Microbially-Enhanced Redox solution Reoxidation for Sour Natural Gas Sweetening

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ABSTRACT

The specific objective of this project are to advance the technology and improve the economics of the commercial iron-based chelate processes such as LO-CAT II and SulFerox process utilizing biologically enhanced reoxidation of the redox solutions used in these processes. The project is based on the use of chelated ferric iron as the catalyst for the production of elemental sulfur, and then oxidizing bacteria, such as Thiobacillus Ferrooxidans (ATCC# 23270) as an oxidizer. The regeneration of Fe^{3+} - chelate is accomplished by the use of these same microbes under mild conditions at 25° - 30°C and at atmospheric pressure to minimize the chelate degradation process. The pH of the redox solution was observed to be a key process parameter. Other parameters such as temperature, total iron concentration, gas to liquid ratio and bacterial cell densities also influence the overall process. The second part of this project includes experimental data and a kinetic model of microbial H_2S removal from sour natural gas using thiobacillus species. In the experimental part, a series of experiments were conducted with a commercial chelated iron catalyst at pH ranges from 8.7 to 9.2 using a total iron concentration range from 925 ppm to 1050 ppm in the solution. Regeneration of the solution was carried out by passing air through the solution. Iron oxidizing bacteria were used at cell densities of 2.3 x 10^7cells/ml for optimum effective performance.

In the modeling part, oxidation of Fe2+ ions by the iron oxidizing bacteria - Thiobacillus Ferrooxidans was studied for application to a continuous stirred tank reactor (CSTR). The factors that can directly affect the oxidation rate such as dilution rate, temperature, and pH were analyzed. The growth of the microorganism was assumed to follow Monod type of growth kinetics. Dilution rate had influence on the rate of oxidation of ferrous iron. Higher dilution rates caused washout of the biomass. The oxidation rate was highly pH sensitive. Specific growth is a function of pH of the both. The specific growth rate had a maximum value at around 2.2. A dynamic model for a packed bed fluid reactor is also developed and simulated. A feedback control loop is proposed to control the production under feed disturbances.

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1.0 EXECUTIVE SUMMARY

About twenty-five percent of natural gas produced in the United States is sour containing significant volumes of hydrogen sulfide. Hydrogen sulfide is a highly undesirable component of natural gas, biogas and various gas streams. Removal of H_2S from sour gas is required for reasons of health, safety and corrosion during transmission and distribution and to prevent sulfur dioxide pollution upon combustion of the gases. At present, well established physicochemical techniques for removal of H_2S dominate the market. Liquid redox processes remove hydrogen sulfide from natural gas. Aqueous solution of chelated ferric ions oxidizes the hydrogen sulfide to elemental sulfur. The reduced iron chelate is then oxidized by contact with air and recycled. This requires expensive equipment for regeneration and the process is usually energy intensive.

The continuing search for more economical processes however, has led to investigation into microbiological solutions for purifying H_2S and SO, containing gases as well as coal and petroleum. The relatively high chemical, catalyst and disposal costs of conventional processes are important drawbacks, which may be overcome partly in a biological process. Microbiological processes operate around ambient temperature and at atmospheric pressure, eliminating the need for high heat and pressurization requirements and thus cutting the energy costs to a minimum.

A series of experiments were conducted with a commercial chelated iron catalyst at pH ranges from 8.7 to 9.2 using a total iron concentration range from 925 ppm to 1050 ppm in the solution. Regeneration of the solution was carried out by passing air through the solution. A 3 to 40% enhancement was observed in the oxidation of H_2S and 10 to 112% enhancement was observed in the regeneration rates of theoretical in the presence of bacteria. Iron oxidizing bacteria were used at cell densities of 2.3×10^7cells/ml for optimum effective performance. The pH of the redox solution was observed to be a key process parameter. Other parameters such as temperature, total iron concentration, gas to liquid ratio and bacterial cell densities also influence the overall process.

In the modeling part, oxidation of Fe2+ ions by the iron oxidizing bacteria- Thiobacillus Ferrooxidans was studied for application to a continuous stirred tank reactor (CSTR). The factors that can directly affect the oxidation rate such as dilution rate, temperature, and pH were analyzed. The growth of the microorganism was assumed to follow Monod type of growth kinetics. Dilution rate had influence on the rate of oxidation of ferrous iron. Higher dilution rates caused washout of the biomass. The oxidation rate was highly pH sensitive. Specific growth is a function of pH of the both. The specific growth rate had a maximum value at around 2.2. A dynamic model for a packed bed fluid reactor is also developed and simulated. A feedback control loop is proposed to control the production under feed disturbances.

2.0 INTRODUCTION

Hydrogen sulfide is a highly undesirable component of natural gas, biogas and various gas streams. Removal of H_2S from sour gas is required for reasons of health, safety and corrosion during transmission and distribution and to prevent sulfur dioxide pollution upon combustion of the gases. At present, well established physicochemical techniques for removal of H_2S dominate the market. The traditional technique for treating this sour gas involves a two step approach of first separating the acidic gases from the natural gas with an amine plant and then either flaring the hydrogen sulfide or recovering the sulfur in a Claus plant. The liquid redox processes are preferred over traditional amine-Claus systems because of their simplicity, higher sulfur recovery and good turn down ratio.

The Liquid Redox Sulfur Recovery Processes absorb hydrogen sulfide from the sour gas stream and produce elemental sulfur. The liquid redox processes may use vanadium, iron or a mixture of iron and quinone as the primary catalysts interacting with hydrogen sulfide. The iron-based processes have been most successful because of their superior performance, simple operation, greater reliability and environmental acceptability [1]. However, the process conditions promote the oxidation reactions that accelerate the decomposition of metal-chelate catalysts resulting in high processing costs, and recirculation power requirements. Moreover, in all the commercial liquid redox processes, expensive redox solution is lost via salt formation and inadequate washing of the sulfur cake produced [2]. The liquid redox process reactions are given below;

Absorption and oxidation:

$$H_2S_{(g)} + H_2O \rightarrow H_2S_{(aq)} + H_2O$$

$$H_2S_{(aq)} + 2OH^- \rightarrow S^{2-} + 2H_2O$$

$$2Fe^{3+} + S^{2-} \rightarrow 2Fe^{2+} + S^0$$

Regeneration:

$$2Fe^{2+} + {}^1/_2\,O_2 + H_2O \rightarrow 2Fe^{3+} + 2OH^-$$

Overall:

$$H_2S + {}^1/_2\,O_2 \rightarrow H_2O + S^0$$

The liquid redox solution contains chelated iron catalyst for the oxidation of hydrogen sulfide. The precipitation of iron in the redox solution is prevented by complexion it with organic chelates which are capable of holding both forms of iron in solution. These organic chelates are classified into two groups: Type A chelates such as ethylenediamine tetra acetic acid (EDTA), N-hydroxyethyl ethylenediamine tri acetic acid (HEDTA) or nitrilotriacetic acid (NTA) which are powerful chelating agents at low pHs; and the type B chelates, consisting of polyhydroxylated

sugars such as sorbitol that are effective at pH above 8. Combination of both types of chelates makes the catalyst stable at any pH from 5 to 9. The selection of a chelant is dependent on the reaction rate of Fe^{3+} chelate with hydrogen sulfide (H_2S), Fe $^{2+}$/Fe^{3+} chelate with oxygen and the rate of degradation of the chelate. Maintaining a reaction temperature below 45 ^{0}C can control ferric ion oxidation of chelate. Other variables that control the oxidative degradation of ferric/ferrous chelates are: pH, chelant concentration, chelant to iron ratio, and the type of degradation products formed.

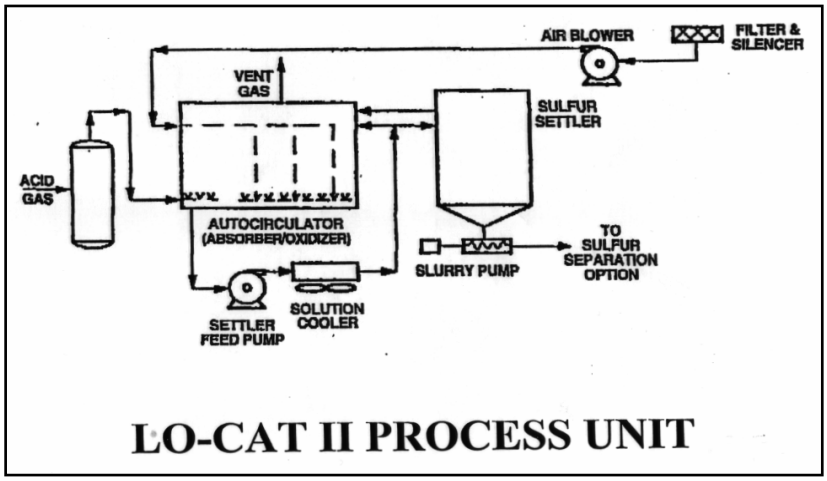

Figure 1 LO-CAT Process

The LO-CAT^{TM} process was originally developed by ARI Technologies, now U.S Filters., to treat sour gas in the absorber at feed gas pressure and relatively low iron concentrations (1000 to 1500 ppm) and high circulation rates. This system referred to as conventional LO-CAT works well for many low-pressure plants; however it results in excessive equipment and pumping costs for high-pressure applications. The continuing search for more economical processes however, has led to investigation into microbiological solutions for purifying H_2S and SO, containing gases as well as coal and petroleum. The ARI-LO-CAT II process as shown in Figure 1 was developed for the high-pressure direct treat applications [3]. The process uses sub-stoichiometric iron chelated catalyst in the absorber and an oxidizer unit that circulates liquid through density differences. The process is described in greater detail in the literature [3, 4]. The process also uses a separate sulfur settler vessel. These features reduce the chemical and operating costs.

The iron-oxidizing bacteria are capable of oxidizing ferrous ions to the ferric state at low pH. According to literature references, these microbes are capable of oxidizing Fe^{2+} to Fe^{3+} state at a 500,000 times faster rate than the purely chemical oxidation process in the absence of bacteria [5]. Microbiological processes operate under ambient temperature at 25-45°C and at atmospheric pressure, eliminating the need for high heat and pressurization requirements and thus cutting the energy costs to a minimum. The main aim of this project are to advance the technology and improve the economics of the commercial iron-based chelate processes utilizing biologically enhanced reoxidation of the redox solutions used in these processes.

3.0 PART I – EXPERIMENTAL INVESTIGATION

The following are the stated deliverables, which includes the tasks performed at UIC.

Task 1: Setup a new laboratory at UIC with a new process Bioreactor along with other laboratory equipment and chemicals. Conduct trail runs with the new setup.

Task 2: Bench scale testing of the new bioreactor with experiments conducted using ARI-310C in mature redox solution (absence of bacteria)

Task 3: Obtaining fresh bacterial cultures and cultivating Thiobacillus ferrooxidans and Thiobacillus species bacteria.

Task 4: Bench Scale Testing of Biological Enhancement of Lo-Cat II Process using Thiobacillus ferrooxidans in mature redox solution system.

Task 5: Bench Scale Testing of Biological Enhancement of Lo-Cat II Process using Thiobacillus species in mature redox solution system.

TASK 1: Setup a new laboratory at UIC with a new process Bioreactor along with other laboratory equipment and chemicals. Conduct trail runs with the new setup.

The laboratory setup was finished in stages with a new bioreactor installed during late October 2002 and with chemicals and other supplementary equipment arriving in November 2002. Some trail runs were performed to operate the new bioreactor using 0.5% H_2S gas in Fe-EDTA solution and in the absence of bacteria. A new microbiology lab was setup within the Chemical Engineering Department at UIC, with partial technical support from the Bioengineering Department at UIC. Fresh cultures of 'Thiobacillus ferrooxidans' (Dr. Blake) and 'Thiobacillus denitrificans' (Dr. Sublette) along with a new 'Mesophilic acidophiles' bacteria (Little Bear Labs Colorado) were obtained for growth in fresh medium and were grown. A new Microbiology lab was set up during April 2003, in the Microbiology & Immunology department at UIC and all the culture preparations were henceforth transferred from the Chemical Engineering lab and were conducted in the new lab. The bioreactor setup is shown in the Figure 3.

Figure 3 Omni culture Bioreactor

TASK 2: Bench scale testing of the new bioreactor with experiments conducted using ARI 310C (absence of bacteria)

Initial experiments were conducted in the new bioreactor using 0.5% H_2S in nitrogen and with no bacteria (microbes) present in the system. A set of one cycle and five cycle experiments were conducted using ARI-310C solution to simulate the experiments conducted at Texas A&M – Kingsville (TAMUK). The total iron in the redox iron system was determined using the atomic absorption method using a known quantity of ARI-310C Sample.

Reagents and analysis: Analytical-grade reagents were used for all experiments. The concentration of Fe^{2+}, total iron, was determined using HACH Pocket Colorimeter™ Analysis System (HACH Co., Loveland, CO.). The pH and ORP were determined using a HACH sensION2 Portable pH/ISE Meter (HACH Co., Loveland, CO).

TASK 3: Obtaining fresh bacterial cultures and cultivating Thiobacillus ferrooxidans and Thiobacillus species bacteria.

Fresh samples of Thiobacillus ferrooxidans were obtained from Dr. Robert Blake at Xavier University and were cultivated as follows:

Microorganism, maintenance, and sub culturing: The bacterial culture used in this study was a strain of *Thiobacillus ferrooxidans* (ATCC# 23270), which was maintained in ferrous sulfate solution along with other basal salts. The organism was maintained in ferrous sulfate solution grown shake flask cultures. The bacteria were maintained in the exponential growth phase by sub culturing

on a daily basis. The inoculums were replaced at regular intervals by culture from the original sample to reduce the possibility of contamination.

Growth media and experimental procedure: In order to maintain supply of iron in its reduced state for growth and respiration of the bacteria, an orbital shaker bath was used in the experiments. The growth medium was prepared by combining four parts of basal salts solution with one part of ferrous sulfate solution along with the inoculums. Given below is the chart used in making the medium.

Basal Salts

Reagents	100 ml	400 ml	500 ml	1000 ml
$K_2HPO_4.3H_2O$	0.067g	0.263 g	0.329 g	0.658 g
$(NH_4)_2SO_4$	0.050 g	0.200 g	0.250 g	0.500 g
$MgSO_4.7H_2O$	0.050 g	0.200 g	0.250 g	0.500 g
Conc. H_2SO_4	0.314 ml	1.300 ml	1.570 ml	3.140 ml

Ferrous Sulfate Solution

	100 ml	400 ml	500 ml	1000 ml
$FeSO_4.7H_2O$	16.7g	42.0 g	84.0 g	167.0 g
1 N H_2SO_4	5.0 ml	12.5 ml	25.0 ml	50.0 ml

The Basal salt solution is prepared by adding all the reagents in part I to deionized water, by adjusting the pH to 1.8-2.0 and then autoclaved to sterilize. The ferrous sulfate solution is prepared by adding ferrous sulfate and 1 N H_2SO_4 to deionized water and then filter sterilized. The final medium obtained from parts II and I are mixed with the inoculums using the ratio below to give the final '*Thiobacillus*' medium

Culture ml)	Part I (ml)	Part II (ml)	Inoculums (ml)
100	72	18	10
500	360	90	50
1000	800	200	100
18 L (carboy)	13.5 L	4.5 L	2 L

Reagents and analysis: Analytical-grade reagents were used for all experiments. Total soluble iron was determined using an atomic absorption spectrometer operated in the flame mode. The concentration of Fe^{2+}, total iron, was determined using HACH Pocket Colorimeter Analysis System (HACH Co., Loveland, CO.). The pH and ORP were determined using a HACH sensION2 Portable pH/ISE Meter (HACH Co., Loveland, CO). The total number of cells in the growth medium was determined using a Petroff-Hausser counting chamber and a phase contrast microscope with a magnification of 400x.

TASK 4: Bench Scale Testing of Biological Enhancement of Lo-Cat II Process using Thiobacillus ferrooxidans (Data presented in Tables 1 - 4)

Five Cycle Experiments with Mature Solution using 0.5% H_2S Gas and Nitrogen blend in presence of *Thiobacillus Ferrooxidans* Bacteria

A series of five cycle experiments were conducted using one-liter solution of the Mature Solution catalyst in a two-liter bioreactor in the presence of *Thiobacillus Ferrooxidans* cultures. A synthetic mature solution was prepared consisting of the following chemicals; dissolved in 100 ml of deionized water:

Potassium bicarbonate	50.60 g
Potassium thiosulfate	42.17 g
Sodium oxalate, anhydrous	10.17 g
Disodium iminodiacetate	7.66 g
Sodium sulfate	42.30 g

The salts solution was added to ARI 310C and its pH were adjusted to 8.0, and the solution volume increased to one liter by addition of deionized water. In a typical run, 0.5% concentration of Hydrogen Sulfide at a rate of 0.26 L/m was passed into the system during the oxidation cycle to oxidize the H_2S to elemental sulfur. The rate of H_2S oxidation is a function of pH, temperature, concentration of Fe^{3+} chelate, gas/redox solution ratio and the degree of agitation.

The progress of the reaction was monitored by measuring the concentration of Fe^{2+}, Fe^{3+} ions, pH, temperature and the redox potential of the reaction. Total iron in the system was determined by using an atomic absorption spectrophotometer. The Ferrous iron was determined in the samples using the HACH procedure. Using the HACH apparatus (cat. no. 46700-22) the sample was diluted to a 0-5 ppm (HI range) range before taking a direct reading in Mg/l or ppm of iron. Similarly, in the determination of the Ferrous iron the sample was diluted to a 0-3 ppm (LO range) range. The HACH calorimeter utilizes a powdered iron pillow (Iron Phenonthroline for Fe^{+2}) as an indicator. Each oxidation cycle was carried out for about 25 - 30 minutes followed by regeneration for about 30 minutes duration, during which air at a predetermined rate of 2.0 – 2.4 L/m was bubbled through the reactor. All the experimental variables: pH, temperature and redox potential were monitored and recorded. The data for these five cycle experiments are presented in tables 1 through 4.

TASK 5: Bench Scale Testing of Biological Enhancement of Lo-Cat II Process using Thiobacillus species (Data presented in Table 5)

Five Cycle Experiments with Mature Solution using 0.5% H_2S Gas and Nitrogen blend in presence of *Thiobacillus Species* Bacteria

Another series of five cycle experiments were conducted in the two-liter bioreactor in the presence of *Thiobacillus Species* bacteria at 30 ^{0}C temperatures in presence of 1000 ppm ARI-310C mature redox solution. In a typical run, 0.5% concentration of Hydrogen Sulfide at a rate of 0.26 L/m was passed into the system during the oxidation cycle to oxidize the H_2S to elemental sulfur. The reduced catalyst was then regenerated by passing air at 2.0-2.4 L/m for 20-

30 minutes. Five cycles consisting of hydrogen sulfide oxidation and ferric ion regeneration were conducted. The hydrogen sulfide oxidation rate data and the ferric ion regeneration rate data are presented in the second set of table 5.

