Microbial Sweetening of Low Quality Sour Natural Gas

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ABSTRACT

About 25 % of natural gas produced in the United States is contaminated with hydrogen sulfide (H_2S) and other contaminants which exceed pipeline specifications of 4 ppmv of sulfur content and the 1990 Clean Air Act Amendments also require its reduction. This paper describes the research effort to remove H_2S from low quality sour natural gas by employing a liquid redox process. Aqueous solution of chelated ferric ions oxidize the hydrogen sulfide to elemental sulfur. The reduced chelated iron is then reoxidized by contact with air and recycled. This requires expensive equipment for regeneration, costly chemicals that degrade readily and the process is usually energy intensive.

Recent studies by Rai et al show that the ferric ion regeneration rates are substantially enhanced in presence of acidophilic bacteria. The objectives of this project jointly supported by the U.S. Department of Energy and the Gas Research Institute 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 such as the LO-CAT II and SulFerox.

Since the inception of the project on June 1, 1994, the oxidation of a synthetic sour gas blend containing 0.50 vol % hydrogen sulfide, 5.0 vol % carbon dioxide and 94.5 vol %

nitrogen was studied. Fe^{3+}•EDTA and LO-CAT 310, a commercial chelated iron catalyst manufactured by Wheelabrator Clean Air Systems, Inc. (WCAS) were used for hydrogen sulfide oxidation and ferric chelate reoxidation by air. LO-CAT 310 contains Fe^{3+}•NTA and sorbitol as the active chelating agents. The process conditions for oxidation of hydrogen sulfide and regeneration of Fe^{3+}•chelate were optimized using Fe^{3+}•EDTA and LO-CAT 310 as outlined in TASKS 1 and 2 of the project. These studies were conducted both in absence of bacterial cultures (blank) and in presence of cultures of Thiobacillus ferrooxidans (T.f.) and Leptospirillium ferrooxidans (L.f.) and mixed cultures (L.f. , T.f.) under various experimental conditions. It was observed that in the presence of these cultures, the hydrogen sulfide oxidation rate was enhanced from 30 to 50% whereas ferric ion regeneration was enhanced by 50 to 150 % as compared to blank experiments having no bacterial cultures.

Thiobacillus ferrooxidans, ATCC # 23270, was grown in high pH medium and used in GRI sponsored studies. The high pH T.f. culture was registered with American Type Culture Collection as Thiobacillus ferrooxidans, ATCC # 55720. Growth studies with T.f. ATCC # 23270 and T.f. ATCC # 55720, were conducted in high pH medium and ARI 310 redox solution both in absence and in presence of glucose and minimal salts solution. There was a substantial increase in growth rate and cell density in presence of glucose and minimal salts solution. The growth characteristics of Leptospirillium ferrooxidans (L.f.) using two strains of L.f. ATCC # 29047 and ATCC # 49881 and Chilean Culture were also studied. The conditions for maximum growth rate for L.f. were established.

Degradation of ARI 310 and pure sorbitol was studied using HPLC in presence of T.f. ATCC # 23270 and T.f. ATCC # 55720, T.a. ATCC # 27807, Leptospirillium ferrooxidans ATCC # 49881 and Chilean Cultures. It was observed that sorbitol and ARI 310 were not degraded by T.a. ATCC # 27807, L.f. ATCC # 49881 or the Chilean Cultures. However T.f. ATCC # 23270 and T.f. ATCC # 55720 readily degraded sorbitol and ARI 310 which contains sorbitol. Attempts were made to isolate the Heterotroph Isolate from T.f. ATCC # 55720 using agarose plates and the isolated contaminant was grown in a nutrient medium. The Heterotrophic Isolate was evaluated for its ferrous oxidizing capability and was found to be incapable of oxidizing ferrous ions. The Iron Oxidizing Isolate from T.f. ATCC # 55720 grown on Fe•EDTA rich medium was found to be very effective for H_2S oxidation and ferric ion regeneration.

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EXECUTIVE SUMMARY

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This project is jointly funded by the U.S. Department of Energy and the Gas Research Institute. Texas A & M University is providing the computer, library and laboratory facilities in support of this project. A sub-contract to Illinois Institute of Technology is aimed at studying the morphological purity of *T.f.* ATCC # 23270., *T.f.* ATCC # 55720 and *L.f.* ATCC # 49881 cultures grown in high pH media and the kinetics of sulfide conversion in presence of these cultures.

INTRODUCTION

About 15 % of the natural gas produced in the United States is sour containing significant volumes of hydrogen sulfide. Liquid redox processes are very popular for sweetening of sour natural gas and they use a chelated iron redox solution for the oxidation of hydrogen sulfide present in sour natural gas. Chelated ferric ion redox solutions such as Fe^{3+}. NTA, Fe^{3+}. EDTA, and commercial catalysts such as : ARI 310 and ARI 340 based on such chelates completely oxidize hydrogen sulfide to elemental sulfur in the pH range of 7 to 8.5. The ferrous ions formed during this reaction can be oxidized to the ferric state with oxygen absorbed from a stream of air bubbled through the redox solution.

Thiobacillus ferrooxidans is capable of deriving its energy for growth by oxidizing ferrous ions to the ferric state. These microbes can oxidize ferrous ions to ferric ions 500,000 times faster than the purely chemical oxidation in the absence of bacteria. [Lacey and Lawson, 1970]. The regeneration of ferric ions in the presence of the iron-oxidizing bacteria under mild conditions 25 - 40 ^{o}C, and atmospheric pressure would minimize the chelate degradation process and thus help in improving the economics of hydrogen sulfide oxidation in natural gas sweetening process. [Rai and Rao, 1995]. The present investigation was undertaken to investigate the ferric iron rate enhancement and to characterize the growth of *T.f.* in such high pH environment.

