. For the initial part of experiment in this bioreactor the sulphide and nitrate concentrations were maintained at the same level as the experiment in the first bioreactor. The sulphide loading rate was varied in the range 0.55 mM/h to 5.26 mM/h, which served to assess the reproducibility of the data generated in the first bioreactor.

The percent conversion of sulphide at steady state ranged from 99.2% to 99.5%, thus confirming the previously achieved conversions in the first bioreactor. The steady state volumetric reaction rate and percent conversion as functions of the volumetric loading rate of sulphide for the first and second bioreactor are compared in Figure 5.18 which reveals the reproducibility of the data.
Figure 5.18. Comparison of the volumetric oxidation rate of sulphide in the first and the second bioreactors

The steady state volumetric reaction rate for reduction of nitrate and percent conversion of nitrate as functions of the volumetric loading rate of nitrate for the first and second bioreactor are compared in Figure 5.19. Volumetric loading rates of nitrate in the second bioreactor ranged from 0.30 mM/h to 3.5 mM/h. The percent conversion of nitrate remained between 98.4% and 99.9%. As can be seen data generated in the first and second bioreactor match very well (variation in conversion around 2.5%) indicating the reproducibility of the data.
Figure 5.19. Comparison of the volumetric reduction rate of nitrate in the first and the second bioreactors.

5.2.4. Effect of Sulphide to Nitrate Ratio

The effect of the sulphide to nitrate ratio was studied in the second bioreactor following the completion of the reproducibility experiment. The bioreactor was operated continuously from the reproducibility experiment to the sulphide to nitrate ratio experiments.

In this part of the experiment the concentration of sulphide in the medium was maintained at $14.9 \pm 2.2$ mM and the nitrate concentrations of $14.4$ mM, $11.0$ mM, $5.4$ mM, and $2.8$ mM were applied to achieve sulphide to nitrate ratios of $1.1$, $1.3$, $3.1$, $5.0$, respectively. The transient data for ports 1 and 3 are shown in Figure 5.20. Lettered sections indicate the period over which a designated sulphide to nitrate ratio was applied.
Figure 5.20. Transient data from ports 1 and 3 during sulphide to nitrate ratio experiments. Lettered sections indicate periods for each ratio (A= 1.3, B= 3.1, C= 5.0, D= 1.1).

In order to avoid severely shocking the bioreactor, the ratio of 1.1 was completed last, as it involved the highest concentration of nitrate (14.4 mM). During the transition from the ratio of 5.0 to the ratio of 1.1, the nitrate concentration in the feed was increased first to 10 mM, and the bioreactor was allowed to reach steady state before increasing the nitrate concentration to 14.4 mM.

As in the first bioreactor, when the feed (medium bag) sulphide concentration exceeded 18 mM, due to inhibitory effect of sulphide, a significant increase in the residual sulphide concentration in the bioreactor was observed. The resulting peaks in sulphide concentration are indicated with arrows. The residual sulphide concentration decreased when the medium concentration was readjusted back to 15-18 mM.

Complete sulphide oxidation occurred at the sulphide to nitrate ratios of 1.1 and 1.3. At a ratio of 1.1 only 7.2% of the sulphide was converted to sulphate, while at a ratio of 1.3, 19.5% of the sulphide was converted to sulphate. This is contrary to normal expectation that presence of a higher level of nitrate (ratio of 1.1) should lead to
production of more sulphate. At the ratio of 1.1, the percent conversion of nitrate dropped to 59.6%. If nitrate reduction had been complete, more sulphate would have been generated. It is necessary to conduct further experiments at sulphide to nitrate ratios lower than 1.1 to determine the cause of the drop in the percent conversion of nitrate. At ratios of 3.1 and 5.0, sulphide oxidation was incomplete, resulting in residual sulphide concentrations of 1.6 mM and 4.9 mM (as measured in port 3), respectively. The percentage of sulphide converted to sulphate and the residual sulphide concentrations at sulphide to nitrate ratios of 1.1, 1.3, 3.1, and 5.0 are shown in Figure 5.21. No sulphate was generated for sulphide to nitrate ratios of 3.1, and 5.0.