3.1 RESULTS AND DISCUSSION (based on the Thesis of A.H. Katkar)

Recycle experiments were conducted in a two-liter vitris omni-culture bioreactor using mature solution. The oxidation rates of hydrogen sulfide present in the synthetic sour gas having 0.5% H_2S concentration was studied by bubbling the gas through the aqueous solution containing the fresh chelated redox liquid catalyst solution as well as mature redox solution. A full description of the procedures used in these experiments is presented in experimental investigation. The rate of hydrogen sulfide oxidation was found to be primarily influenced by pH, temperature, gas/liquid ratio and the concentration of iron chelate in the redox solution. The ferric ion re-oxidation rates were achieved by bubbling air through the reduced catalyst in the bioreactor both in the absence of cultures (blank) and in the presence of acidophilic cultures. It was observed that the air regeneration of catalysts was dependent on the pH, temperature, air/redox solution ratio and bacterial cell concentration in the system.

The experimental runs were conducted at temperatures of 30 ^{0}C and pH in the range of 8.7 to 9.2. The total iron concentration in the redox solution system was maintained between 925ppm to 1050ppm. A cycle consists of passing sour gas through the redox solution followed by regeneration of the ferric ions and separation of elemental sulfur from the redox solution. A cell density of 4.5 x 10^5 to 2.3 x 10^7 cells/ml was typically used in these experiments.

Results of series of five cycle experiments are given in Tables 1 through 5. Table 1 to 5 present the data obtained during a typical run, conducted both in the absence and presence of bacteria using 0.5% H_2S gas and nitrogen blend. The regeneration rates of ferric ion in the presence and absence of bacteria were compared. The redox solution in the presence of Thiobacillus ferrooxidans gave an enhancement of 39% against blank experiments and 112% enhancement with Thiobacillus species. When compared with blank experiments the mature redox solution in the presence of Thiobacillus species bacteria showed on an average of 31.5% enhancement in H_2S oxidation rates. Figure 4.1 to 8.2 present the data obtained during a typical five-cycle experiment conducted in the absence and presence of bacteria. This studies shows that these bacteria, especially Thiobacillus species were capable of producing enhanced re-oxidation rates at high pH conditions.

Table no. 1

Five cycle experiments with and without Thiobacillus ferrooxidans bacteria in mature solution using 0.5% H_2S Gas and Nitrogen blend at 30 ^{0}C

Five-cycle redox system using mature solution
Cycle I - (Absence of Bacteria)

The following conditions were maintained during the experiment.
Total iron content = 900 ppm
Temperature = 30 ^{0}C
H_2S flow rate = 0.26 L/m
Air flow rate = 2.1 L/m
Dil.Fact. = (ml H_2O + µL sample)/ (µL sample)
Fe^{+2} = Dil.Fact. * C.R
Gas Composition: H_2S - 0.5 % in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-51.4	8.60	0					
5	-191.8		0					
10	-217.0		0	70	0.26	158.14	41.11	4.11
15	-231.0		0					
20	-235.8		0	70	0.56	158.14	88.55	4.42
25	-251.2		0	70	0.72	158.14	113.86	4.55

Avg.: 4.36

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-251.0	8.42	70	0.72	158.14	113.86	
5	-246.2						
10	-242.4		70	0.58	158.14	91.72	2.21
15	-238.5						
20	-234.5		70	0.41	158.14	64.83	2.45
25	-229.9						
30	-224.3		70	0.30	158.14	47.44	2.21
35	-217.6						
40	-209.2		70	0.15	158.14	23.72	2.25

Avg: 2.28

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle - II

Bacteria cell count = 0.3 X 10^7 cells/ml
Total iron content = 925 ppm
Temperature = 30 ^{0}C
pH = 8.90
H_2S flow rate = 0.26 L/m
Air flow rate = 2.1 L/m
Gas Composition: H_2S - 0.5 % in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-179.6	8.90	0					
5	-217.6		0					
10	-235.4		0	70	0.35	158.14	55.34	5.53
15	-247.2		0					
20	-256.8		0	70	0.60	158.14	94.88	4.74
25	-265.3		0	70	0.81	158.14	128.09	5.12

Avg.: 5.13

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-264.8	8.75	70	0.81	158.14	128.09	
5	-258.0						
10	-251.3		70	0.61	158.14	96.46	3.16
15	-247.6						
20	-240.9		70	0.39	158.14	61.67	3.32
25	-232.1						
30	-220.5		70	0.22	158.14	34.79	3.11
35	-202.7						
38.33	-178.6		70	0.03	158.14	4.74	3.21

Avg.: 3.20

Percentage increase in oxidation compared to the Blank = 18 %
Percentage increase in Regeneration compared to the Blank = 41 %

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle – III

Bacteria cell count = 0.3 X 10^7 cells/ml
Total iron content = 925 ppm
Temperature = 30 ^{0}C
pH = 9.07
H_2S flow rate = 0.26 L/m
Air flow rate = 2.1 L/m
Gas Composition: H_2S - 0.5 % in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-60.8	9.07	0					
5	-212.7		0					
10	-237.5		0	70	0.28	158.14	44.27	4.42
15	-252.0		0					
20	-262.7		0	70	0.61	158.14	96.46	4.82
25	-271.5		0	70	0.78	158.14	123.34	4.93

Avg.: 4.72

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-270.8	8.77	70	0.78	158.14	123.34	
5	-263.7						
10	-258.2		70	0.55	158.14	86.97	3.63
15	-251.5						
20	-243.4		70	0.31	158.14	49.02	3.71
25	-233.1						
30	-218.4		70	0.10	158.14	15.81	3.58

Avg: 3.64

Percentage increase in oxidation compared to the Blank = 9 %
Percentage increase in Regeneration compared to the Blank = 60 %

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle – IV

Bacteria cell count = 0.3 X 10^7 cells/ml
Total iron content = 950 ppm
Temperature = 30 ^{0}C
pH = 9.14
H_2S flow rate = 0.26 L/m
Air flow rate = 2.1 L/m
Gas Composition: H_2S - 0.5 % in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-69.0	9.14	0					
5	-216.1		0					
10	-242.3		0	70	0.29	158.14	45.86	4.58
15	-258.1		0					
20	-267.8		0	70	0.60	158.14	94.88	4.74
25	-276.8		0	70	0.70	158.14	110.69	4.42

Avg.: 4.58

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-275.9	8.83	70	0.70	158.14	110.69	
5	-267.3						
10	-259.8		70	0.41	158.14	64.83	4.58
15	-250.2						
20	-238.1		70	0.24	158.14	37.95	3.63
25	-219.7						
30	-179.4		70	0.03	158.14	4.74	3.53

Avg: 3.91

Percentage increase in oxidation compared to the Blank = 6 %
Percentage increase in Regeneration compared to the Blank = 71 %

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle – V

Bacteria cell count = 0.3 X 10^7 cells/ml
Total iron content = 925 ppm
Temperature = 30 ^{0}C
pH = 9.19
H_2S flow rate = 0.26 L/m
Air flow rate = 2.1 L/m
Gas Composition: H_2S - 0.5 % in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-66.3	9.19	0					
5	-226.9		0					
10	-250.8		0	70	0.28	158.14	44.27	4.42
15	-264.7		0					
20	-274.8		0	70	0.60	158.14	94.88	4.74

Avg.: 4.58

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-273.7	8.81	70	0.60	158.14	94.88	
5	-264.6						
10	-256.7		70	0.36	158.14	56.93	3.79
15	-247.1						
20	-234.6		70	0.21	158.14	33.20	3.08
25	-215.2						
28.33	-161.0		70	0.02	158.14	4.74	3.18

Avg: 3.35

Percentage increase in oxidation compared to the Blank = 6 %
Percentage increase in Regeneration compared to the Blank = 47 %

Table no. 2

Five cycle experiments with and without Thiobacillus ferrooxidans bacteria in mature solution using 0.5% H_2S Gas and Nitrogen blend at 30 ^{0}C

Five-cycle redox system using mature solution
Cycle I - (Absence of Bacteria)

The following conditions were maintained during the experiment.

Total iron content = 975 ppm
Temperature = 30 ^{0}C
H_2S flow rate = 0.26 L/m
Air flow rate = 2.2 L/m
Dil.Fact. = (ml H_2O + µL sample)/ (µL sample)
Fe^{+2} = Dil.fact. * C.R
Gas Composition: H_2S – 0.5 % in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-72.8	8.60	0					
5	-200.3		0					
10	-224.0		0	70	0.72	158.14	113.86	11.39
15	-238.9		0					
20	-252.0		0	70	1.39	158.14	219.81	10.99

Avg.: 11.19

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-250.2	8.42	70	1.39	158.14	219.81	
5	-245.3						
10	-241.2		70	1.14	158.14	180.28	3.95
15	-236.6						
20	-231.3		70	0.80	158.14	126.51	4.66
25	-225.5						
30	-218.1		70	0.52	158.14	82.23	4.59
35	-207.9						
40	-194.2		70	0.23	158.14	36.37	4.59

Avg.: 4.45

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle - II

Bacteria cell count = 0.45 X 10^6 cells/ml
Total iron content = 1000 ppm
Temperature = 30 ^{0}C
pH = 8.94
H_2S flow rate = 0.26 L/m
Air flow rate = 2.2 L/m
Gas Composition: H_2S – 0.5 % in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. Factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-181.0	8.94	0					
5	-221.2		0					
10	-239.4		0	70	0.78	158.14	123.35	12.35
15	-251.7		0					
20	-261.9		0	70	1.50	158.14	237.21	11.86

Avg.: 12.10

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-261.4	8.72					
5	-255.2		70	1.50	158.14	237.21	
10	-250.6						
15	-245.0		70	1.15	158.14	181.86	5.53
20	-238.9						
25	-230.5		70	0.75	158.14	118.60	5.93
30	-217.5						
35	-146.4		70	0.38	158.14	60.10	5.90

Avg.: 5.79

Percentage increase in oxidation compared to the Blank = 8 %
Percentage increase in Regeneration compared to the Blank = 31 %

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle - III

Bacteria cell count = 0.45 X 10^6 cells/ml
Total iron content = 1000 ppm
Temperature = 35 ^{0}C
pH = 9.09
H_2S flow rate = 0.26 L/m
Air flow rate = 2.2 L/m
Gas Composition: H_2S – 0.5 % in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. Factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-82.1	9.09	0					
5	-212.8		0					
10	-242.0		0	70	0.56	158.14	88.56	8.86
15	-256.7		0					
20	-267.8		0	70	1.13	158.14	178.7	8.94

Avg.: 8.90

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-266.2	8.83	70	1.13	158.14	178.7	
5	-256.6						
10	-246.8		70	0.69	158.14	109.11	6.95
15	-232.2						
20	-206.7		70	0.20	158.14	31.62	7.35
25	-158.6		70	0.02	158.14	3.16	7.02

Avg.: 7.10

Percentage increase in oxidation compared to the Blank = -
Percentage increase in Regeneration compared to the Blank = 60

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle – IV

Bacteria cell count = 0.45 X 10^6 cells/ml
Total iron content = 1025 ppm
Temperature = 30 ^{0}C
pH = 9.16
H_2S flow rate = 0.26 l/m
Air flow rate = 2.0 l/m
Gas Composition: H_2S – 0.5 % in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. Factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-94.7	9.16	0					
5	-222.8		0					
10	-247.5		0	70	0.66	158.14	104.87	10.48
15	-261.8		0					
20	-272.5		0	70	1.40	158.14	221.40	11.07

Avg.: 10.75

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-271.9	8.86	70	1.40	158.14	221.4	
5	-263.1						
10	-255.0		70	0.87	158.14	137.58	8.38
15	-245.1						
20	-231.6		70	0.42	158.14	66.42	7.75
25	-208.8						
30	-149.7		70	0.02	158.14	3.16	7.52

Avg.: 7.80

Percentage increase in oxidation compared to the Blank = -
Percentage increase in Regeneration compared to the Blank = 76

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle – V

Bacteria cell count = 0.45 X 10^6 cells/ml
Total iron content = 1000 ppm
Temperature = 30 ^{0}C
pH = 9.18
H_2S flow rate = 0.26 L/m
Air flow rate = 2.1 L/m
Gas Composition: H_2S – 0.5 % in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. Factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-87.6	9.18	0					
5	-233.8		0					
10	-255.2		0	70	0.77	158.14	121.76	12.17
15	-268.7		0					
20	-278.2		0	70	1.30	158.14	205.58	10.27

Avg.: 11.22

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-277.9	8.87	70	1.30	158.14	205.58	
5	-268.7						
10	-262.1		70	0.92	158.14	145.48	6.01
15	-253.9						
20	-244.8		70	0.60	158.14	94.88	5.53
25	-231.8						
30	-211.6		70	0.23	158.14	36.37	5.64
35	-173.3		70	0.02	158.14	3.16	5.95

Avg.: 5.78

Percentage increase in oxidation compared to the Blank = -
Percentage increase in Regeneration compared to the Blank = 30

Table no. 3

Five cycle experiments with and without Thiobacillus ferrooxidans bacteria in mature solution using 0.5% H_2S Gas and Nitrogen blend at 30 ^{0}C

Five-cycle redox system using mature solution
Cycle I - (Absence of Bacteria)

The following conditions were maintained during the experiment.
Total iron content = 1000 ppm
Temperature = 30 ^{0}C
H_2S flow rate = 0.26 L/m
Air flow rate = 2.3 L/m
Dil.Fact. = (ml H_2O + µL sample)/ (µL sample)
Fe^{+2} = Dil.fact. * C.R
Gas Composition: H_2S – 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-109.0	8.68	0					
5	-230.7		0					
10	-247.8		0	70	1.13	158.14	178.69	17.87
15	-259.7		0					
20	-269.3		0	70	2.04	158.14	322.60	16.13

Avg.: 17.00

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-269.0	8.42	70	2.04	158.14	322.60	
5	-260.2						
10	-257.0		70	1.63	158.14	257.77	6.48
15	-252.6						
20	-247.0		70	1.19	158.14	188.16	6.72
25	-240.5						
30	-231.9		70	0.67	158.14	105.95	7.22
35	-220.5						
40	-201.6		70	0.22	158.14	34.79	7.19

Avg.: 6.90

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle - II

Bacteria cell count = 0.72 x 10^7 cells/ml
Total iron content = 1050 ppm
Temperature = 30 ^{0}C
pH = 8.78
H_2S flow rate = 0.26 L/m
Air flow rate = 2.2 L/m
Gas Composition: H_2S – 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-187.0	8.78	0					
5	-243.1		0					
10	-261.6		0	70	1.18	158.14	186.60	18.60
15	-279.5		0					
20	-291.5		0	70	2.07	158.14	327.34	16.36

Avg.: 17.51

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-290.0	8.51	70	2.07	158.14	327.34	
5	-281.9						
10	-275.2		70		158.14	249.86	7.74
15	-268.5						
20	-261.2		70		158.14	181.86	7.27
25	-253.2						
30	-243.1		70		158.14	96.40	7.69
35	-230.2						
40	-210.1		70		158.14	22.13	7.63

Avg.: 7.58

Percentage increase in oxidation compared to the Blank = 3 %
Percentage increase in Regeneration compared to the Blank = 10 %

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle – III

Bacteria cell count = 0.72 x 10^7 cells/ml
Total iron content = 1050 ppm
Temperature = 30 ^{0}C
pH = 9.01
H_2S flow rate = 0.26 L/m
Air flow rate = 2.2 L/m
Gas Composition: H_2S – 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-114.8	9.01	0					
5	-245.7		0					
10	-264.5		0	70	0.97	158.14	153.39	15.34
15	-274.5		0					
20	-284.0		0	70	1.61	158.14	254.60	12.73

Avg.: 14.03

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-283.7	8.74	70	1.61	158.14	254.60	
5	-272.9						
10	-266.5		70	1.01	158.14	143.90	9.48
15	-259.1						
20	-252.1		70	0.53	158.14	83.81	8.53
25	-241.9						
30	-229.9		70	0.26	158.14	41.11	7.11
35	-213.0						
40	-182.7		70	0.02	158.14	3.16	6.98

Avg.: 8.02

Percentage increase in oxidation compared to the Blank = --
Percentage increase in Regeneration compared to the Blank = 17

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle – IV

Bacteria cell count = 0.72 x 10^7 cells/ml
Total iron content = 1050 ppm
Temperature = 30 ^{0}C
pH = 9.06
H_2S flow rate = 0.26 L/m
Air flow rate = 2.3 L/m
Gas Composition: H_2S – 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-115.4	9.06	0					
5	-260.1		0					
10	-282.3		0	70	1.26	158.14	199.26	19.92
15	-294.2		0					
20	-305.6		0	70	2.12	158.14	335.26	16.76

Avg.: 18.34

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-305.0	8.81	70	2.12	158.14	335.26	
5	-295.1						
10	-288.3		70	1.42	158.14	224.55	11.07
15	-282.1						
20	-274.5		70	1.21	158.14	191.34	7.19
25	-266.1						
30	-256.2		70	0.57	158.14	90.13	8.17
35	-244.0						
40	-222.4		70	0.26	158.14	41.11	7.35
45	-187.5		70	0.03	158.14	4.74	7.34

Avg.: 8.22

Percentage increase in oxidation compared to the Blank = 7
Percentage increase in Regeneration compared to the Blank = 20

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle – V

Bacteria cell count = 0.72 x 10^7 cells/ml
Total iron content = 1050 ppm
Temperature = 30 ^{0}C
pH = 9.18
H_2S flow rate = 0.26 L/m
Air flow rate = 2.1 L/m
Gas Composition: H_2S – 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-113.8	9.18	0					
5	-267.3		0					
10	-285.1		0	70	1.07	158.14	169.21	16.92
15	-296.6		0					
20	-307.8		0	70	2.08	158.14	328.93	16.45

Avg.: 16.68

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-307.8	8.78	70	2.08	158.14	328.93	
5	-294.3						
10	-286.2		70	1.62	158.14	256.19	7.27
15	-280.1						
20	-272.5		70	1.20	158.14	189.77	6.96
25	-264.3						
30	-253.9		70	0.48	158.14	75.91	8.43
35	-240.6						
40	-220.3		70	0.22	158.14	34.79	7.35