This study was sponsored by Gas Research Institute . During the course of investigation a US Patent entitled, “Regeneration of Liquid Redox Systems using *Thiobacillus ferrooxidans*” (US Patent # 5,508,014) has recently been issued [Rai, 1996]. This patent is

assigned to GRI. It was observed that a commercial redox catalyst, ARI 310, was partially degraded, whereas ARI 340 was not affected. This led to the discovery that *T.f. # 23270* obtained from the American Type Culture Collection contained a heterotrophic contaminant that degraded hexitol present in ARI 310. The isolation of the iron-oxidizing and heterotrophic components present in ATCC T.f. # 23270 cultures is described in the paper presented at the 1997 Spring National Meeting of AIChE entitled, "Isolation of *Thiobacillus ferrooxidans* from a Mixed Culture of *T.f.* ATCC # 23270" by C. M.. Diaz, J. M. Pierce and C. Rai, 1997.

This paper describes the growth of *Thiobacillus ferrooxidans # 23270* in high pH medium, determination of *T.f.* growth parameters and experiments to show the effectiveness of *T.f.* # 23270, *T.f.* # 55720, *T.f.* 55720 *Isolates*, both ferrous oxidizing and non iron-oxidizing heterotrophic isolate for hydrogen sulfide oxidation as well as ferric ion regeneration. Use of iron-oxidizing microbes in redox processes is being investigated for improving the overall gas processing economics.

Redox Process Chemistry

The redox process involves in contacting an iron aminopolycarboxylic acid chelate with the sour gas stream where the following reactions take place :

$$H_2S + OH^- \leftrightarrow HS^- + H_2O$$

$$HS^- + OH^- \leftrightarrow S^{2-} + H_2O$$

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

The colloidal sulfur produced is probably S_8 originating through polysulfide intermediates. The ferrous chelate can then be regenerated by air oxidation according to the following reaction :

$$2Fe^{2+} + 0.5O_2 + H_2O \rightarrow 2Fe^{3+} + 2\ OH^-$$

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 is prevented by using a chelating agent. A chelating agent or chelant is a special type of ligand that contains more than one electron donor group. It competes with the sulfide or hydroxide to form a complex with the iron. The most commonly used chelating agents are aminopolycarboxylic acids such as nitrilotriacetic acid (NTA), ethlylenediaminetetracetic acid (EDTA) and hydroxyethylethylenediaminetriacetic acid (HEDTA). Sometimes polyhydroxylated sugars, such as sorbitol are used for improving the catalyst stability in high pH environment.

Mechanism Of Iron Oxidation

The mechanism of iron oxidizing ability of *T. f.* has been postulated by John Ingledew [Ingledew, 1986] as consisting of an iron-oxidase system, a membrane-bound enzyme complex that spans the cytoplasmic membrane of the organism. The Fe^{2+} oxidizing portion of the respiratory chain is short, consisting of only four redox proteins, namely rusticyanin (a copper protein) and three cytochromes. Figure 1 shows a model proposed by Ingledew to explain the process of electron transfer to oxygen which is coupled to oxidative phos phorylation in *T. f.* The electron transfer components are organized in

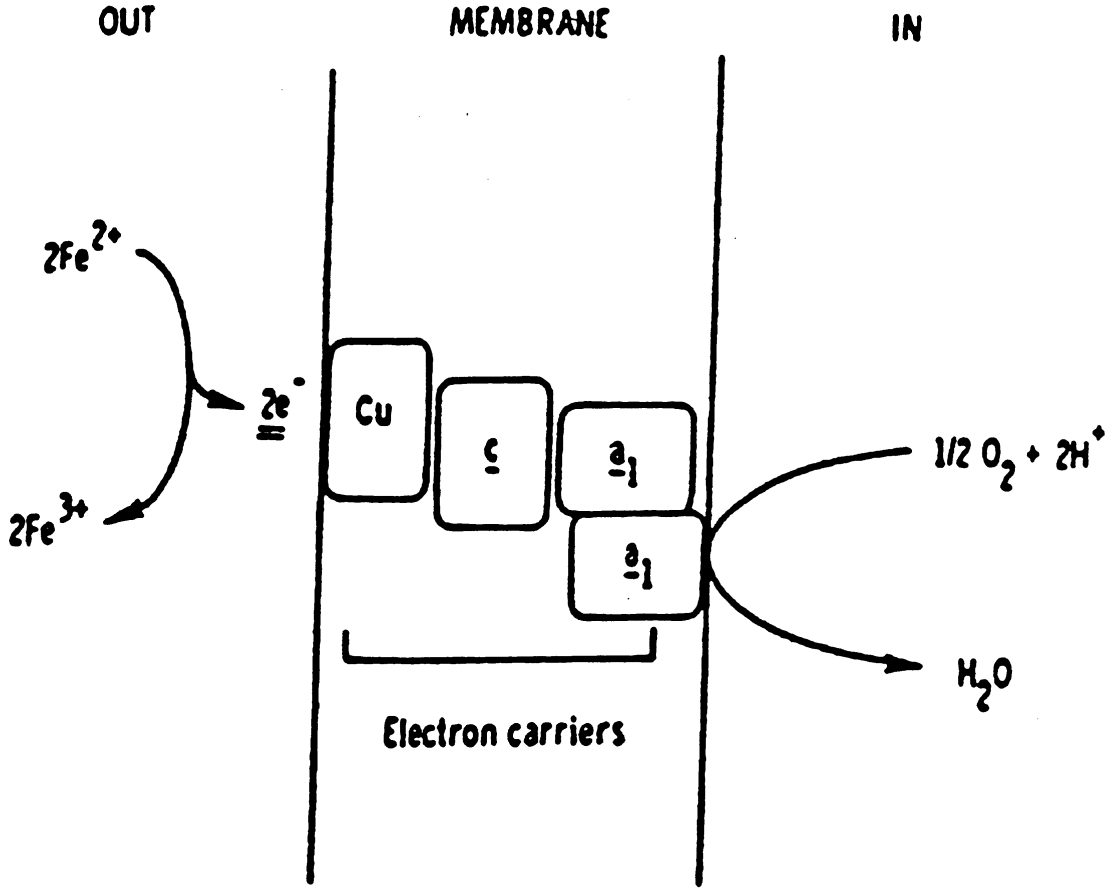


Figure 1 : Components of the Iron Oxidase are identified by their prothetic groups and are arranged from left to right in order of increasing redox potential [Cobley and Haddock, 1975; Ingledew and Cobley, 1980]. "Out" and "In" refer to the bulk phase and cytoplasm, respectively.