![Graph](image)

**Figure 5.21.** The effect of the initial sulphide to nitrate ratio. The percentage of sulphide converted to sulphate and the residual sulphide concentration for sulphide to nitrate ratios of 1.1, 1.3, 3.1, and 5.0 are shown.

For the sulphide to nitrate ratios of 3.1 and 5.0, it is apparent that nitrate was limiting, and thus sulphide oxidation was not complete. The volumetric oxidation rate of sulphide and percent conversion of sulphide at each of the ratios are shown in Figure 5.22. The sulphide reaction rate at a ratio of 1.1 was 3.20 mM/h, with a percent conversion of 99.8%. At the sulphide to nitrate ratio of 1.3, the sulphide reaction rate was 2.37 mM/h, with a percent conversion of 99.8%. The percent conversion of
sulphide did not decrease between the ratios of 1.1 and 1.3, which indicates that the decrease in the sulphide reaction rate between the ratios of 1.1 and 1.3 is due to a decrease in the sulphide loading rate.

The volumetric reduction rate of nitrate and percent conversion of nitrate at each of the ratios are shown in Figure 5.23. At the sulphide to nitrate ratio of 1.1, the percent conversion of nitrate was 59.6%, with a residual nitrate concentration of 5.8 mM. At the sulphide to nitrate ratio of 1.3, the percent conversion of nitrate was 96.7%, with a residual nitrate concentration of 0.4 mM. For ratios of 3.1 and 5.0, the percent conversion of nitrate was 100%. The decrease in the nitrate reaction rates at higher ratios was due to a decrease in the nitrate loading rates. At the ratios of 3.1 and 5.0, the percent conversion of sulphide drops to 90.4 % and 65.0 %, respectively. This indicates that for sulphide to nitrate ratios exceeding 1.3, nitrate was a limiting reactant.

![Figure 5.22. Effect of the sulphide to nitrate ratio on the volumetric oxidation rate and percent conversion of sulphide.](image)
Figure 5.23. Effect of sulphide to nitrate ratio on volumetric reduction rate and percent conversion of nitrate.

5.2.5. Comparison to Previous Work

Table 5.5 compares the results of previous works on biooxidation of sulphide using both free cell reactors and immobilized cell (biofilm) reactors. Oncharit et al. (1990) studied sulphide oxidation by freely suspended cells of *Thiobacillus denitrificans* in a continuously stirred tank reactor with a biomass recycle, and was able to achieve a maximum sulphide oxidation rate of 3.2 mM/h. Gadekar et al. (2006) achieved a maximum sulphide oxidation rate of 3.2 mM/h by *Thiomicrospira* sp. CVO in a continuously stirred tank reactor. The maximum sulphide oxidation rate of the present work, in an immobilized cell bioreactor was 30.30 mM/h (98.5% conversion), showing an improvement of approximately 9.5 times over the previous work in continuously stirred tank reactors with freely suspended cells.

Datta et al. (2007), Krishnakumar et al. (2005), Ma et al. (2006), and Lee et al. (2006) all studied sulphide oxidation in various forms of immobilized cell reactors. The maximum sulphide oxidation rate reported in these works was 19.7 mM/h achieved in a packed-bed reactor, using *Acidithiobacillus thiooxidans* (Lee et al., 2006).
maximum sulphide oxidation rate by the enrichment culture used in this work, 30.30 mM/h (98.5% conversion), is around 50% higher than that reported in the literature.

By comparison to the previous works, it is clear that the present work has achieved a significant improvement as far as sulphide oxidation rate is concerned. The improvement in the sulphide oxidation rate could be, in part, attributed to the utilization of a novel microbial community, capable of oxidizing sulphide at a concentration of 15 mM. In addition, immobilization of the cells has provided a high concentration of bacteria within the bioreactor, leading to a significant increase of sulphide oxidation arte when compared with a continuous bioreactor with freely suspended cells.