Avg.: 7.50

Percentage increase in oxidation compared to the Blank = --
Percentage increase in Regeneration compared to the Blank = 10

Table no. 4

Five cycle experiments with and without Thiobacillus ferrooxidans bacteria in mature solution using 0.5% H_2S Gas and Nitrogen blend at 30 ^{0}C

Five-cycle redox system using mature solution
Cycle I - (Absence of Bacteria)

The following conditions were maintained during the experiment.
Total iron content = 1025 ppm
Temperature = 30 ^{0}C
H_2S flow rate = 0.26 L/m
Air flow rate = 2.3 L/m
Dil.Fact. = (ml H_2O + µL sample)/ (µL sample)
Fe^{+2} = Dil.fact. * C.R
Gas Composition: H_2S - 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-26.5	8.57	0					
5	-177.0		0					
10	-201.3		0	70	0.32	158.14	50.62	5.06
15	-216.4		0					
20	-228.3		0	70	0.60	158.14	94.88	4.74
25	-237.0		0	70	0.79	158.14	124.93	4.99

Avg.: 4.93

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-236.4	8.31	70	0.79	158.14	124.93	
5	-229.6						
10	-224.3		70	0.62	158.14	98.04	2.68
15	-216.8						
20	-209.1		70	0.49	158.14	77.48	2.62
25	-199.6						
30	-186.3		70	0.28	158.14	44.27	2.68

Avg.: 2.66

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle - II

Bacteria cell count = 0.39 X 10^7 cells/ml
Total iron content = 1025 ppm
Temperature = 30 ^{0}C
pH = 8.98
H_2S flow rate = 0.26 L/m
Air flow rate = 2.3 L/m
Gas Composition: H_2S - 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-92.6	8.98	0					
5	-146.6		0					
10	-171.3		0	70	0.38	158.14	60.09	6.00
15	-186.2		0					
20	-197.9		0	70	0.74	158.14	117.02	5.85
25	-208.5		0	70	0.95	158.14	150.23	6.00
							Avg.:	5.95

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-208.3	8.71	70	0.95	158.14	150.23	
5	-202.4						
10	-198.0		70	0.72	158.14	113.86	3.63
15	-193.4						
20	-188.9		70	0.54	158.14	85.39	3.24
25	-181.6						
30	-177.8		70	0.32	158.14	50.60	3.32
						Avg.:	3.39

Percentage increase in oxidation compared to the Blank = 21 %
Percentage increase in Regeneration compared to the Blank = 28 %

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle – III

Bacteria cell count = 0.39 X 10^7 cells/ml
Total iron content = 1025 ppm
Temperature = 30 ^{0}C
pH = 9.26
H_2S flow rate = 0.26 L/m
Air flow rate = 2.2 L/m
Gas Composition: H_2S - 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-138.1	9.26	0					
5	-200.6		0					
10	-224.3		0	70	0.37	158.14	58.51	5.85
15	-238.3		0					
20	-249.4		0	70	0.76	158.14	120.18	6.00
25	-258.5		0	70	0.96	158.14	151.81	6.07
								Avg.: 5.97

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-258.3	8.87	70	0.96	158.14	151.81	
5	-251.7						
10	-244.4		70	0.72	158.14	117.02	3.74
15	-237.3						
20	-229.5		70	0.52	158.14	82.23	3.47
25	-220.9						
30	-211.2		70	0.26	158.14	41.11	3.69
							Avg.: 3.64

Percentage increase in oxidation compared to the Blank = 21 %
Percentage increase in Regeneration compared to the Blank = 36 %

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle – IV

Bacteria cell count = 0.39 X 10^7 cells/ml
Total iron content = 1025 ppm
Temperature = 30 ^{0}C
pH = 9.12
H_2S flow rate = 0.26 L/m
Air flow rate = 2.2 L/m
Gas Composition: H_2S - 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	+6.8	9.12	0					
5	-145.4		0					
10	-173.5		0	70	0.30	158.14	47.44	4.74
15	-189.4		0					
20	-202.4		0	70	0.62	158.14	98.04	4.90
25	-213.7		0	70	0.80	158.14	126.51	5.06

Avg.: 4.90

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-213.5	8.83	70	0.80	158.14	126.51	
5	-208.2						
10	-201.3		70	0.58	158.14	91.72	3.47
15	-194.2						
20	-185.3		70	0.28	158.14	44.27	4.11
25	-174.3						
30	-158.4		70	0.12	158.14	18.97	3.58
35	-126.6						

Avg.: 3.72

Percentage increase in oxidation compared to the Blank = --
Percentage increase in Regeneration compared to the Blank = 40 %

Five-cycle redox system using mature solution with Thiobacillus ferrooxidans (T. f)

Cycle – V

Bacteria cell count = 0.39 X 10^7 cells/ml
Total iron content = 1025 ppm
Temperature = 30 ^{0}C
pH = 9.21
H_2S flow rate = 0.26 L/m
Air flow rate = 2.2 L/m
Gas Composition: H_2S - 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	+2.3	9.21	0					
5	-155.4		0					
10	-186.3		0	70	0.30	158.14	47.44	4.74
15	-199.7		0					
20	-216.9		0	70	0.66	158.14	104.37	5.21
25	-228.5		0	70	0.85	158.14	134.42	5.37

Avg.: 5.10

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-227.8	8.78	70	0.85	158.14	134.42	
5	-219.2						
10	-211.4		70	0.65	158.14	102.79	3.16
15	-201.3						
20	-194.2		70	0.31	158.14	49.02	4.26
25	-183.6						
30	-173.5		70	0.13	158.14	20.55	3.79

Avg.: 3.74

Percentage increase in oxidation compared to the Blank = 3 %
Percentage increase in Regeneration compared to the Blank = 41 %

Table no. 5

Five cycle experiments with and without Thiobacillus sp bacteria in mature solution using 0.5% H_2S Gas and Nitrogen blend at 30 ^{0}C

Five-cycle redox system using mature solution
Cycle I - (Absence of Bacteria)

The following conditions were maintained during the experiment.
Total iron content = 975 ppm
Temperature = 30 ^{0}C
H_2S flow rate = 0.26 L/m
Air flow rate = 2.4 L/m
Dil.Fact. = (ml H_2O + µL sample)/ (µL sample)
Fe^{+2} = Dil.fact. * C.R
Gas Composition: H_2S – 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	14.3	8.68	0					
5	-174.4		0					
10	-200.8		0	70	0.83	158.14	131.26	13.13
15	-219.0		0					
20	-231.2		0	70	1.65	158.14	260.93	13.05

Avg.: 13.09

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-229.8	8.51	70	1.65	158.14	260.93	
5	-217.5						
10	-214.0		70	1.46	158.14	230.88	3.00
15	-206.4						
20	-200.3		70	1.01	158.14	159.72	5.06
25	-193.9						
30	-187.4		70	0.59	158.14	93.30	5.59
35	-173.4						
40	-146.6		70	0.08	158.14	12.65	6.21

Avg: 4.96

Five-cycle redox system using mature solution with Thiobacillus species bacteria

Cycle - II

Bacteria cell count = 2.3 x 10^7 cells/ml
Total iron content = 1000 ppm
Temperature = 30 ^{0}C
pH = 8.93
H_2S flow rate = 0.26 L/m
Air flow rate = 2.4 L/m
Gas Composition: H_2S – 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-118.4	8.93	0					
5	-195.5		0					
10	-219.4		0	70	1.19	158.14	188.18	18.81
15	-236.4		0					
20	-249.2		0	70	2.24	158.14	354.23	17.71

Avg.: 18.26

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-248.4	8.74	70	2.24	158.14	409.58	
5	-236.3						
10	-227.8		70	1.45	158.14	317.86	12.50
15	-218.1						
20	-207.8		70	0.88	158.14	237.21	10.75
25	-193.8						
30	-172.0		70	0.35	158.14	170.79	9.96
32	-155.6		70	0.10	158.14	15.81	10.57

Avg.: 10.94

Percentage increase in oxidation compared to the Blank = 40 %
Percentage increase in Regeneration compared to the Blank = 121 %

Five-cycle redox system using mature solution with Thiobacillus species bacteria

Cycle – III

Bacteria cell count = 2.3 x 10^7 cells/ml
Total iron content = 1000 ppm
Temperature = 30 ^{0}C
pH = 8.89
H_2S flow rate = 0.26 L/m
Air flow rate = 2.4 L/m
Gas Composition: H_2S – 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-12.6	8.89	0					
5	-194.1		0					
10	-221.6		0	70	1.01	158.14	159.72	15.97
15	-238.5		0					
20	-253.2		0	70	1.86	158.14	294.14	14.70

Avg.: 15.33

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-251.8	8.72	70	1.86	158.14	294.14	
5	-240.0						
10	-230.6		70	1.34	158.14	211.90	8.22
15	-220.9						
20	-208.2		70	0.68	158.14	107.53	9.33
25	-189.7						
30	-141.6		70	0.05	158.14	7.90	9.54

Avg: 9.03

Percentage increase in oxidation compared to the Blank = 17 %
Percentage increase in Regeneration compared to the Blank = 82 %

Five-cycle redox system using mature solution with Thiobacillus species bacteria

Cycle – IV

Bacteria cell count = 2.3 x 10^7 cells/ml
Total iron content = 1000 ppm
Temperature = 30 ^{0}C
pH = 9.02
H_2S flow rate = 0.26 L/m
Air flow rate = 2.3 L/m
Gas Composition: H_2S – 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-20.3	9.02	0					
5	-201.5		0					
10	-229.8		0	70	1.15	158.14	181.86	18.18
15	-249.4		0					
20	-262.6		0	70	2.20	158.14	347.90	17.39

Avg.: 17.78

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-262.4	8.84	70	2.20	158.14	347.90	
5	-248.1						
10	-237.2		70	1.42	158.14	224.55	12.33
15	-225.2						
20	-209.5		70	0.72	158.14	113.86	11.70
25	-183.1						
29	-110.2		70	0.08	158.14	12.65	11.59

Avg.: 11.87

Percentage increase in oxidation compared to the Blank = 35 %
Percentage increase in Regeneration compared to the Blank = 140 %

Five-cycle redox system using mature solution with Thiobacillus species bacteria

Cycle – V

Bacteria cell count = 2.3 x 10^7 cells/ml
Total iron content = 1000 ppm
Temperature = 30 ^{0}C
pH = 8.97
H_2S flow rate = 0.26 L/m
Air flow rate = 2.3 L/m
Gas Composition: H_2S – 0.5% in Nitrogen

Oxidation of H_2S

Time (m)	ORP (mV)	pH	H_2S (ppm)	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-20.4	8.97	0					
5	-205.9		0					
10	-234.7		0	70	1.20	158.14	189.76	18.97
15	-250.4		0					
20	-263.4		0	70	2.05	158.14	324.18	16.20

Avg.: 17.58

Regeneration of catalyst

Time (m)	ORP (mV)	pH	µL inj.	C.R	Dil. factor	Fe^{+2}(Mg/l)	Rate (Mg/l-m)
0	-263.4	8.78	70	2.05	158.14	324.18	
5	-251.6						
10	-241.4		70	1.52	158.14	240.37	8.38
15	-230.0						
20	-213.2		70	0.68	158.14	107.53	10.83
25	-182.5						
27	-148.3		70	0.08	158.14	12.65	11.53

Avg.: 10.24

Percentage increase in oxidation compared to the Blank = 34 %
Percentage increase in Regeneration compared to the Blank = 106 %

Comparison of Reaction Rates (Data from Tables 1-5)

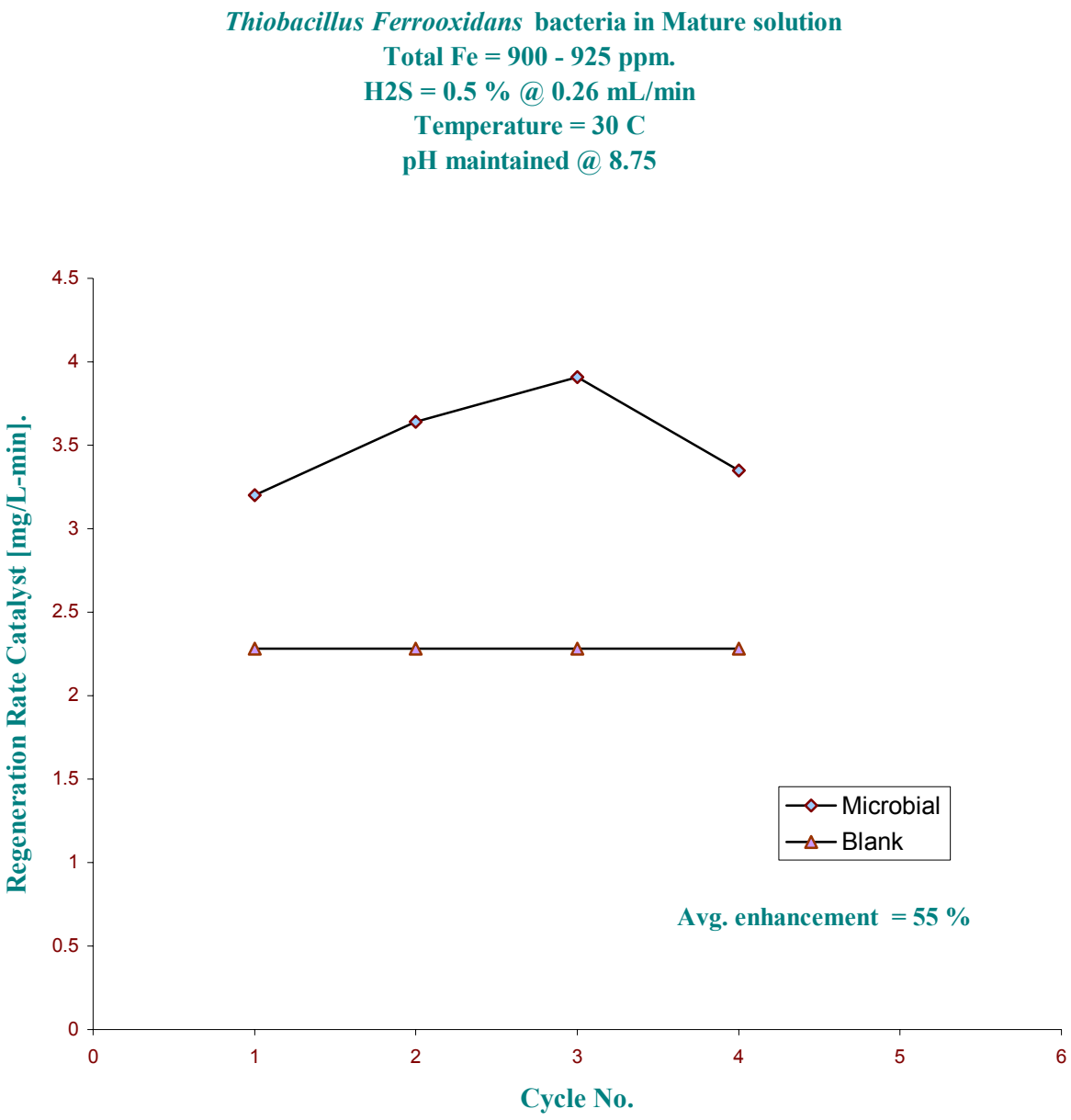

Figure 4.1 (Data values from Table 1)

Catalyst Regeneration, Blank Vs Bacteria

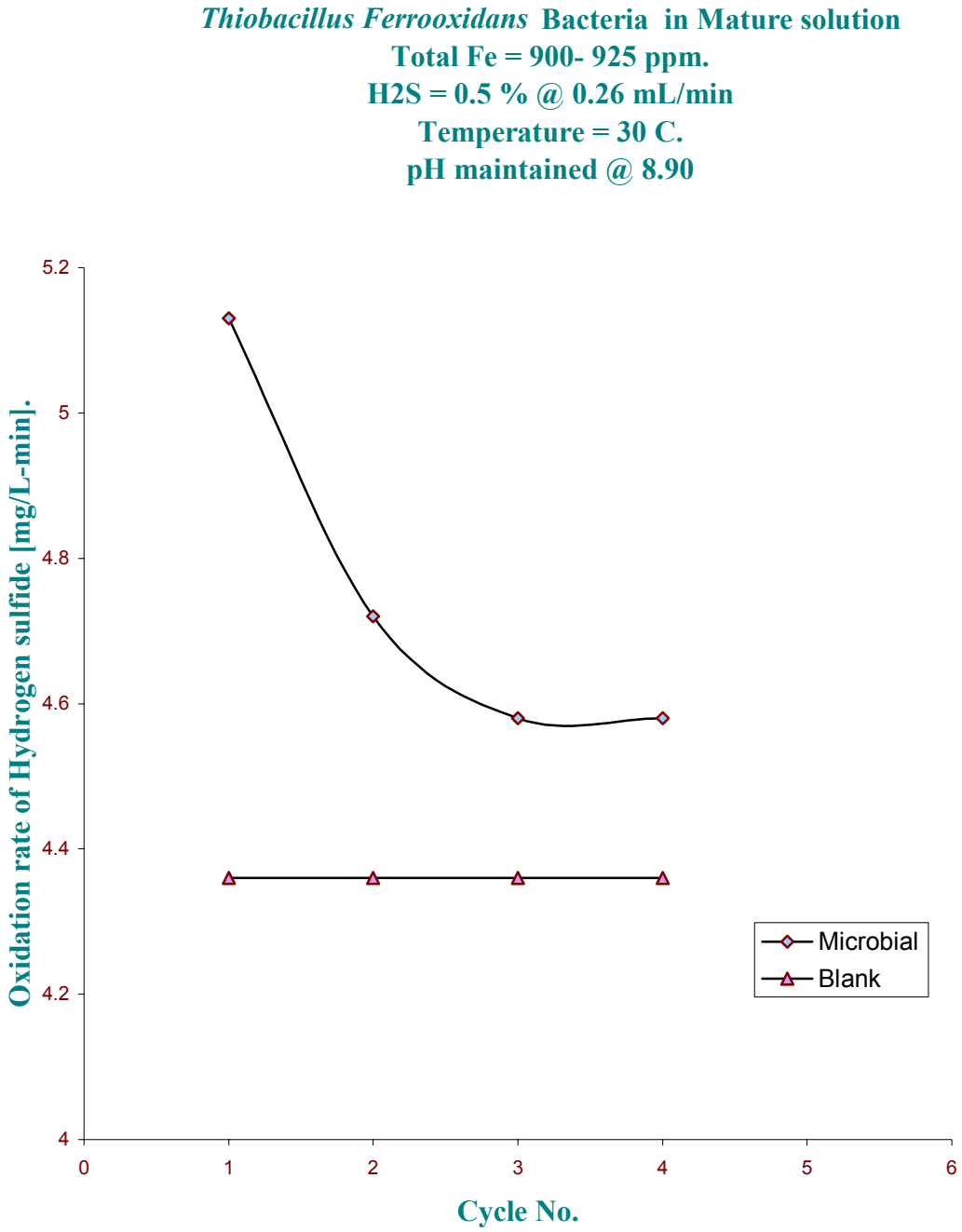

Figure 4.2 (Data values from Table 1)