the cytoplasmic membrane in such a fashion as to couple Fe^{2+} oxidation to the generation of a transmembrane proton electrochemical potential (ΔP),which has been measured to be 250 mV. In the iron-oxidase complex, the copper protein, rusticyanin is thought to be the initial acceptor of electrons from Fe^{2+} . The midpoint potential (E_m) of rusticyanin has been measured to be 680 mV at pH 3.2 . During the growth of *T.f.* the electrical potential (E_h) of Fe^{2+} /Fe^{3+} couple has been found to increase from approximately 500 mV to 800 mV as Fe^{2+} is oxidized by the cells.

Growth of *T.f.* has also been observed at low substrate concentrations. Two theories have been postulated to explain Fe^{2+} ion oxidation, and hence growth, at lower substrate concentrations, particularly in a glucose media containing small amounts of Fe^{2+} concentrations. These are as follows: (1) The presence of binding proteins in the membrane, or periplasmic space, which interact with rusticyanin and effectively scavenges Fe^{2+} from the growth medium and (2) A reaction is observed between iron and rusticyanin by monitoring EPR signals of the copper center and by monitoring oxidation/reduction of rusticyanin spectrophotometrically.

Reaction :

$$\text{rusticyanin } (Cu^{2+}) + Fe^{2+} \text{ rusticyanin } \rightarrow (Cu^{+}) + Fe^{3+}$$

They conclude that the growth of *T.f.* is limited when the Fe^{2+} concentration of the growth medium falls below the level that reacts sufficiently with rusticyanin.

Kinetics of Iron Oxidation

The growth of *Thiobacillus ferrooxidans* in batch cultures has been found to be logarithmic and the rate of growth is given by

$$\frac{dN}{dt} = \mu N \qquad (1)$$

where N is the number of cells per liter, μ (hr^{-1}) is the specific growth rate, and t is the time. The rate of iron oxidation is also found to be directly proportional to the growth rate.

$$\frac{dN}{dt} = -\frac{dP}{dt} = -\frac{1}{Y}\frac{dN}{dt} \qquad (2)$$

where S and P are the concentrations of ferrous and ferric ions, respectively and Y is the growth yield of cells produced per gram of iron oxidized. Assuming the Y is constant, equation (2) can be integrated to get

$$N = Y\,(P - P_o) + N_o \qquad (3)$$

where P_o is the initial product concentration and N_o is the initial cell concentration. The specific growth rate is a function of limiting substrate concentration (i.e. Fe^{2+} ion concentration), temperature and pH. This occurs in batch culture if the initial concentration of ferrous iron is small. Based on the above discussed mechanism of iron oxidation, the specific growth rate can be represented by the Michaelis-Menten equation :

$$\mu = \mu_{max}\left[\frac{S}{K_S + S}\right] \qquad (4)$$

where μ_{max} (hr^{-1}) is the maximum specific growth rate and K_S (g/l) is the saturation constant. From equations (1), (2) and (3) the rate of product formation is given by

$$\frac{dP}{dt} = \mu (P - P_o + N_o/Y) \qquad (5)$$

The specific growth rate is calculated by determining dP/dt as a function of time over the growth phase of the bacteria. The accuracy in measuring P is more than that of N and thus equation (5) is more useful that equation (1) in determining μ. This method however assumes that there is no lag between the iron oxidation and the growth of the organism and Yield, Y is independent of time. Thus, in this work Y was averaged over the growth phase of the cells and this average value was used in equation (5). The μ obtained from equation (5) was used in the Lineweaver-Burk equation

$$\frac{1}{\mu} = \frac{K_S}{\mu_{max}} \frac{1}{S} + \frac{1}{\mu_{max}} \qquad (6)$$

The slope (K_S/μ_{max}) and intercept $1/\mu_{max}$ were determined by linear regression of the plot of $1/\mu$ against 1/S.

RESULTS AND DISCUSSION

Materials And Methods

Bacterial Cultures

T.f. is gram negative, rod shaped bacterium and is obligately autotrophic. It derives energy for its growth from the oxidation of reduced sulfur compounds and from the oxidation of Fe^{2+} to Fe^{3+} ions in the presence of air. It is iron-oxidizing with an optimum pH within the range of 1.5 to 2.5 but has shown successful growth in a more alkaline environment in the pH range of 7 to 8.5 [Rai and Rao, 1995].

Pure cultures of *Thiobacillus ferrooxidans* (ATCC 23270) and (ATCC 55720) were obtained from American Type Culture Collection (ATCC). They were maintained by serial transfers into fresh growth media every week. The cells were maintained into two media, a ferrous sulfate based low pH basal salt solution (64K) and a high pH nutrient media. *T.f.* # 23270 and *T.f.* # 55720 were also grown in high pH Fe.EDTA rich medium. Shaker baths maintained at 30^{o}C and 53^{o}C were used for bacterial growth studies.