**Table 5.5.** Comparison of Sulphide Oxidation Rate to Previous Works.

<table>
<thead>
<tr>
<th>Species</th>
<th>Reactor Configuration</th>
<th>pH</th>
<th>T (°C)</th>
<th>Electron Acceptor</th>
<th>Maximum Sulphide Oxidation Rate</th>
<th>End Product</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Thiobacillus denitrificans</em></td>
<td>Packed column</td>
<td>6.8-7.4</td>
<td>30-35</td>
<td>O₂</td>
<td>0.817 mM/h</td>
<td>Sulphur</td>
<td>Ma et al., 2006</td>
</tr>
<tr>
<td>immobilized on</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>activated carbon</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microbial community in</td>
<td>Biotrickling filter</td>
<td>4-5</td>
<td>70</td>
<td>O₂</td>
<td>1.1 mM/h</td>
<td>-</td>
<td>Datta et al. 2007</td>
</tr>
<tr>
<td>hot pools around Lake Rotorua</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>in New Zealand</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thiobacillus denitrificans</em></td>
<td>Reverse fluidized loop reactor</td>
<td>9.5</td>
<td>-</td>
<td>O₂</td>
<td>2.3 mM/h</td>
<td>Sulphur and sulphate</td>
<td>Krishnakumar et al., 2005</td>
</tr>
<tr>
<td><em>Acidithiobacillus thiooxidans</em></td>
<td>Packed-bed reactor</td>
<td>-</td>
<td>-</td>
<td>O₂</td>
<td>19.7 mM/h</td>
<td>Sulphate</td>
<td>Lee et al., 2006</td>
</tr>
<tr>
<td><em>Thiobacillus denitrificans</em></td>
<td>CSTR with total biomass</td>
<td>-</td>
<td>-</td>
<td>O₂</td>
<td>3.2 mM/h</td>
<td>Sulphate</td>
<td>Oncharit et al., 1990</td>
</tr>
<tr>
<td>reactor</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thiomicrospira</em> sp. CVO</td>
<td>Continuously Stirred Tank</td>
<td>7.0</td>
<td>-</td>
<td>NO₃</td>
<td>3.2 mM/h</td>
<td>No Sulphate formed</td>
<td>Gadekar et al., 2006</td>
</tr>
<tr>
<td>enrichment culture</td>
<td>Reactor (CSTR)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thiomicrospira</em> sp. CVO</td>
<td>Immobilized Cell Bioreactor</td>
<td>6.8-7.2</td>
<td>22</td>
<td>NO₃</td>
<td>30.30 mM/h</td>
<td>Sulphur and Sulphate</td>
<td>Present Work</td>
</tr>
<tr>
<td>(packed column)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
6. CONCLUSIONS AND RECOMMENDATIONS

6.1. Conclusion

The objective of the present work was to study the biooxidation of sulphide under denitrifying conditions in batch system and a continuous immobilized cell reactor. The culture used was a mixed microbial culture, enriched from the produced water of a Canadian oil reservoir.

6.1.1. Batch Experiments

In the batch experiments, the effect of sulphide concentration on the activity of the microbial culture and biooxidation of sulphide under denitrifying conditions was studied. Batch experiments were conducted in duplicate at sulphide concentrations of approximately 2 (1.7, 1.9), 5, (5.4, 5.5), and 15 (14.4, 15.8), and 20 mM. The culture was not able to oxidize sulphide at 20 mM. For sulphide concentrations in the range of 1.7 to 15.8 mM, sulphide oxidation was complete. For sulphide concentrations ranging from 1.7 to 5.5 mM, an increase in the sulphide initial concentration led to an increase in the sulphide oxidation rate, and thus an increase in the nitrate reduction rate during the exponential phase of bacterial activity. An extended lag phase was observed when initial sulphide concentrations of 14.4 and 15.8 mM were used; given that the culture could not oxidize sulphide at 20 mM one could conclude that sulphide at concentrations above 15 mM could impose a strong inhibitory effect on the bacterial activity.

The effect of the initial sulphide to nitrate ratio on the sulphide oxidation and nitrate reduction rates, as well as the composition of the end-products was also studied. These experiments were carried out at initial sulphide to nitrate ratios ranging from 0.3 to 4.0, in duplicate. It was found that as the initial sulphide to nitrate ratio decreased, the sulphide oxidation and nitrate reduction rates increased. The increase in nitrate reduction rates was more pronounced than that of the sulphide oxidation rates; the increasing trend was observed for initial nitrate concentrations in the range of 1.3 to 7.3
mM, corresponding to ratios of 4.08 to 0.83. At nitrate concentrations higher than 7.3 mM (ratios lower than 0.83), the nitrate reduction rate remained relatively constant. It was found that the percentage of sulphide that was oxidized to sulphate increased as the initial sulphide to nitrate ratio decreased. At the sulphide to nitrate ratio of 0.42, the sulphide was completely converted to sulphate, indicating that at ratios lower than 0.42, nitrate would be in excess. At the ratio of 4.08, 2.4% of the sulphide was converted to sulphate, indicating that at ratios greater than 4.08, nitrate would be limiting.