Oxidation Rate of H2S, Blank Vs Bacteria

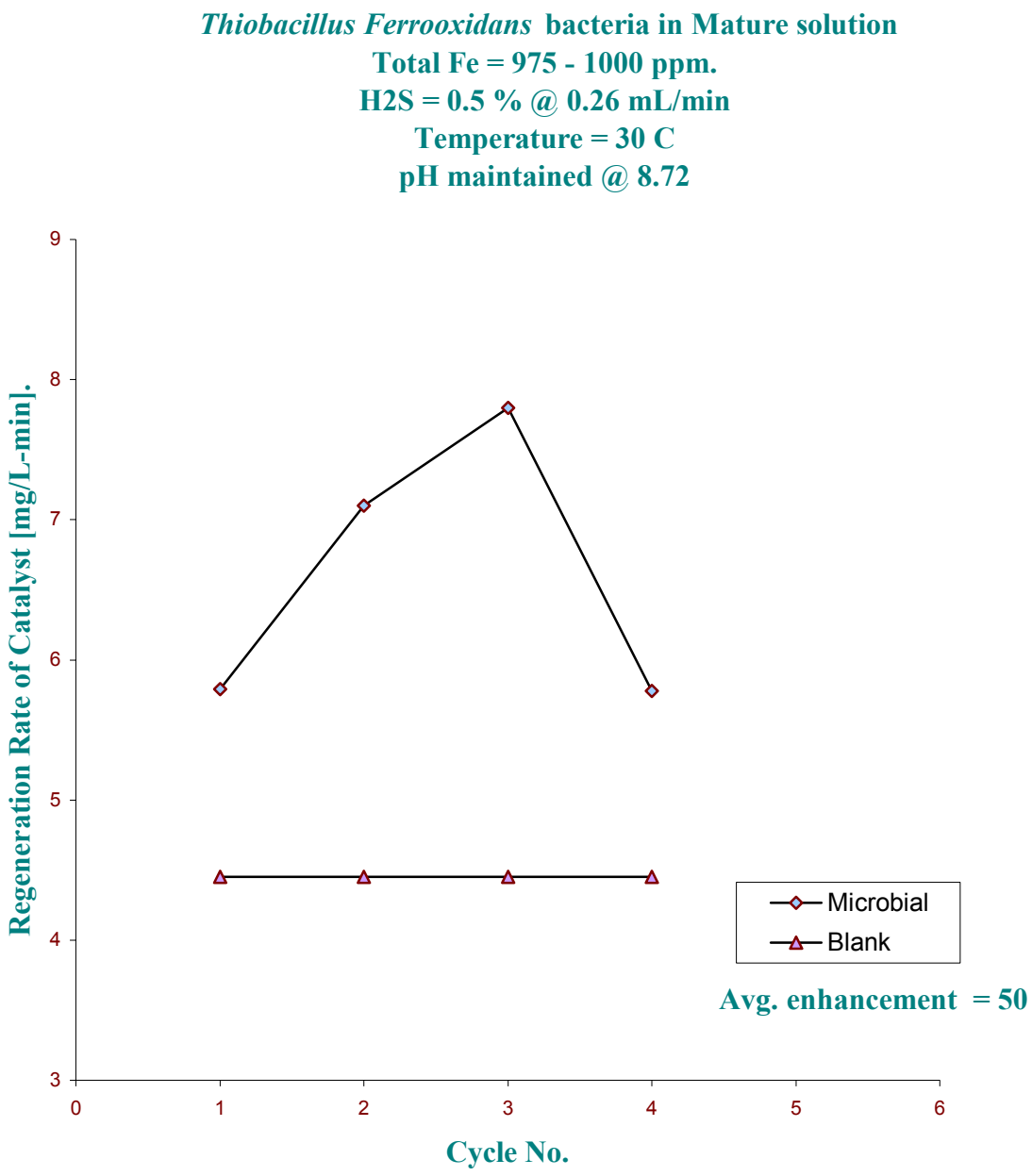

Figure 5.1 (Data values from Table 2)

Catalyst Regeneration, Blank Vs Bacteria

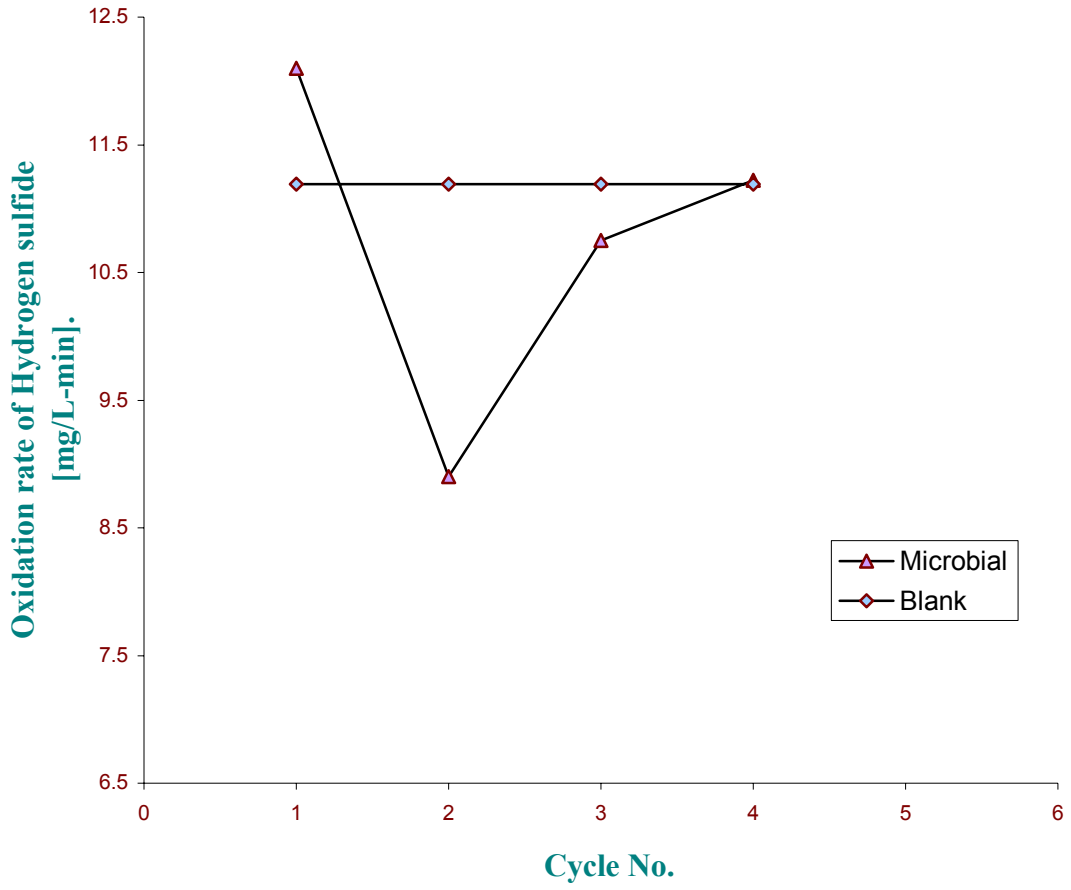

Figure 5.2 (Data values from Table 2)

Oxidation Rate of H2S, Blank Vs Bacteria

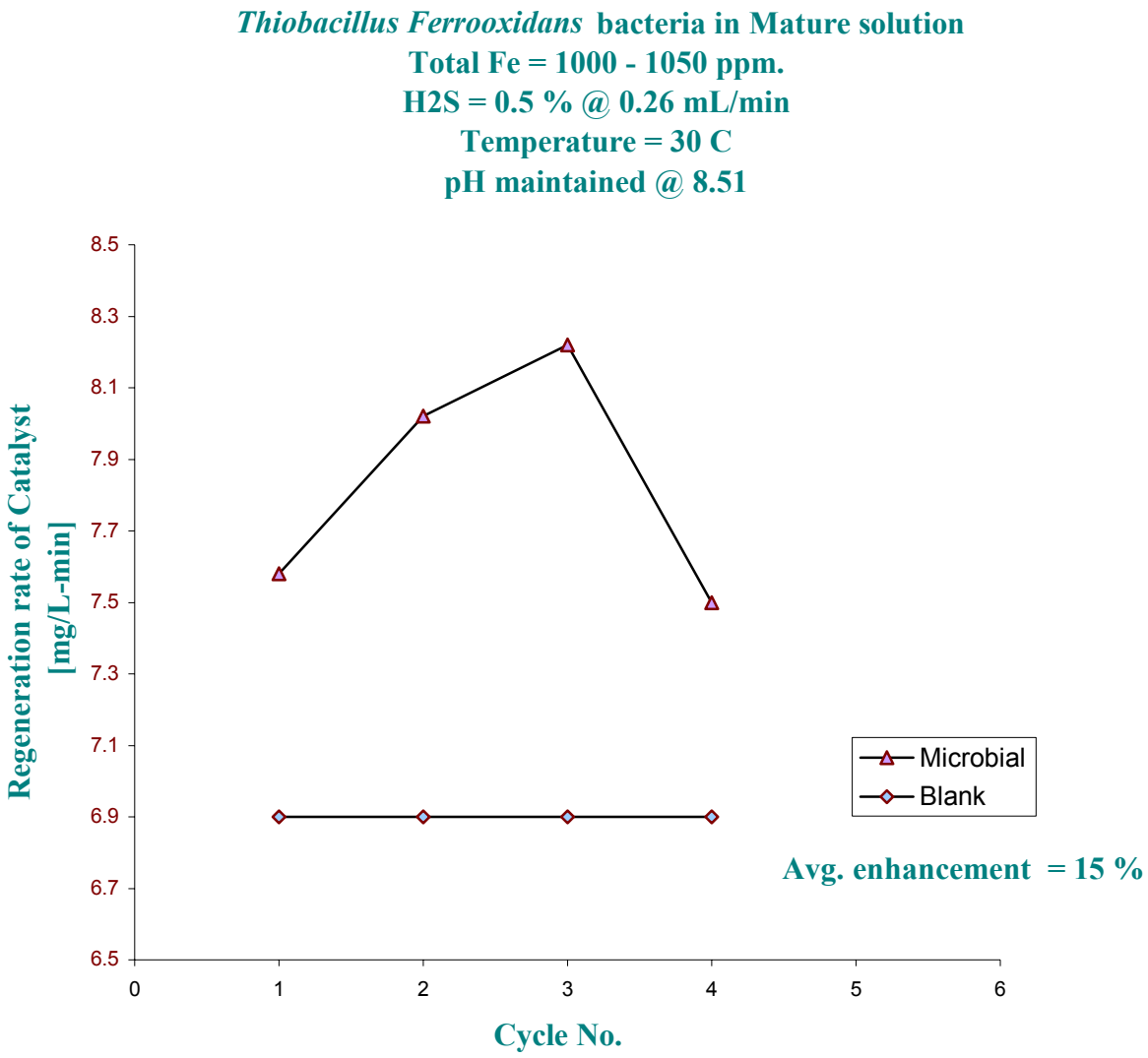

Figure 6.1 (Data values from Table 3)

Catalyst Regeneration, Blank Vs Bacteria

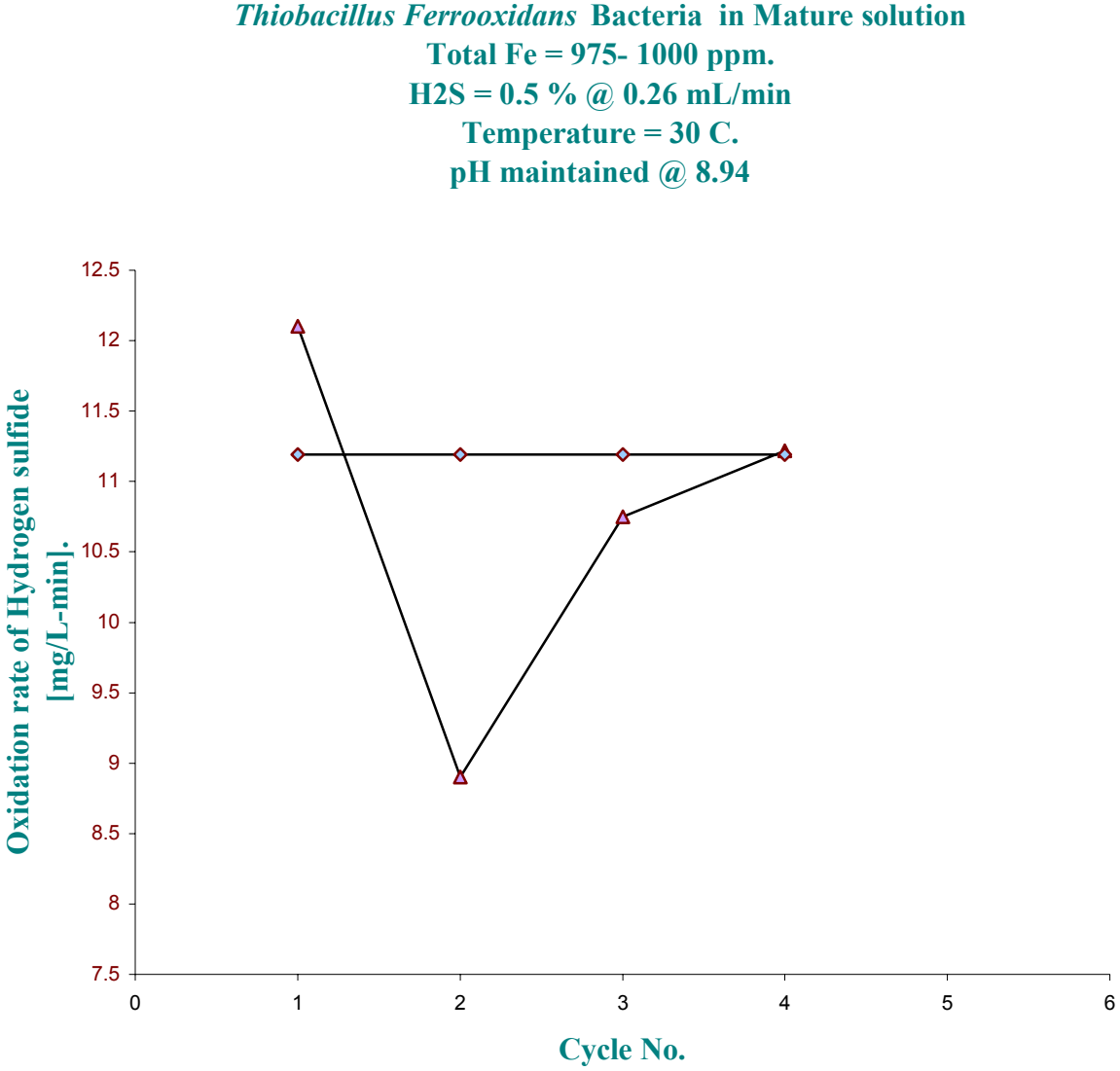

Figure 6.2 (Data values from Table 3)

Oxidation Rate of H2S, Blank Vs Bacteria

Thiobacillus Ferrooxidans **bacteria in Mature solution**
Total Fe = 1025 ppm.
H2S = 0.5 % @ 0.26 mL/min
Temperature = 30 C
pH maintained @ 8.71

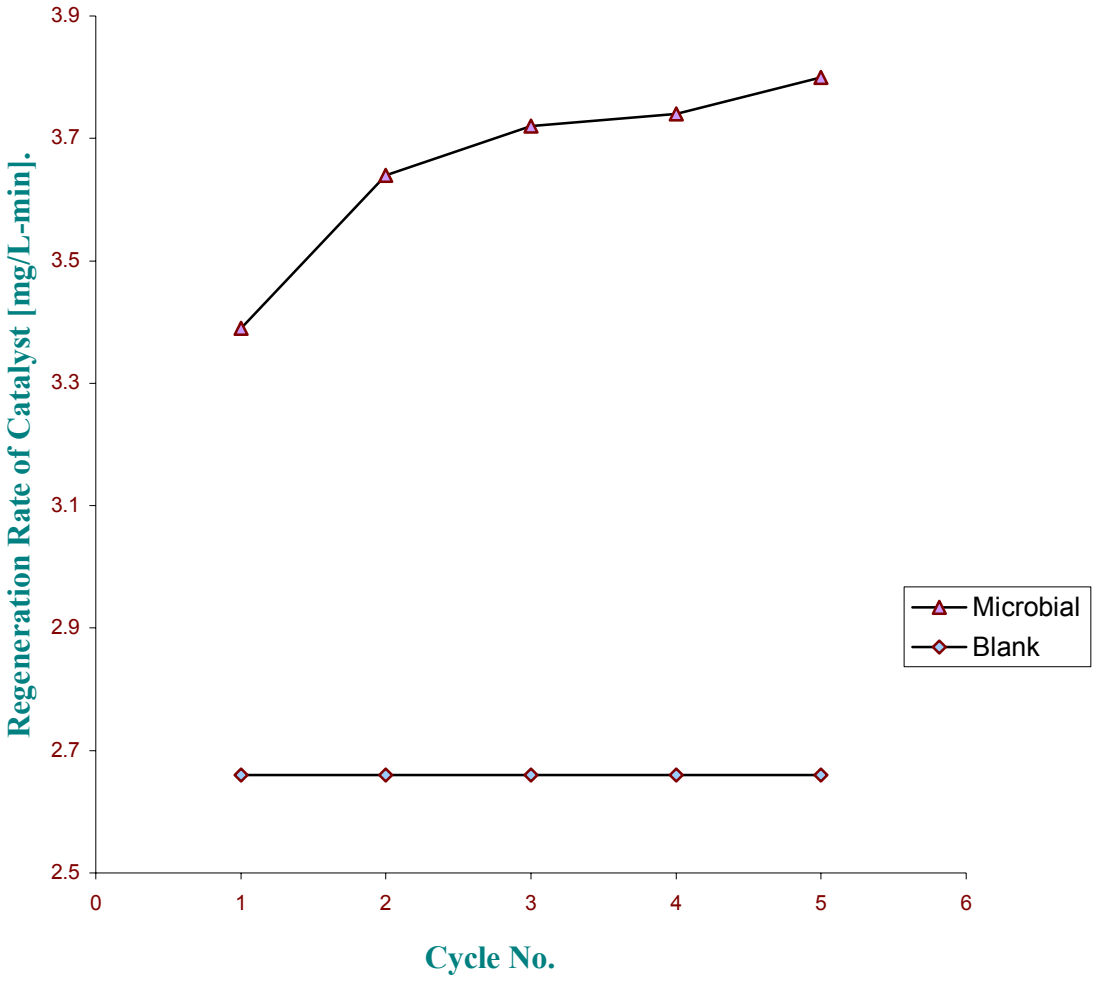

Figure 7.1 (Data values from Table 4)