The growth rate of bacteria was established by counting cells from the cultures with a Petroff-Hausser counting chamber under a phase contrast microscope at 20X and 40X magnifications at definite time intervals. Total number of cells per milliliter of the culture were calculated by using a factor of 2×10^{7} to the observed cell count. A logarithmic growth rate was established. The maximum cell growth typically occurred in 25 to 35 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 x 10^{11} cells/l were achieved in the redox system solutions. Cell densities of 1.0 to 1.5 x 10^{9} cells/l were used in experiments carried out with the bacteria.

The ability to obtain and maintain pure cultures of a microorganism is a prerequisite fundamental for research purposes. This is very much true for studies involving *Thiobacillus ferrooxidans* because of their vulnerability to contamination from various iron-oxidizing heterotrophs. Hence all flasks and equipment used in the growth of *Thiobacillus ferrooxidans* were sterilized by autoclaving at 132 ^{o}C and 20 psig for 15 minutes. The deionized water used for the preparation of the media was filter sterilized using 0.2 μm, 250 ml Nalgene filters.

Preparation of Media

High pH nutrient media was used throughout this work. The ferrous sulfate was added as the sole source of energy. Composition and preparation of the high pH media consisted of the following compounds (gram per liter) : $Na_2S_2O_3$ $5H_2O$ 10; Na_2HPO_4 $7H_2O$ 7.9; sodium formate 6.8; glucose 3.6; KH_2PO_4 1.5; NH_4Cl 0.3, $MgSO_4$ $7H_2O$ 0.1, trace metal solution 5 ml. These components in the stated quantity were added to de-ionized water till the volume was brought up to 1 litter. Its pH was adjusted to 7.6 - 8.5. It was filter sterilized and asceptically distributed into sterile tubes or flasks.

The composition of the trace metal solution was as follows (grams per liter) : disodium EDTA 50; NaOH 11; $CaCl_2$ $2H_2O$ 7.2; $FeSO_4$ $7H_2O$ 5; $MnCl_2$ $2H_2O$ 2.5; $ZnSO_4$ $7H_2O$ 2.2; $CoCl_2$ $6H_2O$ 0.5; $(NH_4)_6Mo_7O_{24}$ $4H_2O$ 0.5; $CuSO_4$ $5H_2O$ 0.2. To prepare the trace

metal solution disodium EDTA was added to de-ionized water till the volume was brought up to 1 liter. The pH was adjusted to 6.0 with NaOH. The remaining components were added one by one. After complete dissolution of all the salts, the pH was adjusted to 4.0 with HCl. Then it was filter sterilized and stored at 4 ^{o}C.

High pH Fe·EDTA media used in this study has the following composition (grams per liter) : $Na_2HPO_4 \cdot 7H_2O$ 8; sodium formate 7; KH_2PO_4 1.6; NH_4Cl 0.4; $MgSO_4$ 0.1; $FeSO_4$ 22.11; EDTA 26.6; These components in the stated quantity were added to de-ionized water till the volume was brought up to 1 litter. Its pH was adjusted to 7.6 - 8.5. It was filter sterilized and asceptically distributed into sterile tubes or flasks.

Gas Samples

The synthetic sour gas cylinders were purchased from Air Liquide and had following composition :

H_2S : 0.5 vol. %

CO_2 : 5 vol. %

N_2 : 94.5 vol. %

The chelated iron catalyst, ARI 310, is a proprietary product and was provided by Wheelabrator Clean Air Systems, Inc. The remaining chemicals were purchased from Fisher Scientific Company.

Analytical Procedures

Ferrous iron and the total iron concentrations in the media and the chelated catalyst solutions were determined by volumetric analysis and verified with atomic absorption spectroscopy. The ferric content was determined by the difference of ferrous and total iron.

In the volumetric analysis the samples were titrated against a standard (0.025 N) potassium dichromate with di-phenylamine sulfonate as the indicator. The end point is very sharp and stable. A standard procedure for the estimation of total iron is described by Roland Young [Young, 1971]. The total iron was also estimated using a Perkin Elmer 3100 Atomic Absorption Spectrophotometer operated at 248.5 nm wavelength, 0.2 nm slit, 25amp lamp current and 30 second reading cycle. The iron was estimated by the following relationship :

$$1 \text{ ml of } 0.025 \text{ N } K_2Cr_2O_7 = 0.001396 \text{ g Fe}$$

Experimental Procedure

A two liter Virtis Omni-Culture Bioreactor was used in the oxidation of H_2S and the subsequent regeneration of the catalyst. It consists of a temperature sensor with a heating element and a compressed air supply unit. Adequate mixing is achieved by a magnetic agitator. The stirring rate can be varied from a low rpm to 600 rpm. Typically the experiments were carried out at 300 rpm. The reactor vessel and the agitator were autoclaved at 133^{o}C and 20 psig for 15 minutes prior to each experiment to avoid contamination of the catalyst solution.

Experimental runs were carried out at 30^{o}C to 45^{o}C, a pH of 8.5 and at optimized total iron concentration and air flow rates. All the experiments were conducted on a batch basis. In the first step hydrogen sulfide from the synthetic sour gas blend was passed through the chelated iron catalyst , when the ferric ion (Fe^{3+}) is reduced to the ferrous ion (Fe^{2+}) and the hydrogen sulfide is oxidized to elemental sulfur. In the second step the solution was regenerated by bubbling air through the reduced redox solution under controlled experimental conditions. The elemental sulfur produced was removed by filtration.

The rate of hydrogen sulfide oxidation is a function of the pH, temperature, concentration of Fe^{3+} chelate, concentration of oxidant, the gas/liquid ratio, and the degree of agitation. These variables were carefully controlled and have been optimized. Similarly, the rate of ferric ion regeneration is a function of the pH of the redox solution, the temperature, concentration of the chelated iron, air to liquid ratio and the degree of agitation. The progress of the reaction was monitored by measuring the concentration of Fe^{2+}, Fe^{3+}, pH, temperature of the reaction mixture in the reactor.