6.1.2. Continuous Experiments

In the continuous bioreactor systems, the effects of the loading rate of sulphide and the loading rate of nitrate on the performance of the bioreactor with respect to sulphide oxidation and nitrate reduction were investigated. At sulphide loading rates ranging from 0.26 to 30.30 mM/h, sulphide conversion remained in the range of 97.6% to 99.7%. A linear increase in the volumetric oxidation rate of sulphide was observed as the sulphide loading rate was increased. Similarly, at nitrate loading rates ranging from 0.19 to 24.44 mM/h, the nitrate conversion ranged from 97.2% to 100%. A linear increase in volumetric reduction rate of nitrate was observed as the nitrate loading rate was increased. The maximum sulphide oxidation rate was 30.30 mM/h with 98.5% conversion, and the maximum nitrate reduction rate was 24.44 mM/h with 99.7% conversion. Operating a second bioreactor under conditions similar to the first one for volumetric loading rates of sulphide in the range 0.55 to 5.26 mM/h confirmed the reproducibility of the data with respect the oxidation of sulphide and reduction of nitrate. These results demonstrate that the utilization of immobilized cells significantly improved the oxidation rate of sulphide over free cell systems.

The effect of the ratio of sulphide to nitrate on the performance of the bioreactor and the composition of the end products was studied at sulphide to nitrate ratios of 1.1, 1.3, 3.1, and 5.0. Sulphide conversion was complete at sulphide to nitrate ratios of 1.1 and 1.3, but decreased to 90.5% at the ratio of 3.1 and 65.0% at the ratio of 5.0. This indicates that nitrate was limiting for sulphide to nitrate ratios of 3.1 and 5.0. It was found that the increase in the sulphide to nitrate ratio (and the resulting limitation of
nitrate) caused a decrease in the volumetric oxidation rate of sulphide. The nitrate reduction rate decreased as the ratios of sulphide to nitrate increased. This may have been due to the decrease in nitrate concentration, rather than the change in sulphide to nitrate ratio.

At sulphide to nitrate ratios of 1.3, 3.1, and 5.0, nitrate conversion was complete; however, at a ratio of 1.1, the conversion of nitrate dropped to 59.6%, indicating that nitrate was in excess, and sulphide was the limiting reactant. For ratios of 1.3, 3.1, and 5.0, it was determined that the volumetric reaction rate of nitrate decreased as the sulphide to nitrate ratio increased. Given that nitrate reduction was complete at these ratios, the decrease in nitrate reduction rate is largely attributed to the decrease in the nitrate loading rate. At the sulphide to nitrate ratios of 1.1 and 1.3, 7.2% and 19.6% of the sulphide was converted to sulphate, respectively. There was no residual sulphide in the bioreactor effluent at these ratios. At ratios of 3.1 and 5.0, no sulphate was generated, and the residual sulphide concentrations were 1.6 and 4.9 mM, respectively, indicating that for ratios between 1.3 and 5.0, an increase in the ratio caused a decrease in the generation of sulphate.

6.2. Recommendations for Future Work

The following is a list of recommendations for future work regarding the oxidation of sulphide by the enriched microbial culture.

- The biological oxidation of gaseous H₂S should be studied in both continuously stirred tank reactors and immobilized cell reactors.

- Various reactor configurations, such as a fluidized bed reactor, should be examined to determine the optimum configuration for biological sulphide oxidation.
• The performance of the bioreactor at higher sulphide concentrations should be studied to determine the maximum tolerable sulphide concentration in the bioreactor.

• The performance of the bioreactor with other immobilization matrices, such as activated carbon, should be examined.