Catalyst Regeneration, Blank Vs Bacteria

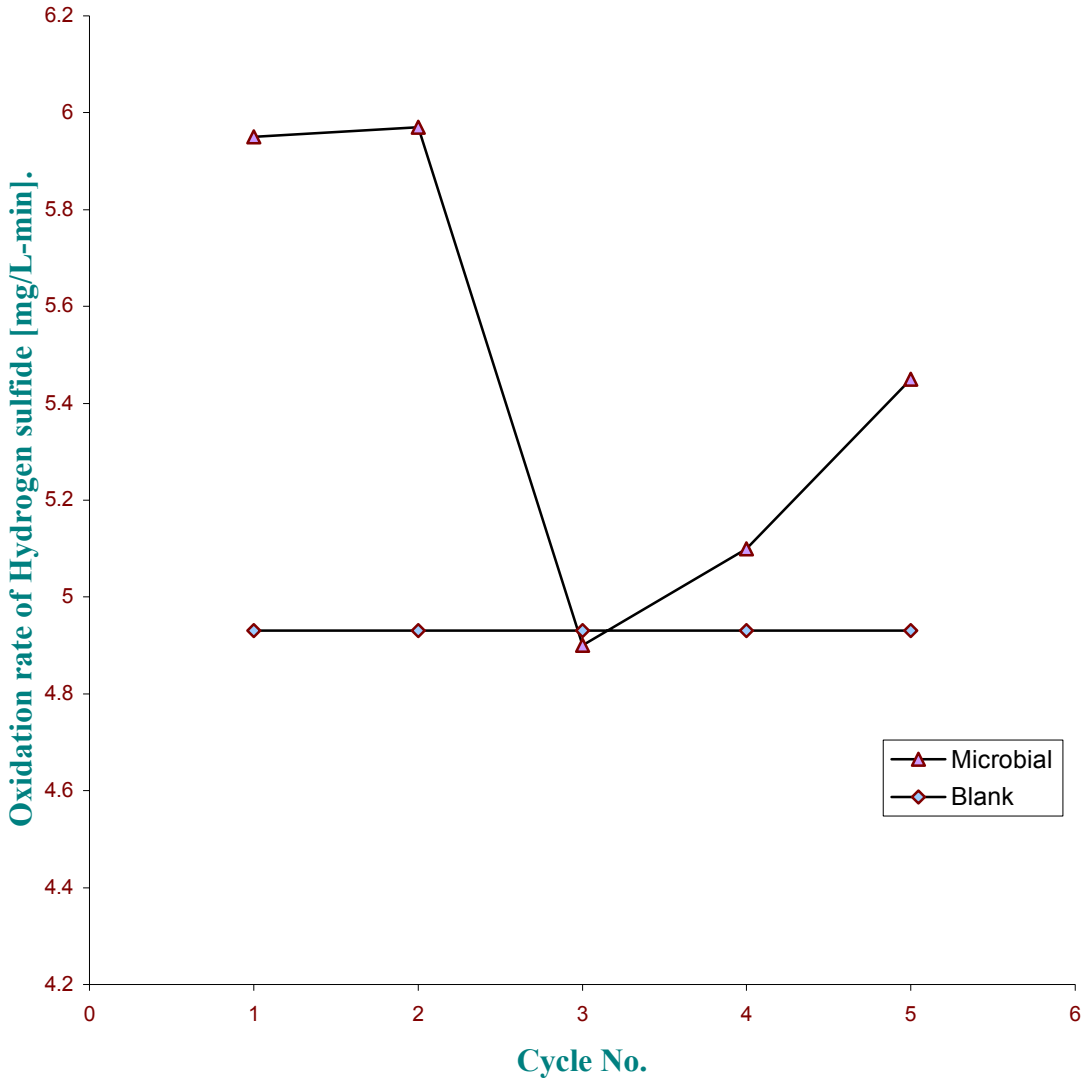

Figure 7.2 (Data values from Table 4)

Oxidation Rate of H2S, Blank Vs Bacteria

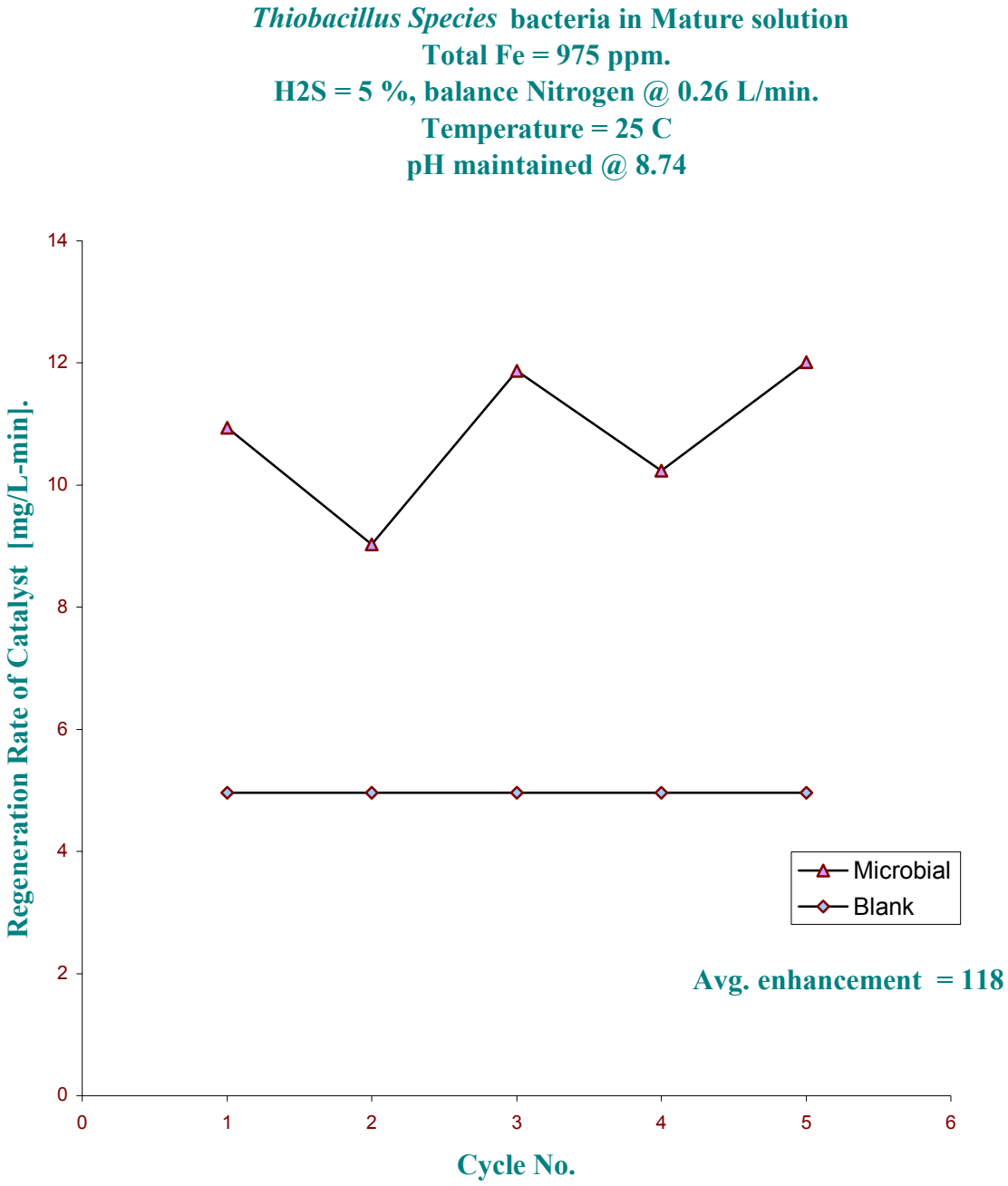

Figure 8.1 (Data values from Table 5)

Catalyst Regeneration, Blank Vs Bacteria

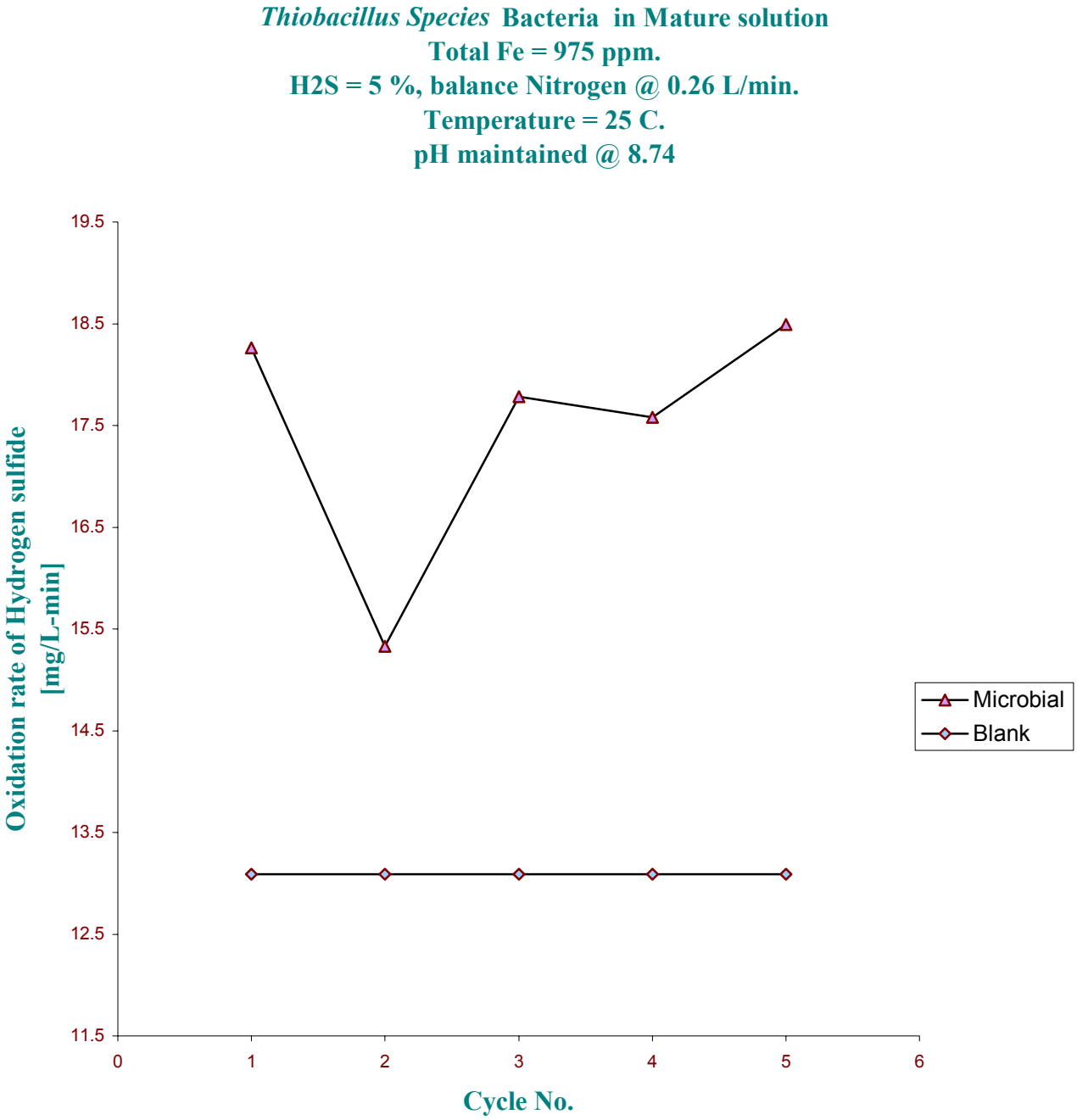

Figure 8.2 (Data values from Table 5)

Oxidation Rate of H2S, Blank Vs Bacteria

3.2 CONCLUSION

The five-cycle experiments carried out in this study conclusively showed that in the presence of bacteria, the chelated ferric iron regeneration rates in the redox system of the mature solution are enhanced by 10% to 112% as compared to the experiments in the absence of bacterial cultures. A 9% to 31.5% increase was observed in the oxidation rate of hydrogen sulfide using Thiobacillus ferrooxidans and Thiobacillus species against absence of bacteria. The mature solution is a high solute catalyst containing the degradation products of commercial iron chelant.

The reoxidation rates of ferric iron were observed to be good with 0.5 H_2S at 30 ^{0}C. This may be due to the fact that the bacteria go into a state of shock when contacted with 0.5% concentrations of hydrogen sulfide. It is believed that acclimatization of bacteria in high H_2S concentration may result in improved reoxidation rates. At higher agitation speeds the reoxidation rate of ferric iron increased significantly. However for the blank experiments the effect of agitation was negligible. Temperature also has an effect on the performance of the bacteria. It was observed that a temperature range of 25 ^{0} C to 35 ^{0} C was favorable. At 45 ^{0} C, the rate enhancement in the presence of bacteria was not as high as in the temperature rang of 25 ^{0} C to 35 ^{0} C.

4.0 PART II - MODELING OF MICROBIAL SWEETENING OF SOUR NATURAL GAS

The biological-oxidation of ferrous iron is an important industrial process for the regeneration of ferric iron in the removal of Hydrogen Sulfide (H_2S). Iron oxidizing bacteria can achieve oxidation of ferrous (Fe^{2+}) ion to ferric (Fe^{3+}) ion under acidic conditions. The second part of this project is to develop kinetic model for the process including bacterial growth, conversion of ferrous to ferric ion by biological pathways. The kinetics of the H_2S absorption and catalyst regeneration using microbial cultures of *T.F.* depend on the concentration of specific ions in the solution. The pH of the solution is an important parameter in determining the stability of the catalytic ferric solution. The pH also is an important factor in determining the specific growth rates of the microbial cultures of *Thiobacillus ferrooxidans*. Therefore it is worthwhile to examine the ion equilibrium balances and use them to determine the pH of the catalyst solution. To model this complex process, we worked on the following two subjects: (I) CSTR model; (ii) Packed Bed Fluid Reactor Model.

4.1 REACTOR DESIGN

The principal factor affecting the effectiveness of industrial process is the reaction rate. Thus reactor design with biological contacting devices is very important. Most reactor designs involving Thiobacillus Ferrooxidans include

1. Rotating Biological Contactors, i.e. CSTR
2. Packed Bed Reactors
3. Fluidized Bed Reactors.

The first part of the project focuses on biological oxidation of Ferrous Sulfate oxidation in a CSTR.

4.2 KINETICS OF THE BIOLOGICAL OXIDATION

$$\text{Cells} + \text{Substrate} \rightarrow \text{More cells} + \text{Product}$$

$$X + S \rightarrow X + P \qquad (6)$$

The growth rate (r_g) of the cells is given by

$$r_g \propto X$$

$$\Rightarrow r_g = \mu X \qquad (7)$$

Where X is the concentration of the biomass containing the microorganisms. In equation (7), μ is called as the specific growth rate of the biomass. The most common equations used to explain the specific growth rate include Monod Equation, Tessier equation, Moser equation [9]. This project will make use of the Monod type of growth model to explain the growth of thiobacillus ferrooxidans and rate of biological oxidation of Fe^{2+}.

The Monod model for growth kinetics is

$$\mu = \mu_{max} \frac{[Fe^{2+}]}{K_S + [Fe^{2+}]} \qquad (8)$$

In the above equation, K_S is a constant.

$$r_g = \mu_{max} X \qquad (9)$$

Product Inhibition: If the formation of a product retards the growth of the cells and substrate utilization, the rate law for the cell growth becomes

$$K = \left(1 - \frac{[Fe^{2+}]}{[Fe^{3+}]}\right)^n \qquad (10)$$

In the above equation, $[Fe^{3+}]$ is the product concentration at which the metabolism stops. K and n are inhibition parameters.

Stoichiometry

The most important factors to be considered while discussing cell growth include

1. pH
2. Redox potential
3. Temperature
4. Presence of two limiting reactants

Recalling equation (6) in terms of yield coefficients (stoichiometric coefficients),

$$\text{Cells} + \text{Substrate} \rightarrow \text{More cells} + \text{Product} \qquad (11a)$$

$$X + S \rightarrow X + P \qquad (11b)$$

$$S \xrightarrow{\text{Cells}} Y_{C/S} X + Y_{P/S} P \qquad (11c)$$

$$Y_{C/S} = \frac{\text{Mass of new cells formed}}{\text{Mass of substrate consumed to produce new cells}}$$

$$Y_{P/S} = \frac{\text{Mass of product formed}}{\text{Mass of substrate consumed to form product}}$$

Product Formation
When only the product is formed from the cells, then

$$r_p = Y_{P/C}\ r_g \qquad (12)$$

Where r_p is the rate of product formation.

The relationship between r_p and $-r_s$

$$r_p = Y_{P/S}\ (-r_s) \qquad (13)$$

$-r_s$ is the substrate utilization rate.