Oxidation of Hydrogen Sulfide

1. Baseline Experiments Using ARI 310 for the Oxidation of Hydrogen Sulfide

A set of one cycle experiments using one liter solution of the commercial iron chelate catalyst ARI 310, at a total iron concentration of about 1000 mg/l was carried out in the absence of bacteria (baseline) at a fixed pH, and a H_2S gas flow rate of 0.00066 scf/s. A two liter Virtis Omni-Culture Bio-reactor containing about one liter of the redox cata-

lyst was used. The temperature and the agitation rate were set at a fixed temperature and 300 rpm respectively. The ferrous, ferric iron and the outlet hydrogen sulfide gas concentrations were determined at every 10 minute intervals. At the end of 30 minutes the pH was readjusted to initial pH by the addition of 10N NaOH. The reduced catalyst solution was regenerated by bubbling air through it at a rate of 0.0138 scf/s. The rate of regeneration was estimated by the difference in ferrous iron content at every three or four minute interval.

2. Microbial Experiments Using ARI 310 and T.f.# 23270 Culture

The baseline (blank) experiments were followed by an identical set of one cycle experiments using one liter solution of redox catalyst ARI 310 under the same conditions of pH, temperature, total iron concentration, gas and air flow rates in the presence of bacteria. The bacteria was used at cell concentrations ranging from 10^{09} to 10^{11} cells/L. The same-experimental procedure and analytical techniques were employed. The following bacterial cultures were studied in these experiments:

1. *T.f. ATCC # 23270*
2. *T.f. ATCC # 55720*
3. *Isolated T.f. 55720* (isolated from *T.f. 55720* and grown in Fe.EDTA medium)
4. *Isolated T.f. 55720* (isolated from *T.f. 55720* and grown in a nutrient broth)

DISCUSSION

Growth Kinetics of *T.f.* 23270 in High pH Medium

1. *Growth of T.f. 23270 in High pH Medium*

The bacterial cultures were grown using a constant temperature bath. The growth medium was inoculated with the appropriate volume of culture in 250 ml conical flasks. It was observed that peak growth of *Thiobacillus ferrooxidans 23270* cells in high pH (Thiobacillus Media) was achieved in 30 hours with a cell concentration of 5.0 x 10^{11} cells/l at a pH of 7.6 and at 35^{o}C. Whereas at 30^{o}C and a pH of 8.4, the peak growth was achieved in 42 hours with a cell concentration of 8.0 x 10^{10} cells/l as shown in Fig 2. This growth curve was obtained by inoculating fresh medium with *T.f. ATCC # 23270* culture having a cell concentration of 5 x 10^{8} cells/l.

2. *Growth of T.f. 23270 in ARI 310 and ARI 340*

T. f. 23270 cells were also grown in redox solutions such as ARI 310 and ARI 340. This approach was adopted because *T. f.* cultures are to be used in redox systems such as ARI 310 and ARI 340. When *T.f.* 23270 was grown in ARI 310 at pH of 7.7 and at 50^{0}C , it achieved a peak growth after 12 hours with a maximum cell concentration of 2.42 x 10^{11} cells/l. When it was grown in ARI 340 at pH of 7.7 and at 50^{0}C, it achieved a peak growth after 15 hours with a maximum cell concentration of 2.25 x 10^{11} cells/l . The Fe^{2+} concentration was recorded and the maximum specific growth rate ($æ_{max}$, hr^{-1}) and the saturation constant (K_S ,g/l) were determined from the Lineweaver Burk plot. Typical data for the growth of *T.f.* 23270 in ARI 310 (Table 1), and the Lineweaver Burk Plot used to determine the maximum specific growth rate and the saturation constant are presented.in Fig. 3.

The maximum specific growth rate for *Thiobacillus ferrooxidans* in various media used by previous investigators and the data from this study using ARI 310 and ARI 340 media are reported in Table 2. The maximum specific growth varied from 0.15 to 0.3 hr^{-1} and the saturation constants varied from 1 to 5 g/l.

Figure 2 : Growth Curve of *T.f.* 23270
(Thiobacillus Media)