• The effect of temperature and pH on the performance of the bioreactor and the kinetics of sulphide oxidation should be studied.
7. REFERENCES


A. Sample Calculations

A.1. Sulphide Oxidation Rate and Nitrate Reduction Rate

In batch experiments, the sulphide oxidation rate was determined using the slope of the experimental data during the exponential phase of bacterial activity which corresponded to a significant decrease in concentration of sulphide (i.e. data collected during the lag phase corresponding to a relatively stable sulphide concentration was excluded). A similar approach was used to calculate the reduction rate of nitrate. In Figure A.1.1, complete sulphide and nitrate data for an initial sulphide concentration of 14.4 mM is shown. Figure A.1.2. shows only the data utilized in the rate calculations, along with the linear trendlines for the period. Equations for the trendlines and their respective $R^2$ values are shown.

Figure A.1.1. Complete sulphide and nitrate data for batch experiment with initial sulphide concentration of 14.4 mM.
Figure A.1.2. Sulphide and nitrate data selected for rate calculations. Linear trendlines and equations are shown for both sulphide and nitrate.

A.2. Volumetric Loading Rate

In the continuous experiments, the volumetric loading rate was calculated for both sulphide and nitrate. The equation for the volumetric loading rate is as follows:

$$VLR = \frac{C_i}{HRT}$$  \hspace{1cm} \text{A.2.1.}

Where: \( VLR \) is the volumetric loading rate (mM/h)

- \( C_i \) is the inlet concentration (mM)
- \( HRT \) is the hydraulic retention time (h)

For an inlet medium sulphide concentration of 16.36 mM, and a hydraulic retention time of 12.63 h, the VLR of sulphide is calculated as follows:
The hydraulic retention time was determined using the following equation:

$$\text{HRT} = \frac{\text{void volume (mL)}}{\text{flow rate (mL/h)}} = \frac{35 \text{ mL}}{0.5 \text{ mL/h}} = 70 \text{ h}^{-1} \quad \text{A.2.2.}$$

The void volume of the bioreactors could not be measured during the experiment. Prior to beginning the experiments, the void volume of the bioreactors was found to be 60 mL, and at the end of the experiment, the void volume of the first bioreactor was measured to be 10 mL. The initial void volume of the bioreactor was determined by filling the bioreactor packed with the sand matrix with liquid medium and allowing the liquid to drain completely from the bioreactor. The volume of the drained liquid was equivalent to the void volume. Following the completion of the experimental run the liquid content of the bioreactor was measured again by allowing the liquid to drain for a long period of time. Given that the experiment was run over an extended period of time, an average value of 35 mL was used in the calculations. The same value was used for the second bioreactor as the specification of this bioreactor is exactly the same as the first one.

**A.3. Volumetric Reaction Rate**

In the continuous experiments, the volumetric reaction rate was calculated for both sulphide and nitrate. The equation for the volumetric reaction rate is as follows:

$$\text{VRR} = \frac{C_i - C_o}{\text{HRT}} \quad \text{A.3.1.}$$

Where:  
VRR is the volumetric reaction rate (mM/h)  
$C_i$ is the inlet medium concentration (mM)  
$C_o$ is the outlet medium concentration (mM)
HRT is the hydraulic retention time (h)

For an inlet medium nitrate concentration of 10.22 mM, an outlet nitrate concentration of 0.32 mM, and a hydraulic retention time of 20.76 h, the VRR is calculated as follows:

\[
VRR = \frac{C_i - C_o}{HRT} = \frac{10.22 \text{ mM} - 0.32 \text{ mM}}{20.76 \text{ h}} = 0.48 \text{ mM/h}
\]

**A.4. Percent Conversion**

The percent conversion for both sulphide and nitrate in the continuous bioreactors was determined using the following equation:

\[
\text{% Conversion} = \frac{VRR}{VLR} \times 100\% \quad \text{A.3.2.}
\]

Where: % Conversion is the percent conversion

VRR is the volumetric reaction rate (mM/h)

VLR is the volumetric loading rate (mM/h)

For a sulphide VRR of 0.8804 mM/h and a sulphide VLR of 0.8847 mM/h, the percent conversion of sulphide is calculated as follows:

\[
\text{% Conversion} = \frac{VRR}{VLR} \times 100\% = \frac{0.8804}{0.8847} \times 100\% = 99.51\%
\]
B. Experimental Data from Batch Experiments

B.1. Effect of Initial Sulphide Concentration

Complete data sets for initial sulphide concentrations of 5.5 mM and 15.8 mM are shown in Figure B.1.1 and B.1.2, respectively.