Connecting r_p, $-r_s$ and r_g by doing a mass balance of the substrate [6] leads to

[Net Rate of Substrate Consumption Rate] = [Rate consumed by cells] + [Rate consumed to form product]

$$-r_s = Y_{S/C}\ r_g + Y_{S/P}\ r_p \qquad (14)$$

$$Y_{S/C} = \frac{\text{Mass of substrate consumed}}{\text{Mass of more cells formed}}$$

$$Y_{S/P} = \frac{\text{Mass of substrate consumed}}{\text{Mass of more product formed}}$$

4.3 MASS BALANCE IN A 'CSTR'

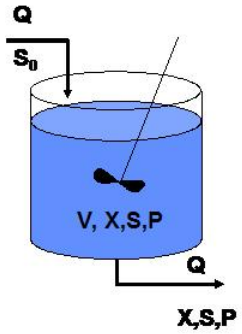

Figure 9: Mass Balance in a CSTR

Cell Balance

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of cells}\end{pmatrix}=\begin{pmatrix}\text{Rate of cells}\\ \text{entering}\end{pmatrix}-\begin{pmatrix}\text{Rate of cells}\\ \text{leaving}\end{pmatrix}+\begin{pmatrix}\text{Net rate of generation}\\ \text{due to chemical reaction}\end{pmatrix} \tag{15}$$

$$V\frac{dX}{dt}=F\,X_0-F\,X+r_sV$$

$\div$ by V;

$$\Rightarrow\quad \frac{dX}{dt}=D\,X_0-D\,X-r_s \tag{16}$$

If the microorganisms are suspended, then $F\,X_0$ is zero.

$$\frac{dX}{dt}=0-D\,X-r_s \tag{17}$$

Substrate Balance

The substrate is being consumed to form cells and product.

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of substrate}\end{pmatrix}=\begin{pmatrix}\text{Rate of substrate}\\ \text{entering}\end{pmatrix}-\begin{pmatrix}\text{Rate of substrate}\\ \text{leaving}\end{pmatrix}-\begin{pmatrix}\text{Net rate of substrate}\\ \text{utilization}\end{pmatrix} \tag{18}$$

$$V\frac{dS}{dt}=F\,S_0-F\,S-r_sV \tag{19}$$

$$-r_s=Y_{S/C}\,r_g+Y_{S/P}\,r_g \tag{20}$$

$$\frac{dS}{dt}=D.S_0-DS-\left(Y_{S/C}\,r_g+Y_{S/P}\,r_g\right) \tag{21}$$

Product Balance

$$\begin{pmatrix}\text{Rate of}\\ \text{accumulation}\\ \text{of product}\end{pmatrix} = \begin{pmatrix}\text{Rate of product}\\ \text{entering}\end{pmatrix} - \begin{pmatrix}\text{Rate of product}\\ \text{leaving}\end{pmatrix} - \begin{pmatrix}\text{Rate of product}\\ \text{generation}\end{pmatrix} \tag{22}$$

$$V\frac{dP}{dt} = 0 - F\,P + r_p \tag{23}$$

$$r_p = Y_{P/C}\,r_g \tag{24}$$

$$\frac{dP}{dt} = 0 - DP + Y_{P/C}\,r_g \tag{25}$$

5.0 RESULTS AND DISCUSSION

5.1 EVOLUTION OF TOTAL BIOMASS, FERROUS AND FERRIC IRON IN A CSTR OVER TIME

Figure shows the evolution of the biomass, substrate and product over time. Steady state conditions were attained after 150 hours [7]. Initially, ferrous iron decreased and ferric iron and total biomass increased until the maximum values were achieved. The yield coefficients used in the present kinetic model was extracted only in the growth phase and is a scalar value.

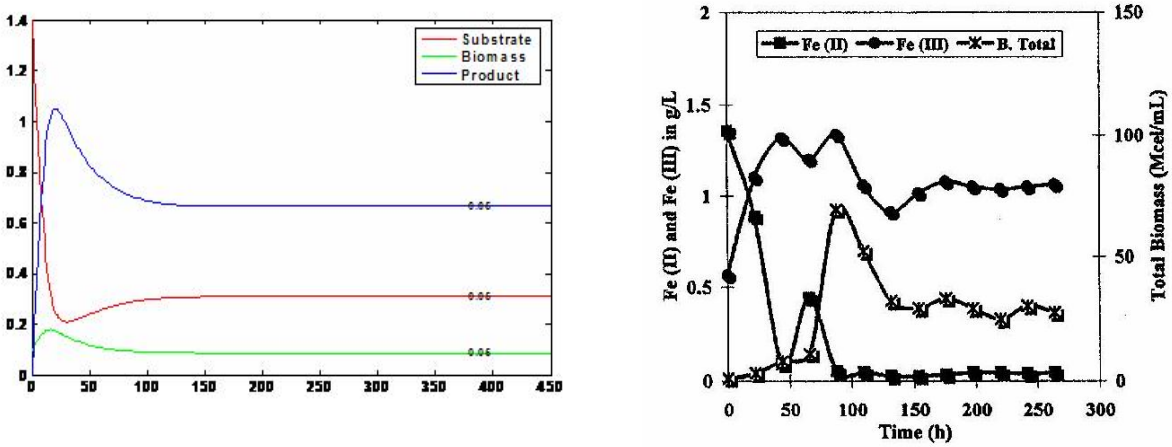

Figure 10: Evolution of total biomass, ferrous and ferric iron in a CSTR over time (left- present model, right-experimental data by Gomez and Cantero, 2003

5.2 EFFECT OF DILUTION RATE

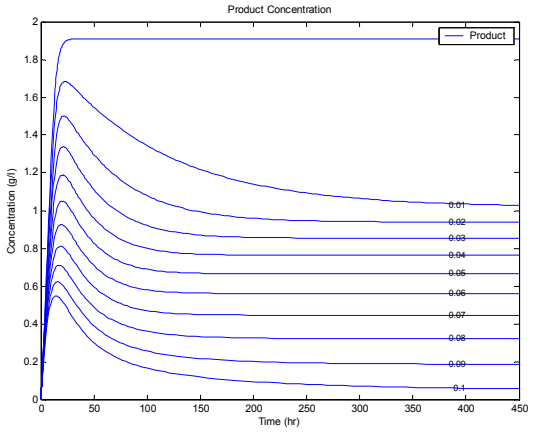

Figure 11: Product Concentration with varying dilution rates

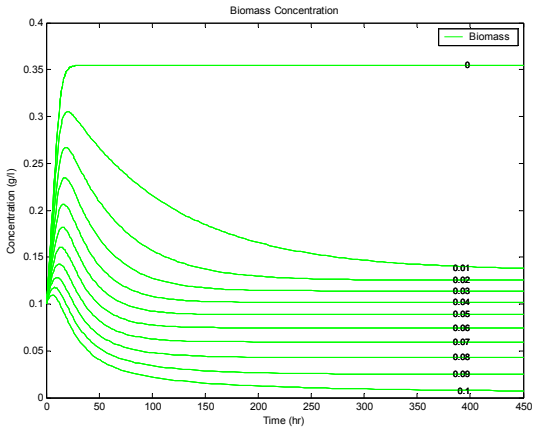

Figure 12: Biomass Concentration with varying dilution rates

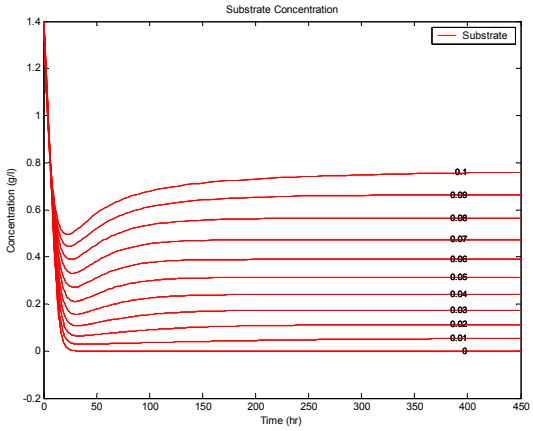

Figure 13: Substrate Concentration with varying dilution rates

When the dilution rate increases, there is a washout of the biomass. This effect was more pronounced at higher dilution rates that significantly reduced the rate of oxidation of the ferrous iron. In a CSTR, the cells are not immobilized unlike in a plug flow reactor and are freely suspended like a batch reactor. Dilution rate seems to be the most important design variable that needs to be properly tuned to achieve the maximum growth of the biomass to yield more products. However, there exists a trade-off in the rate of cell generation due to wash out.

5.3 EFFECT OF pH

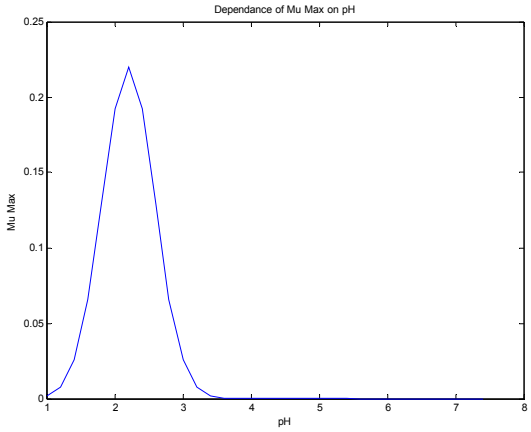

Figure 14: Maximum specific growth rate as a function of pH

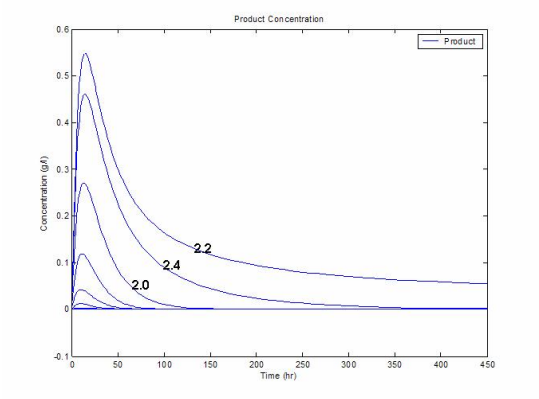

Figure 15: Product Concentration as a function of pH

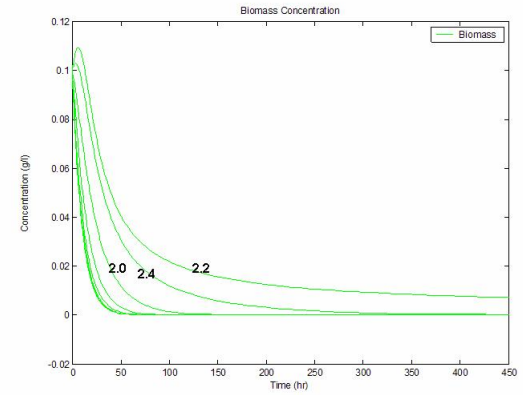

Figure 16: Biomass Concentration as a function of pH

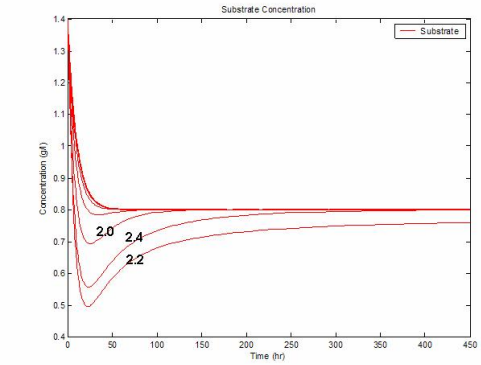

Figure 17: Substrate Concentration as a function of pH

The maximum specific growth rate as a function of pH of the solution had a strong dependence. There exists an optimum pH of 2.2 at which the growth rate was found to be a maximum as shown in Figure 14. Any slight deviations from the optimal value significantly influenced the product formation (the rate of biological oxidation) because the optimal ph at which the biomass grows is around 2.0.

A model accounting for this effect is given as

$$\mu^*_{\max} = \mu_0 \exp\left(-k_0 (pH - pH_0)^2\right) \qquad (26)$$

The parameter values were identified as follows: pH0= 2.2, $k_0 = 3.349$ and $\mu_0 = 0.183$ hr^{-1}
Figure 15, Figure 16 and Figure 17 justify the existence of an optimum pH yielding more product formation.

5.4 PFR AND FEEDBACK CONTROL

A novel process for H_2S gas treatment is based on two steps corresponding to absorption with chemical reaction of the gas in a ferric solution (whereas the ferric ion is converted to ferrous), and biological oxidation of ferrous ions in the solution to produce ferric ions again. The biological oxidation of ferrous ion, produced during the absorption step, to ferric ion involves the biocatalyst activity of the bacterium Thiobacillus Ferrooxidans and may be regarded as the regeneration of the absorbing solution. Advantages of this process with respect to conventional treatment processes for H_2S abatement (e.g., Claus) are mild pressure and temperature conditions (typical of biotechnological processes), lower costs and closed-loop operation without input of chemicals or output of wastes.

5.4.1 Packed Bed Fluid Reactor Model

The packed bed reactor is modeled as a series of perfectly mixed volumes CSTR models. The schematic of the PFR model is displayed in Figure 1818. The CSTR differential volumes can be represented by equations (27) – (30). Equation (27) solves for the biomass concentration where the accumulation of biomass is equal to the biomass growth. No biomass enters or leaves the volume because it is considered to be fixed to the bed. Equations (28), (29) and (30) solve for the product and substrate concentrations, and pH dependence.

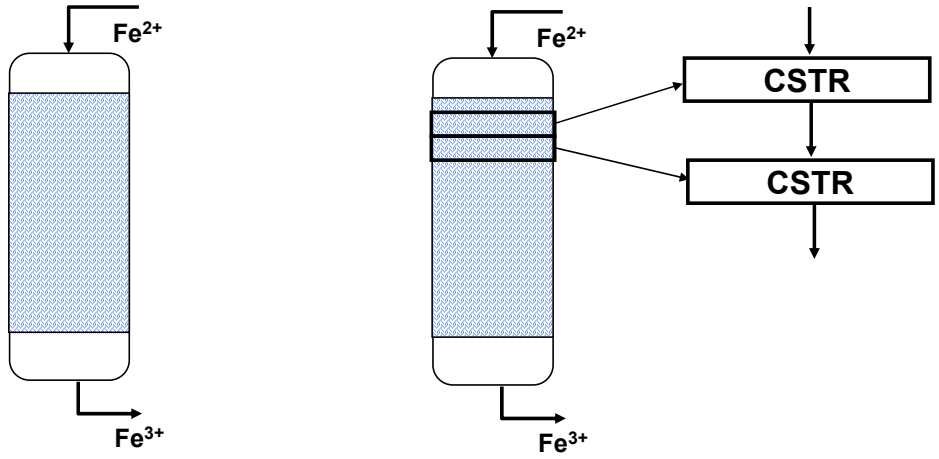

Figure 18- Left: Packed Bed Reactor. Right: Reactor modeled as a series of CSTRs.

$$\frac{dX_i}{dt} = \frac{\mu^*_{\max} X_i}{K_s + \left[Fe_i^{2+}\right] + K_I \left[Fe_i^{3+}\right]} \tag{27}$$

$$\frac{d\left[Fe_i^{3+}\right]}{dt} = Y_{P/X} \frac{\mu^*_{\max} X_i}{K_s + \left[Fe_i^{2+}\right] + K_I \left[Fe_i^{3+}\right]} - \frac{Q}{V_i}\left(\left[Fe_{i-1}^{3+}\right] - \left[Fe_i^{3+}\right]\right) \tag{28}$$

$$\frac{d\left[Fe_i^{2+}\right]}{dt} = -Y_{S/X} \frac{\mu^*_{\max} X_i}{K_s + \left[Fe_i^{2+}\right] + K_I \left[Fe_i^{3+}\right]} + \frac{Q}{V_i}\left(\left[Fe_{i-1}^{2+}\right]^0 - \left[Fe_i^{2+}\right]\right) \tag{29}$$

With $\mu_{\max}^{*} = \mu_0 \exp\left(-k_0 (pH - pH_0)^2\right)$ (30)

The system of equations (27) – (30) is solved with the initial and boundary conditions. Figure19 displays the product concentration profiles along the reactor length. In this figure we can observe the same trend then in the CSTR, where the product observes a maximum. In the case of the PFR the maximum expands as a plateau for positions closer to the outlet. This could be beneficial for the operation since it would ensure maximum product outlet.

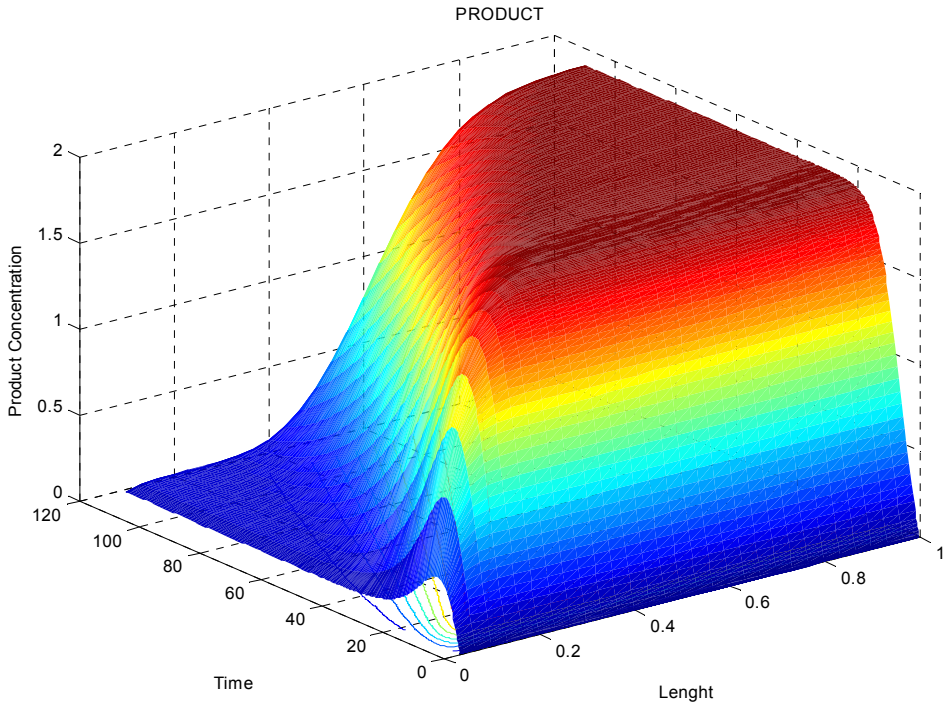

Figure19. Product concentration profile

Figure20 depicts the evolution of the biomass concentration. In this plot we can observe that the biomass concentration establishes in a steady state condition as for positions far from the inlet. This can be explained with the results displayed in Figure 21 where the substrate is depleted giving a constant biomass concentration.