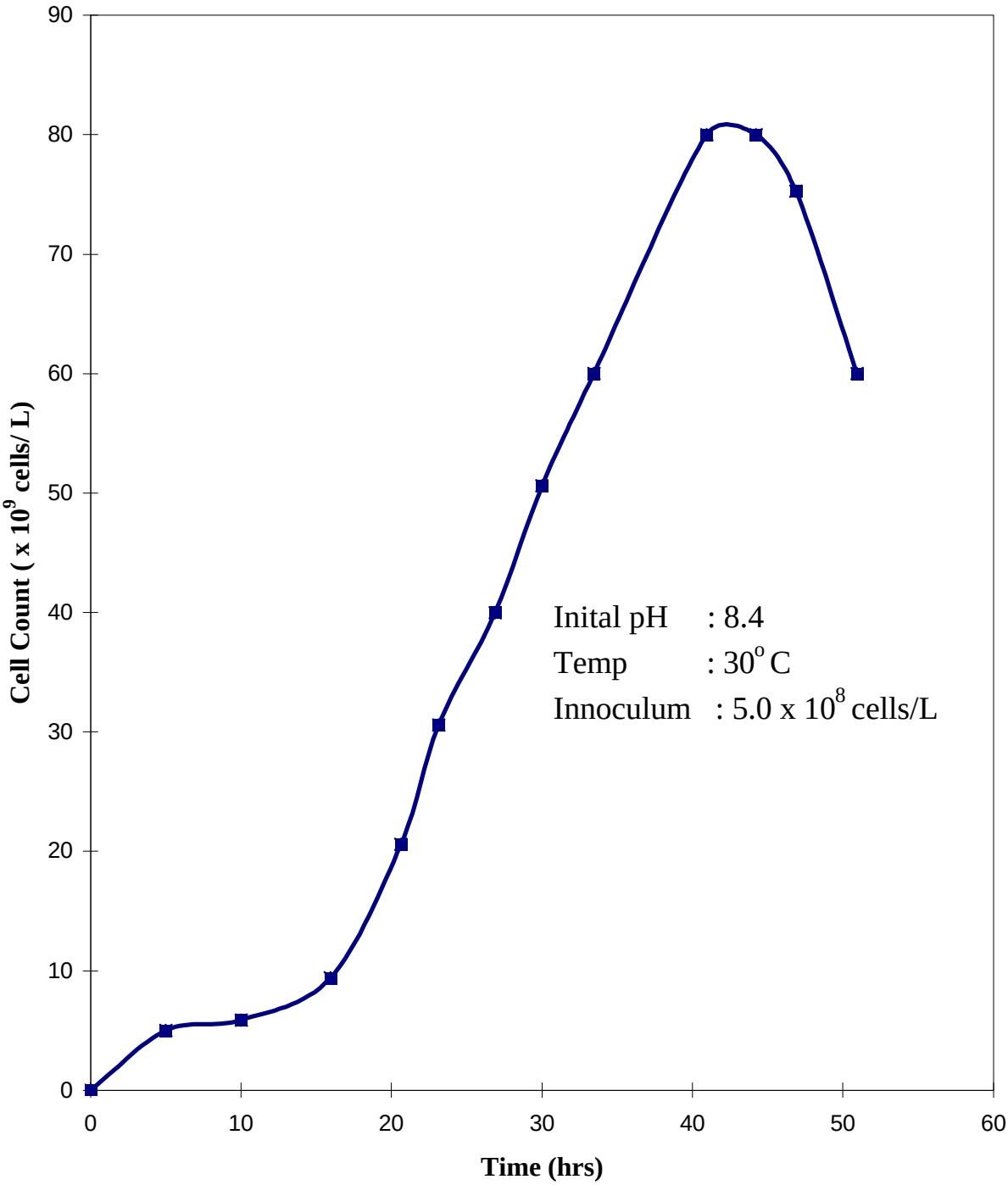


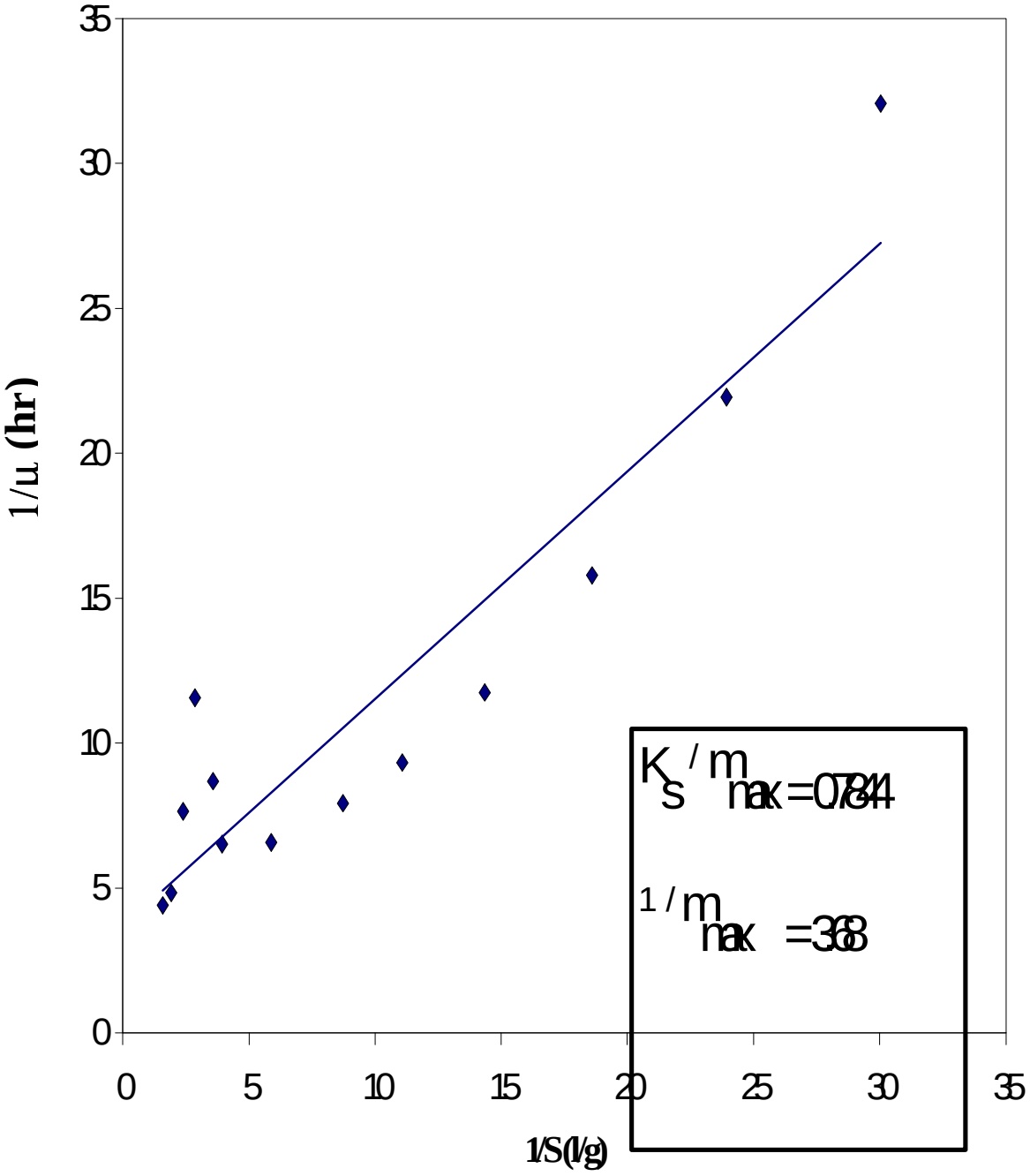
Table 1 : Kinetic Data For the Growth of *T.f. 23270* in ARI 310 Media (Temp = 50^0C, pH =7.7, Total iron = 0.97734 g/l, Innoculum = 1.96 x 10^{12} cells/l)