![Graph showing concentration changes over time for different ions](image)

**Figure B.1.1.** Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide concentration of 5.5 mM. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.1.2. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide at an initial sulphide concentration of 15.8 mM. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

B.2. Effect of Initial Sulphide to Nitrate Ratio

Experimental data for the first 52 hours of the experiments at initial sulphide to nitrate ratios of 4.08, 2.30, 2.17, 1.14, 1.12, 0.83, 0.77, 0.59, 0.56, 0.46, and 0.42 are shown in figures B.2.1, B.2.3, B.2.5, B.2.7, B.2.9, B.2.11, B.2.13, B.2.15, B.2.17, B.2.19, and B.2.21 respectively. Complete data for the duration of the experiments (290 hours) are shown for all ratios. Initial sulphide to nitrate ratios of 4.08, 2.30, 2.17, 1.14, and 1.12 are shown in Figures B.2.2, B.2.4, B.2.6, B.2.8, and B.2.10, respectively. Complete data for initial sulphide to nitrate ratios of 0.83, 0.77, 0.59, 0.56, 0.46, and 0.42 are shown in Figures B.2.12, B.2.14, B.2.16, B.2.18, B.2.20, and B.2.22, respectively.
Figure B.2.1. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 4.08. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.2.2. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 4.08. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.2.3. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 2.30. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.2.4. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 2.30. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
**Figure B.2.5.** Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 2.17. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

**Figure B.2.6.** Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 2.17. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.2.7. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 1.14. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.2.8. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 1.14. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.2.9. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 1.12. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.2.10. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 1.12. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.2.11. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 0.83. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.2.12. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.83. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.2.13. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 0.77. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.2.14. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.77. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.2.15. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 0.59. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.2.16. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.59. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.2.17. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 0.56. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.2.18. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.56. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.2.19. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 0.46. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.2.20. Complete set (290 h) of data for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.46. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.2.21. Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile during the oxidation of sulphide (first 52 h) at an initial sulphide to nitrate ratio of 0.42. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.2.22. Complete set of data (290 h) for sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile at an initial sulphide to nitrate ratio of 0.42. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
B.3. Control Experiments

A control experiment was completed for an initial sulphide concentration of 6.2 mM and a nitrate concentration of 10.54 mM. This functioned as a control experiment for the initial sulphide concentration near 5 mM, as well as the initial sulphide to nitrate ratio of 0.59. The data set for this experiment is shown in figure B.3.1. The remaining control experiments were conducted for initial sulphide to nitrate ratios of 4.16, 2.20, 0.77, and 0.39. The complete data sets for these experiments are shown in figures B.3.2, B.3.3, B.3.4, and B.3.5, respectively.

Figure B.3.1 Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at an initial sulphide concentration of 6.2 mM, and an initial sulphide to nitrate ratio of 0.59. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.3.2 Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at an initial sulphide to nitrate ratio of 4.16. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.3.3 Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at an initial sulphide to nitrate ratio of 2.20. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
Figure B.3.4 Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at an initial sulphide to nitrate ratio of 0.77. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.

Figure B.3.5 Sulphide, sulphate, nitrate, nitrite, and thiosulphate concentrations profile for control experiment at an initial sulphide to nitrate ratio of 0.39. Error bars indicate one standard deviation; some error bars are not visible as the associated error is small.
C. Calibration Curves for Analytical Methods

C.1. Calibration Curve for Sulphide Measurement

The calibration curve for the measurement of sulphide is shown in Figure C.1.1. The equation of the best fit line ($R^2=0.9936$) was the following:

$$SulphideConcentration = 9.88 \times A_{480}$$

![Figure C.1.1 Calibration curve for sulphide measurement. Error bars indicate one standard deviation. Some error bars are not visible as the associated error is small.](image)

C.2. Calibration Curve for Total Protein Measurement

The calibration curve for the measurement of protein concentration is shown in figure C.2.1. The equation of the best fit line ($R^2=0.9755$) was the following:

$$ProteinConcentration = 44.2 \times A_{595}$$
Figure C.2.1. Calibration curve for protein measurement. Error bars indicate one standard deviation. Some error bars are not visible as the associated error is small.