Figure

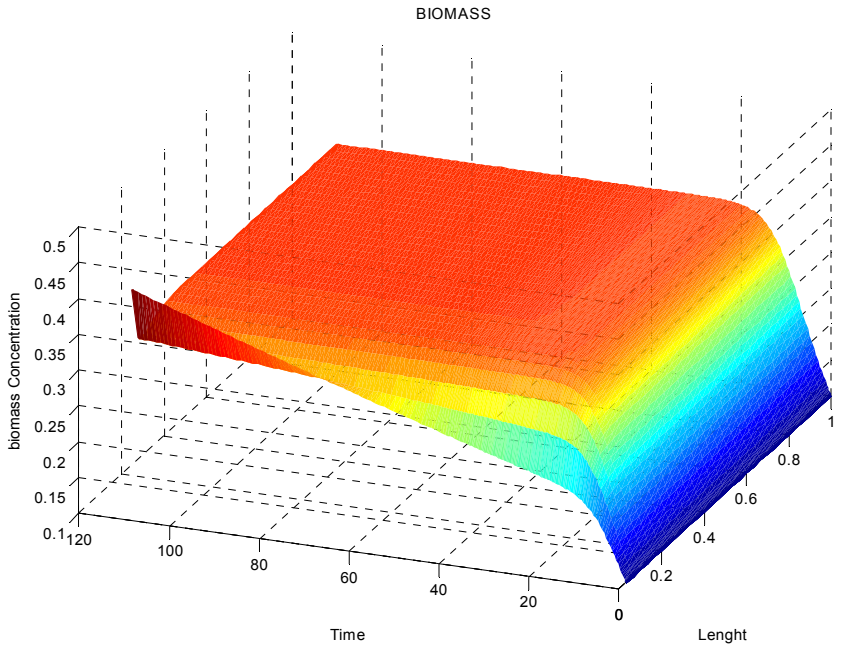

Figure 20. Biomass concentration profiles

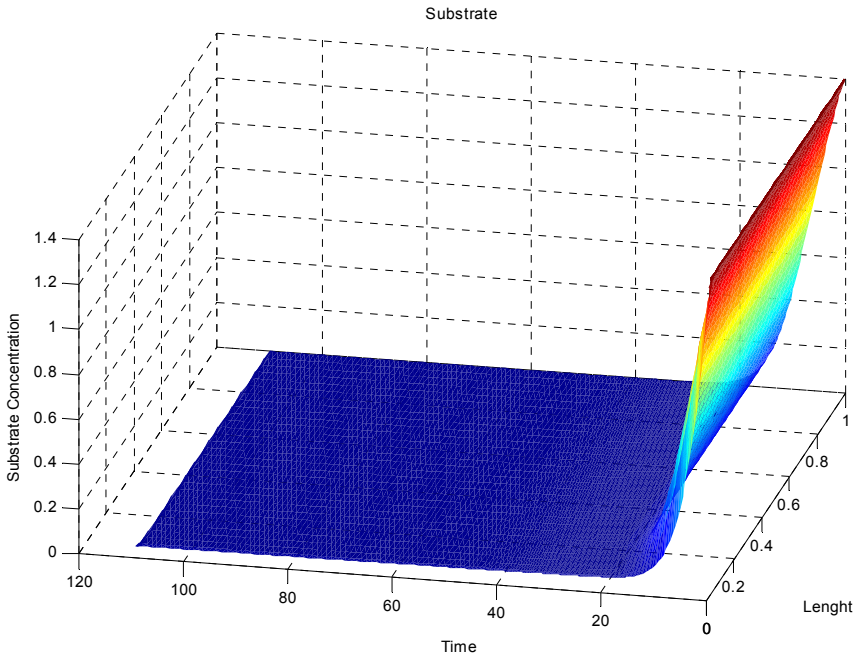

Figure 21 Substrate concentration profiles

5.4.2 CSTR Bioreactor Control

With the objective of supplying a constant amount of Fe^{3+} to the chemical step, a feedback control was devised to control the production of Fe^{3+} illustrated in Figure 22. The control is implemented by measuring the Fe^{3+} concentration and implementing a PI feedback control. The selected control variable is the dilution rate; this rate is controlled by simultaneously manipulating the feed and outlet flow.

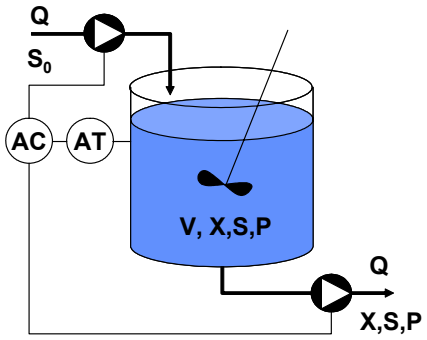

Figure 22 - Proposed feedback production control

To evaluate the control loop efficiency the system was subjected to disturbances in the inlet substrate concentration. Two different disturbances were applied: a step change and a sinusoidal change of the inlet substrate concentration. Figure 23 displays the disturbance and the corresponding controlled system evolution. In this figure we can see that the production tracks the set-point really accurately even when subjected to a 20% change in the inlet concentration. Figure 24 shows the evolution of the controlled system under a periodic disturbance. In this figure we can also see that the control handles the disturbance almost perfectly. Thus, we can conclude that the selected control scheme is an adequate way to control this system under inlet disturbances.

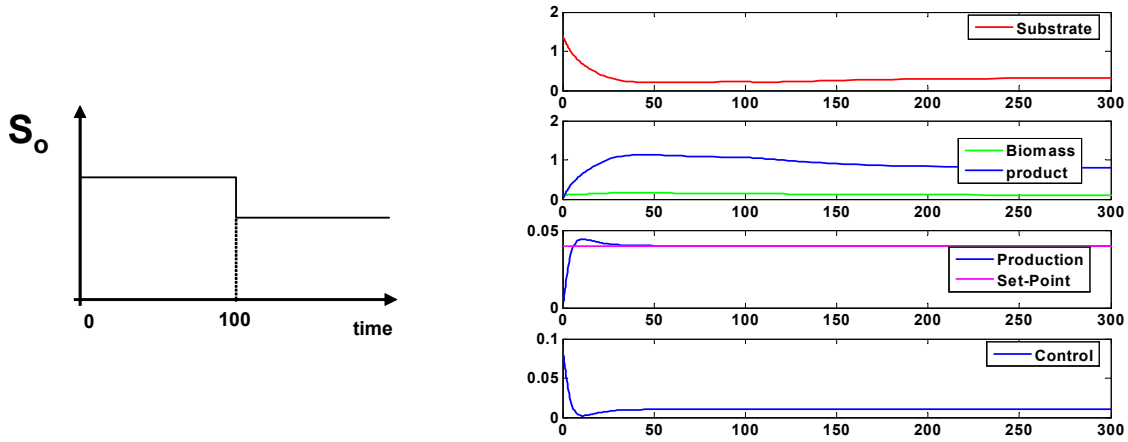

Figure 23 - Left: Inlet substrate concentration evolution. Right: Reactor performance

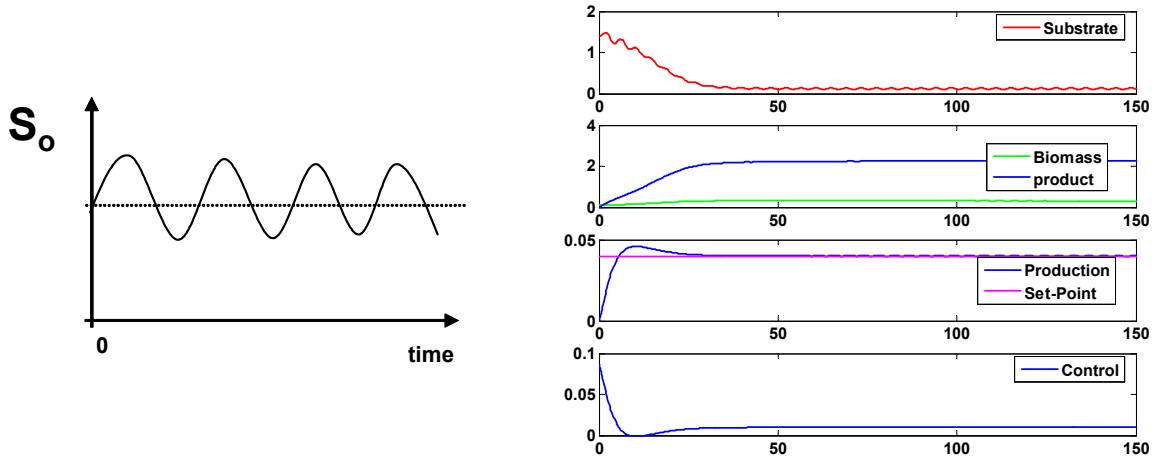

Figure 24 - Left: Periodic inlet substrate concentration. Right: Reactor performance under inlet disturbance.

6.0 CONCLUSIONS FROM THE MODELING AND FUTURE WORK

The models presented in the literature lacked engineering rigor. The design of a CSTR for the biological oxidation of ferrous iron was presented using published kinetic experimental data. A Monod type of growth model was used for the growth of biomass. CSTR and Batch reactor models (with zero dilution rates) agree with experimental data. Dilution rate had an influence on the rate of oxidation of ferrous iron. Higher dilution rates caused washout of the biomass. The oxidation rate was highly pH sensitive. Specific growth is a function of pH of the broth. The specific growth rate had a maximum value at around 2.2. A packed bed fluid reactor dynamic model was developed using a finite volume discretization approach. This model can predict the product, biomass, and substrate concentrations as a function of time and length. To control the product production a feedback loop was implemented in the CSTR reactor. This feedback loop showed excellent behavior under different inlet concentrations. All the simulations were conducted at constant pH. For future work it would be reasonable to model the pH dependence on the product and substrate concentrations. Also it would be worthwhile to consider multiple feed in the PFR reactor and also develop a distributed control system to the plug flow reactor.

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APPENDIX – A

Microbial Sweetening of Sour Natural Gas Using Mixed Cultures

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About twenty-five percent of natural gas produced in the United States is sour containing significant volumes of hydrogen sulfide. Liquid redox processes remove hydrogen sulfide from natural gas. Aqueous solution of chelated ferric ions oxidize the hydrogen sulfide to elemental sulfur. The reduced iron chelate is then oxidized by contact with air and recycled. This requires expensive equipment for regeneration and the process is usually energy intensive.

A microbial process for regeneration of chelated ferric ions may offer an economical alternative to commercial liquid redox processes. The present study investigates the use of a mixed culture of iron oxidizing bacteria to regenerate commercial iron chelate catalysts. The objective of this study is to quantify an increase in the biologically enhanced redox solution reoxidation rates. It was observed that the presence of bacterial cultures enhance the reoxidation rates and sulfur removal significantly. The proprietary mixed cultures of iron oxidizing bacteria used in this study derive the energy required for their growth from the oxidation of reduced sulfur compounds and from the oxidation of Fe(II) to Fe(III) ions in presence of air.

A series of experiments were conducted with a commercial chelated iron catalyst at a constant pH of 7.5 using a total iron concentration of 1000 ppm in the solution. Regeneration of the solution was carried out by passing air through the solution. Sulfur produced was removed by centrifuging in the case of baseline experiments and by vacuum filtration in the presence of bacteria. A 50 to 125% increase was observed in the regeneration rates whereas sulfur yields were 80 to 100% of theoretical in the presence of bacteria as compared to 35 to 50% in the absence of bacteria. Iron oxidizing bacteria were used at cell densities of 1.5×10^9 cells/l to be effective. The pH of the redox solution was observed to be a key process parameter. Other parameters such as temperature, total iron concentration, gas to liquid ratio and bacterial cell densities also influence the overall process.

INTRODUCTION

The natural gas industry has long been interested in sulfur recovery technology for applications to streams resulting from treatment of sour natural gas resources [*1*]. About 14% of U.S. natural gas reserves contain hydrogen sulfide (H_2S) and other contaminants (2). The sour gas is very hazardous to human health and could cause extensive damage to natural gas pipelines if not properly processed [*3*]. In order to reduce its health and environmental hazards, and meet the pipeline industry specifications, the H_2S concentrations in natural gas are reduced to less than 4 ppmv. About twenty-five percent of natural gas produced in the United States is sour containing significant volumes of H_2S and other sulfur compounds. The traditional technique for treating this sour gas involves a two-step approach of first separating the acidic gases from the natural gas with an amine plant and then either flaring the hydrogen sulfide or recovering the sulfur in a Claus plant [*4*]. The liquid redox processes are preferred over traditional Amine-Claus systems because of their simplicity, higher sulfur recovery and good turn down ratio. Scavenger processes are preferred for natural gas streams where sulfur recovery is not economical [*5*].

The Liquid Redox Sulfur Recovery Processes absorb hydrogen sulfide from the sour gas stream and produce elemental sulfur. The liquid redox processes may use vanadium, iron or a mixture of iron and quinone as the primary catalysts interacting with hydrogen sulfide. The iron-based processes have been most successful because of their superior performance, simple operation, greater reliability and environmental acceptability [*6*]. However, the process conditions promote the oxidation reactions that accelerate the decomposition of metal-chelate catalysts resulting in high processing costs, and recirculation power requirements. Moreover, in all the commercial liquid redox processes, expensive redox solution is lost via salt formation and inadequate washing of the sulfur cake produced [*7*].

Redox Process Chemistry

The iron-based redox processes employ iron in the ferric state (Fe^{3+}) to oxidize hydrogen sulfide to the elemental sulfur (S^o). The ferric ion is reduced to the ferrous state (Fe^{2+}), which is then regenerated to the ferric state by oxidation with air as follows:

$$H_2S_{(l)} + OH^- \leftrightarrow HS^- + H_2O$$

$$HS^- + OH^- \leftrightarrow S^= + H_2O$$

$$S^= + 2Fe^{3+} \leftrightarrow S^o + 2Fe^{2+}$$

$$2Fe^{2+} + 1/2O_2 + H_2O \leftrightarrow 2Fe^{3+} + 2OH^-$$

Typical iron concentrations in the chelated catalysts range from 500 to 2500 ppm as determined by economics involving pumping and chemical costs. The LO-CAT chelated catalysts can handle concentrations of H_2S as high as 100% and there appears to be no lower limits [*9*].

Neither ferrous nor ferric ions are stable in aqueous solutions at neutral or alkaline pH levels and ordinarily will precipitate either as ferrous or ferric hydroxide. This precipitation is prevented by complexing the iron with organic chelates which are capable of holding both forms of iron in solution. These organic chelates are classified into two groups: Type A chelates such as ethylenediamine tetraacetic acid (EDTA) or nitrilotriacetic acid (NTA) which are powerful chelating agents at low pH's; and the type B chelates, consisting of polyhydroxylated sugars that are effective at pH above 8. Combination of both types of chelates makes the catalyst stable at any pH from 5 to 9.0.

The selection of a chelant is dependent on the reaction rate of Fe^{3+}-chelate with H_2S, Fe^{3+}-chelate with oxygen and the rate of degradation of the chelate. The chelate degradation occurs through the oxidation of the chelate by Fe^{3+} ion and the free radical induced oxidation [*8*]. Other variables that control the oxidative degradation are: pH, chelant concentration, chelant to iron ratio, and the type of degradation products formed.

The LO-CAT^{TM} process was originally developed by ARI Technologies, now Wheelabrator Clean Air Systems, Inc., to treat sour gas in the absorber at feed gas pressure and relatively low iron concentrations (1000 to 1500 ppmw) and high circulation rates. This system referred to as conventional LO-CAT works well for many low-pressure plants, however, it results in excessive equipment and pumping costs for high pressure applications. The ARI-LO-CAT II process as shown in Figure 1, was developed for the high pressure direct treat applications [*9*]. The process uses substoichiometric iron chelated catalyst in the absorber and an oxidizer unit that circulates liquid through density differences. The process is described in greater detail in the literature [*9*, *10*]. The process also uses a separate sulfur settler vessel. These features reduce the chemical and operating costs.

The iron-oxidizing bacteria are capable of oxidizing ferrous ions to the ferric state at low pH. According to the literature references these microbes are capable of oxidizing Fe^{2+} to Fe^{3+} state at 500,000 times faster rate than the purely chemical oxidation process in the absence of bacteria [*11*]. The regeneration of Fe^{3+} chelate in the presence of acidophilic microbes under mild conditions at 25–45°C, and atmospheric pressure would minimize the chelate degradation process and thus help in improving the economics of hydrogen sulfide oxidation in the natural gas sweetening process. The Fe^{3+}-chelates are also capable of oxidizing the mercaptans to the insoluble disulfides [*12*]. It is proposed to use these microbes for achieving enhanced ferric ion reoxidation rates in ARI-LO-CAT II process thereby improving the overall sour gas processing economics.

The basic objective of this study, jointly sponsored by U.S. Department of Energy and Gas Research Institute, is to develop information and technology to improve the economics of the commercial iron-based redox processes such as ARI-LO-CAT II and SulFerox, with emphasis on the biologically-enhanced reoxidation of the redox solution used in these processes. In this study, a mixed culture of iron oxidizing bacteria were used to regenerate the commercially used iron chelates for reoxidation of reduced redox solutions. There are more than forty gas processing units nationwide using liquid redox technology. These gas processing plants could use the new technology being developed in this project and thus lower the gas processing costs of sub-quality sour natural gas substantially.

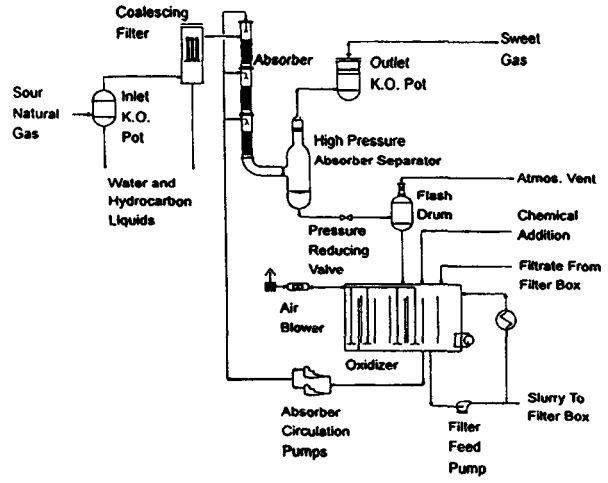

FIGURE 1 Process Flow Diagram for the ARI-LO-CAT II System.