Time (hr)	Fe(II) (g/l)	Fe(III) (g/l)	Cell conc. (x10^{10} cells/l)
0	0.8377	0.1396	6.52
2	0.76791	0.2094	7.52
4	0.6283	0.349	8.0
6	0.4188	0.5583	10.3
8	0.2792	0.6981	15.6
10	0.06981	0.90753	20.1
12	0.02 (e)	0.92734	24.2
14	0.005 (e)	0.95234	23.5
16	0.0001(e)	0.96734	20.2

Table 2 : Comparison of Specific Growth Rates at Low and High pH

Media	Temp (^{o}C)	pH	μ_{max} (hr^{-1})	Source
3K	40	2.0	0.029	Macdonald & Clark (1970)
3K	30	2.0	0.110	Macdonald & Clark (1970)
9K	31	2.0 - 2.3	0.25	Lacey & Lawson (1970)
$FeSO_4$ based mini-mal medium	30	1.9	0.12	Barraon and Lueking (1990)
9K	30	2.0-2.5	0.183	Shrihari et al. (1990)
64 K	21	3.5	0.0784	Gokhran (1993)
64 K	21	2.9	0.152	Gokhran (1993)
LO-CAT 310	40	7.65	0.239	This study
LO-CAT 310	50	7.7	0.272	This study
LO-CAT 340	40	7.65	0.191	This study

Figure 3: Lineweaver Burk Plot for the Growth of *T.f.* 2327O Culture (Table 1)



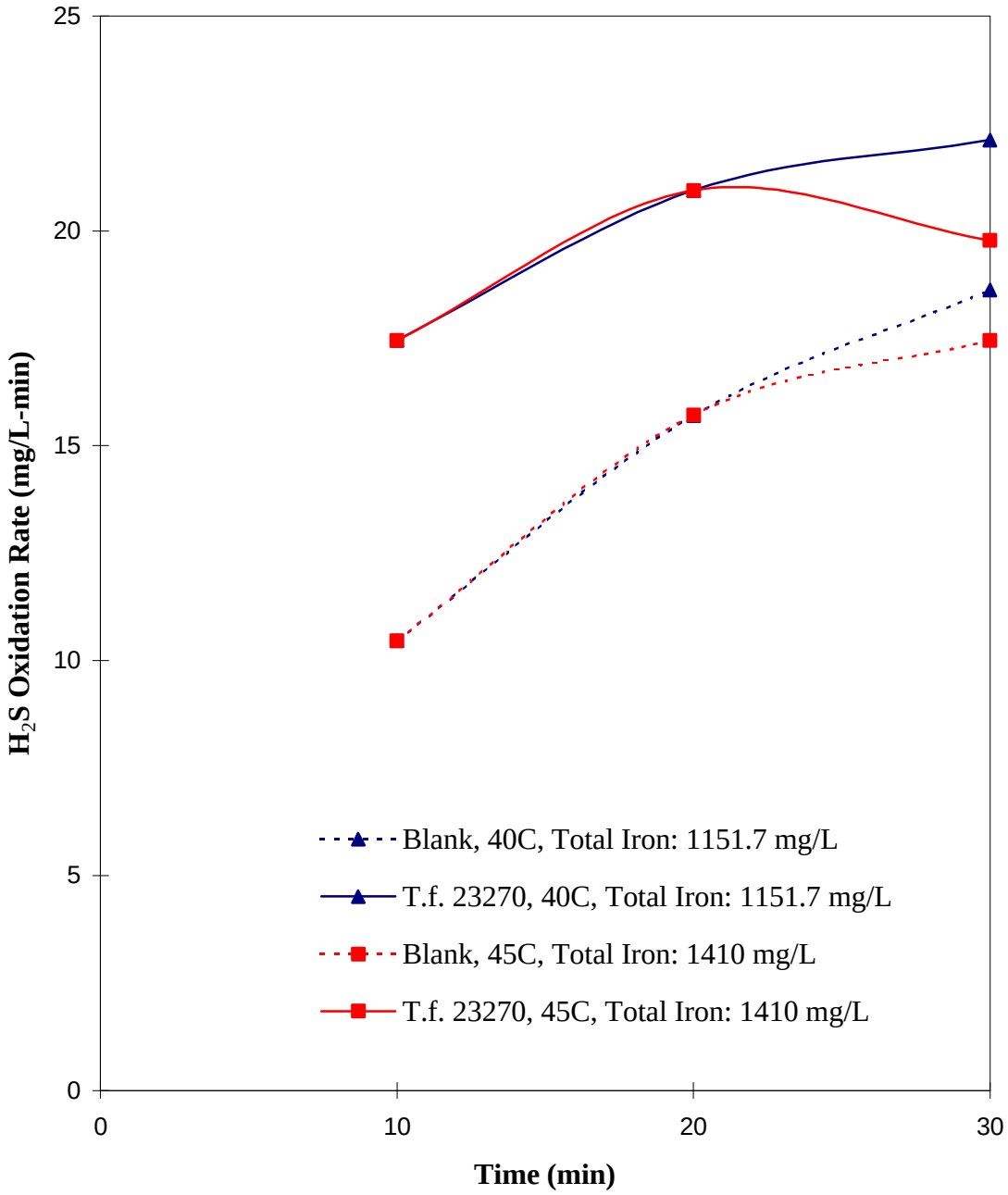
Oxidation of Hydrogen Sulfide Using *T.f. 23270 and T.f. 55720*