MECHANISM OF MICROBIAL OXIDATION OF FERROUS IRON

The iron oxidizing bacteria derive the energy required for their growth from the oxidation of reduced sulfur compounds and from the oxidation of Fe^{2+} to Fe^{3+} ions, using air as an oxidant. The major electron transfer components of the respiratory chain of the iron oxidizing bacteria have been postulated by Ingledew et al. [*13*] and Cox et al. [*14*]. These components are organized in the cytoplasmic membrane in such a way as to couple Fe^{2+} oxidation to generate a transmembrane proton electrochemical potential. This potential is the main driving force for electron transfer. A diagrammatic representation of electron transfer mechanism is shown in Figure 2. The major electron transfer components of the respiratory system of the bacteria are comprised of: a cytochrome oxidase, cytochrome c, cytochrome a and a blue colored copper protein, rusticyanin [*15*]. Ferrous ion oxidation takes place at the cell wall and generates a transmembrane electrochemical potential of 250 mV. The reduction of molecular oxygen is catalyzed by a cytochrome oxidase at a pH of 6.5 on the inside of the cytoplasmic membrane [*16*].

Components of the iron oxidase are identified by their prosthetic groups and are arranged from left to right in order of increasing redox potential (Cobley and Haddock, 1975; Ingledew and Cobley, 1980). "Out" and "in" refer to the bulk phase and cytoplasm, respectively.

MATERIALS AND METHODS

a. Growth Characteristic of Iron Oxidizing Bacteria

The proprietary iron oxidizing bacteria used in this study were maintained in basal salt solutions at a low pH prior to their use in these experiments. One bacteria (Bacteria A)

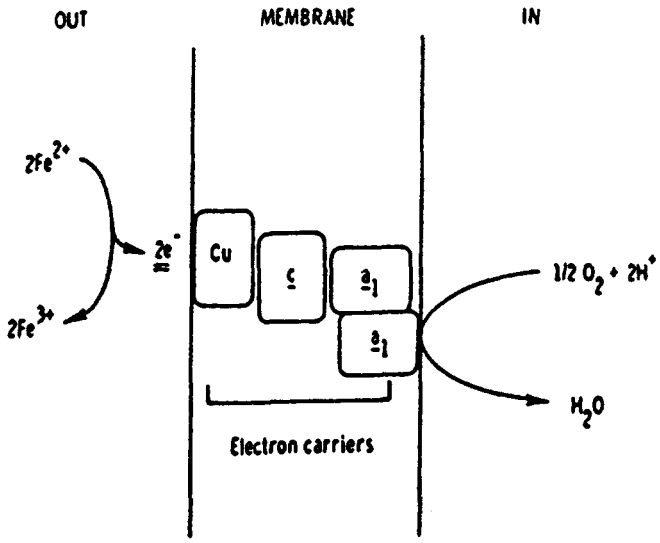

FIGURE 2 Components of the iron oxidase are identified by their prosthetic groups and are arranged from left to right in order of increasing redox potential (Cobley and Haddock, 1975; Ingledew and Cobley, 1980). "Out" and "In" refer to the bulk phase cytoplasm, respectively.

was grown in 9K media and the other bacteria (Bacteria B) was grown in a high pH nutrient media. These bacteria were also grown in a redox solution system for three to five days prior to use in a high pH media maintained at 25° to 45°C in a controlled temperature shaker bath. The composition of nutrient media is shown in Table 1. The iron oxidizing bacterial mixed cultures used in this study were initially obtained from American Type Culture Collection (ATCC), however, they were cultivated either in a high pH media or grown in the redox solution used for the hydrogen sulfide oxidation studies. The cultures were grown separately and then mixed and also were grown in the same media. The maximum cell growth typically occurred in 25 to 50 hours resulting in a cell density of 1.5×10^{11} cells/l in high pH media. The cell densities of 1.0 to 2.0×10^{11} cells/l were achieved in the redox system solutions. Cell densities of $(1.0-1.5) \times 10^{9}$ cells/l were used in the experiments carried out in the presence of the bacteria. Bacterial cell counts were determined using a Petroff-Hauser bacteria counter under a phase contrast microscope.

b. Gas Samples and Chemical

Synthetic sour gas samples used in this study were blended by Alphagaz Inc. of LaPorte, Texas. The synthetic sour gas had the following composition:

$$H_2S - 0.5\ (v/v)$$
$$CO_2 - 5\%\ (v/v)$$
$$N_2 - 94.5\%$$

Two types of commercial catalysts, Catalyst A and Catalyst B, were used in this study. These catalysts contain chelated ferric ion complexes. Precipitation of ferric hydroxide is prevented by chelating the ferric ions with organic chelates. Two types of chelates: type A, such as ethylene diaminetetraacetic acid (EDTA) or nitrilotriacetic acid (NTA) and type B, such as polyhydroxylated sugars keep the catalyst stable at any pH and were used in the commercial chelated catalyst formulations. This paper presents data using catalyst A only. All other chemicals used were obtained from Sigma Chemical Company.

TABLE 1 High pH Medium for Iron Oxidizing Bacteria

Composition per liter:	
$Na_2S_2O_3 \cdot 5H_2O$	10.0g
$Na_2HPO_4 \cdot 7H_2O$	7.9g
Sodium formate	6.8g
Glucose	3.6g
KH_2PO_4	1.5g
NH_4Cl	0.3g
$MgSO_4 \cdot 7H_2O$	0.1g
Trace metals solution	5.0mL
pH 7.6–8.5 at 25°C	

c. Experimental Procedure

The oxidation of hydrogen sulfide present in the synthetic sour gas blend was studied in a two-liter Virtis Omni Culture Bioreactor shown in Figure 3 [*17, 18*]. The hydrogen sulfide is readily oxidized by the chelated ferric ions present in the commercial catalyst A used in this study. The ferric ions (Fe^{3+}) are reduced to the ferrous state (Fe^{2+}) and hydrogen sulfide is oxidized to elemental sulfur. The elemental sulfur is removed by filtration or centrifuging and the ferric ion is regenerated by bubbling air through the reduced redox solution under controlled experimental conditions. The rate of hydrogen sulfide oxidation is a function of the pH, temperature, concentration of Fe^{3+} chelate, the gas/liquid ratio and the degree of agitation. These variables were carefully controlled and optimized. Likewise, the rate of ferric ion regeneration is a function of the pH of the redox solution, the temperature, the concentration of chelated iron, air to redox solution ratio and the degree of agitation. The progress of the reaction was monitored by measuring the concentration of Fe^{2+}, Fe^{3+}, pH, temperature, and redox potential of the reaction medium in the

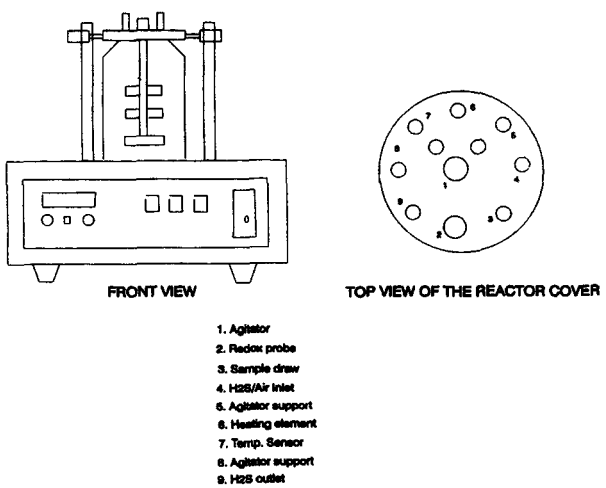

FIGURE 3 Omni culture bio-reactor.

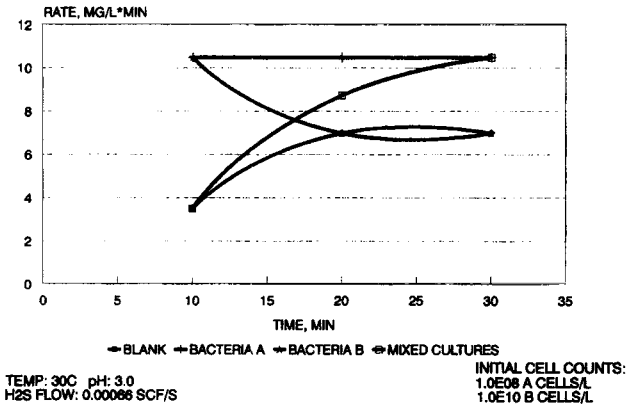

FIGURE 4 Comparison of H2S oxidation rates with and without bacteria.

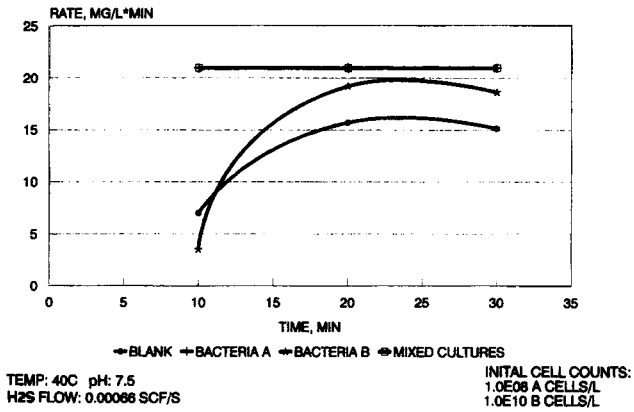

FIGURE 6 Comparison of H2S oxidation rates with and without bacteria.

Virtis Omni Culture Bioreactor. Two sets of experiments were conducted in each case, one in absence of bacteria (blank) and the other one in presence of a single bacteria or a mixed culture.

1. One-Cycle Experiments Using Commercial Chelated Catalysts. (Absence of Bacteria)—Baseline

A set of experiments was conducted at 30°C to 45°C and a pH varying from 3, 5 and 7.6 using 1000 ppm solution of commercial iron-chelate Catalyst A in absence of iron oxidizing bacteria (baseline). A cycle consists of oxidation of hydrogen sulfide by bubbling it through redox solution, filtration of elemental sulfur followed by reoxidation of ferrous ions with air. In a typical experiment, hydrogen sulfide was oxidized by passing the synthetic sour gas mixture through one liter of redox solution in Virtis Omni-Culture Bioreactor, elemental sulfur was centrifuged after each cycle and the redox solution was regenerated by bubbling air through it in absence of bacterial cells (blank run). The data on these experiments is shown in Figures 4 to 6. The redox solution regeneration rates were fairly constant, for a specific pH, temperature and gas to liquid ratio in the control (baseline) experiments and the quantity of elemental sulfur recovered ranged from 35 to 50% of the theoretical amount.

2. One Cycle Experiments Using Commercial Chelated Catalyst A in Presence of Iron Oxidizing Bacteria

In this set of one-cycle experiments the iron oxidizing bacterial cells of bacteria A, B or a mixed culture were used in the redox solution containing 1000 ppm of commercial chelated Catalyst A at 30°C to 45°C and a pH of 3.0 or 7.5. In a typical experiment, hydrogen sulfide was oxidized by bubbling the synthetic sour gas mixture through one liter of redox solution contained in the Virtis Omni-Bioreactor, elemental sulfur produced was filtered (not centrifuged) after each cycle and the redox solution was regenerated by bubbling air through the solution containing iron oxidizing bacteria or a mixed culture at a cell concentration of 1 to 7.5×10^9 cells/liter. The data are presented in Figures 4, 5 and 6 and compared with the baseline experimental data obtained in absence of bacteria. The data show a ferric ion regeneration rate enhancement of 50 to 150% and an increased production of elemental sulfur, 80 to 98% recovery as compared to 35 to 50% recovery in absence of bacteria. The data on rate enhancement is shown in Figures 7 and 8 and the data on sulfur recovery is given in Figures 9 and 10.

DISCUSSION OF RESULTS

The oxidation of hydrogen sulfide present in the synthetic sour gas mixture blended by Alphagaz of LaPorte,

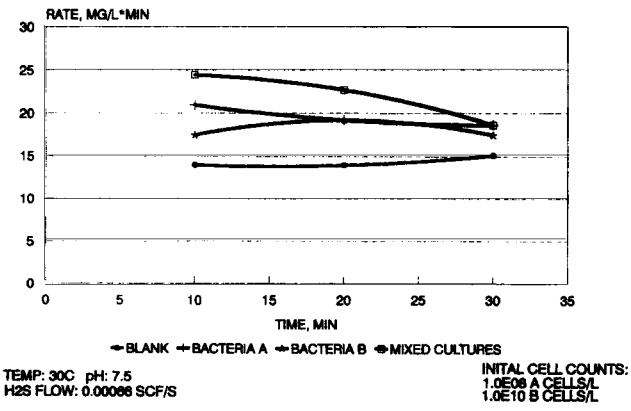

FIGURE 5 Comparison of H2S oxidation rates with and without bacteria.

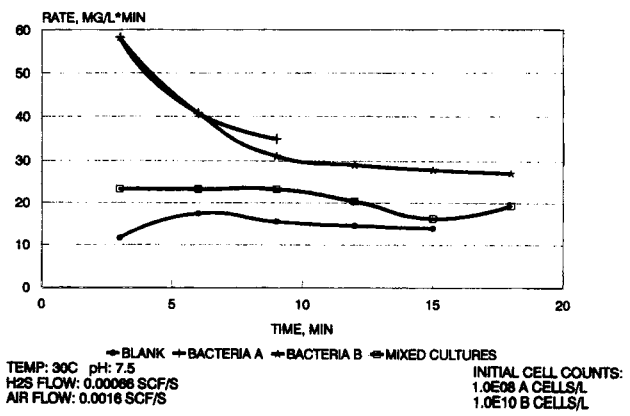

FIGURE 7 Comparison of regeneration rates with and without bacteria.

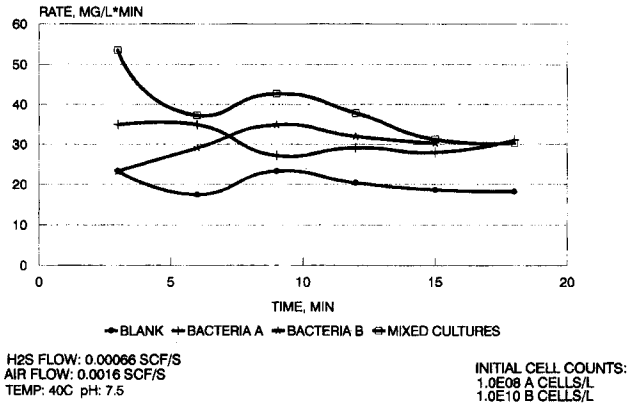

FIGURE 8 Comparison of regeneration rates with and without bacteria.

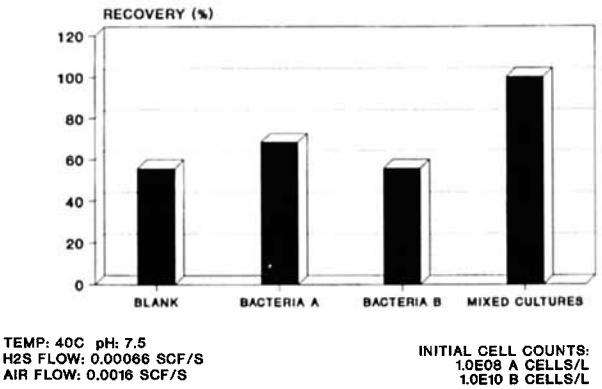

FIGURE 10 Comparison of sulfur recovery with and without bacteria.

Texas has been studied using a commercial chelated iron catalyst in a two-liter Virtis Omni-Culture Bioreactor. The rate of hydrogen sulfide oxidation was found to be primarily influenced by the pH, temperature, gas/liquid ratio and the concentration of iron chelate in the redox solution. Essentially all hydrogen sulfide was oxidized to elemental sulfur in the presence of commercial iron-chelate catalysts at a pH of 7.5 and 30 to 45°C. There was a 20% rate enhancement in hydrogen sulfide oxidation in the presence of mixed cultures.

The regeneration of the ferric ions in the chelated catalysts could be accomplished by bubbling air through the reduced chelated catalyst in the bioreactor. The air regeneration of the chelated ferric ions was dependent on the pH, temperature, air/redox solution ratio and the bacterial cell concentration. Single cycle experiments were carried out both in absence of iron oxidizing bacteria (blank), as well in presence of the bacterial cells. The ferric ion regeneration rates in the reduced redox solution were found to be 50% to 150% higher in presence of bacterial cells at typical cell density of 1 to 5×10^9 cells/l under optimum operating conditions. The data are presented in Figures 7 and 8 with a commercial chelated catalyst in one-cycle experiments.

The sulfur recovery was also studied in single cycle experiments. Invariably, 35 to 50% sulfur was recovered by centrifuging in the controlled blank runs, whereas in presence of mixed cultures of iron oxidizing bacteria the sulfur recovery ranged from 80 to 100% of the theoretical values. It was observed that filtration was the preferred technique for sulfur recovery in the presence of iron-oxidizing bacteria, since centrifuging affected the bacterial cell densities in the redox system. The sulfur recovery data for one-cycle experiments are shown in Figures 9 and 10.

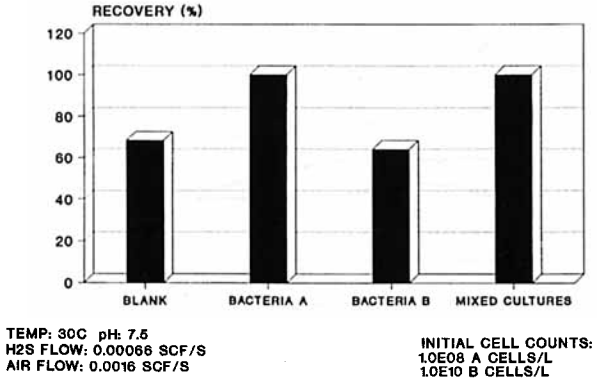

FIGURE 9 Comparison of sulfur recovery with and without bacteria.

CONCLUSIONS

One of the bacterial strain (Bacteria A) was readily grown in 9K media and the other iron-oxidizing bacteria (Bacteria B) used in this study was grown in a high pH medium containing trace metals. Cell densities as high as 2×10^{11} cells/l could be achieved in twenty to fifty hours. Moreover, the high pH medium could be easily replaced by the used redox solution of the commercial catalyst evaluated in this study without adversely affecting the growth characteristics and the bacterial cell densities of the iron-oxidizing bacteria and the mixed cultures.

These experiments conclusively show that in the presence of iron oxidizing bacteria, Bacteria A, or B or the mixed cultures, the rates of hydrogen sulfide oxidation are enhanced by about 20%, the ferric ion reoxidation rates in the redox system of the commercial chelated redox catalyst are enhanced by 50% to 150% as compared to blank runs in absence of bacterial cells at an operating pH of 7.5 and the redox solution temperature varying from 30° to 45°C. Moreover, the iron oxidizing bacteria also induce higher elemental sulfur recoveries ranging from 80 to 100% of theoretical as compared to 35 to 50% in absence of bacteria.

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