The oxidation of H_2S present in the synthetic gas blend was studied using ARI 310 in a two liter Virtis Omni Culture Bioreactor. The rate of oxidation was found to depend on pH, temperature and the concentration of the iron chelate in the redox solution. It was observed that the H_2S oxidation rates improved substantially at a pH of 8.5 and temperature 40^oC in the presence of the bacteria, *T.f. 23270* and *T.f. 55720*, by 28.4% and 103% respectively as compared to the baseline (blank) experiments. Likewise the H_2S oxidation rates for experiments at 45^0C and a pH of 8.5 also improved substantially in presence of the bacteria, with *T.f. 23270* by 33.3 % and with *T.f. 55720* by 86 % as compared to blank experiments in absence of any cultures. The oxidation rate enhancement in presence of bacterial cultures is shown in the figures 4 and 5.

Regeneration of Ferric Ions Using *T.f. 23270 and T.f. 55720*

The regeneration of the ferric ions in the reduced catalyst was achieved by bubbling air through it. The regeneration rate was influenced by pH, temperature and the bacterial cell density. The ferric ion regeneration in the reduced redox solution was enhanced by 56.6 % and 59.1 % as compared to the baseline (blank) experiments in the presence of *T.f. 23270* and *T.f. 55720* respectively at a pH of 8.5 and temperature of 40^oC. Whereas at a pH of 8.5 and 45^o C the regeneration rates improved by 22.1% and 112% in the presence of the bacteria, *T.f. 23270* and *T.f. 55720* respectively. The regeneration rate data are shown in Figures 6 and 7.

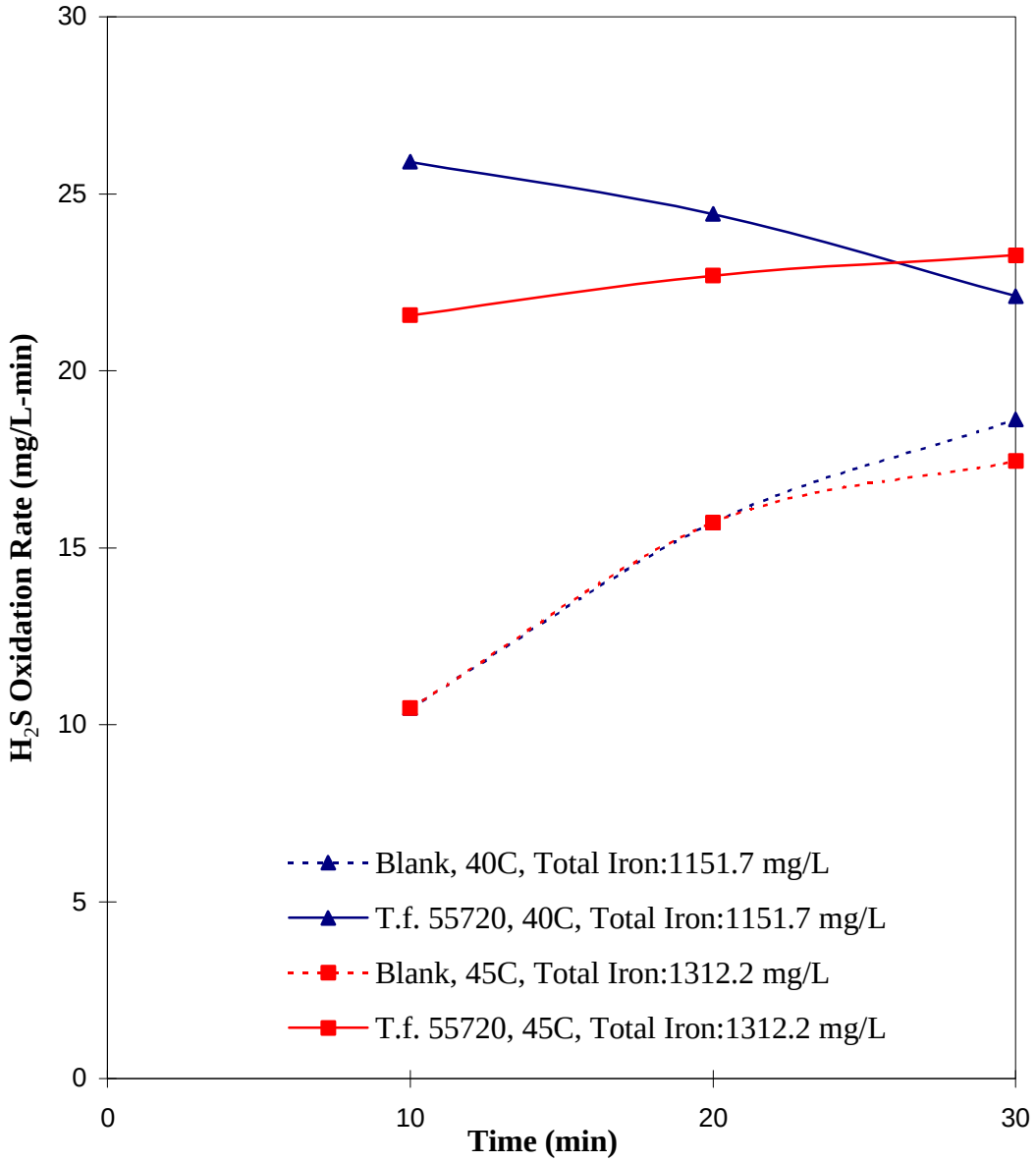
Figure 4 : Comparison of H_2S Oxidation Rate (BLANK v/s *T.f. 23270*)



pH: 8.5 Initial H_2S: 0.5%

BACTERIA HISTORY : Culture from ATCC,
Grown in High pH EDTA,
Temperature: 37C, pH: 7.5-8.0

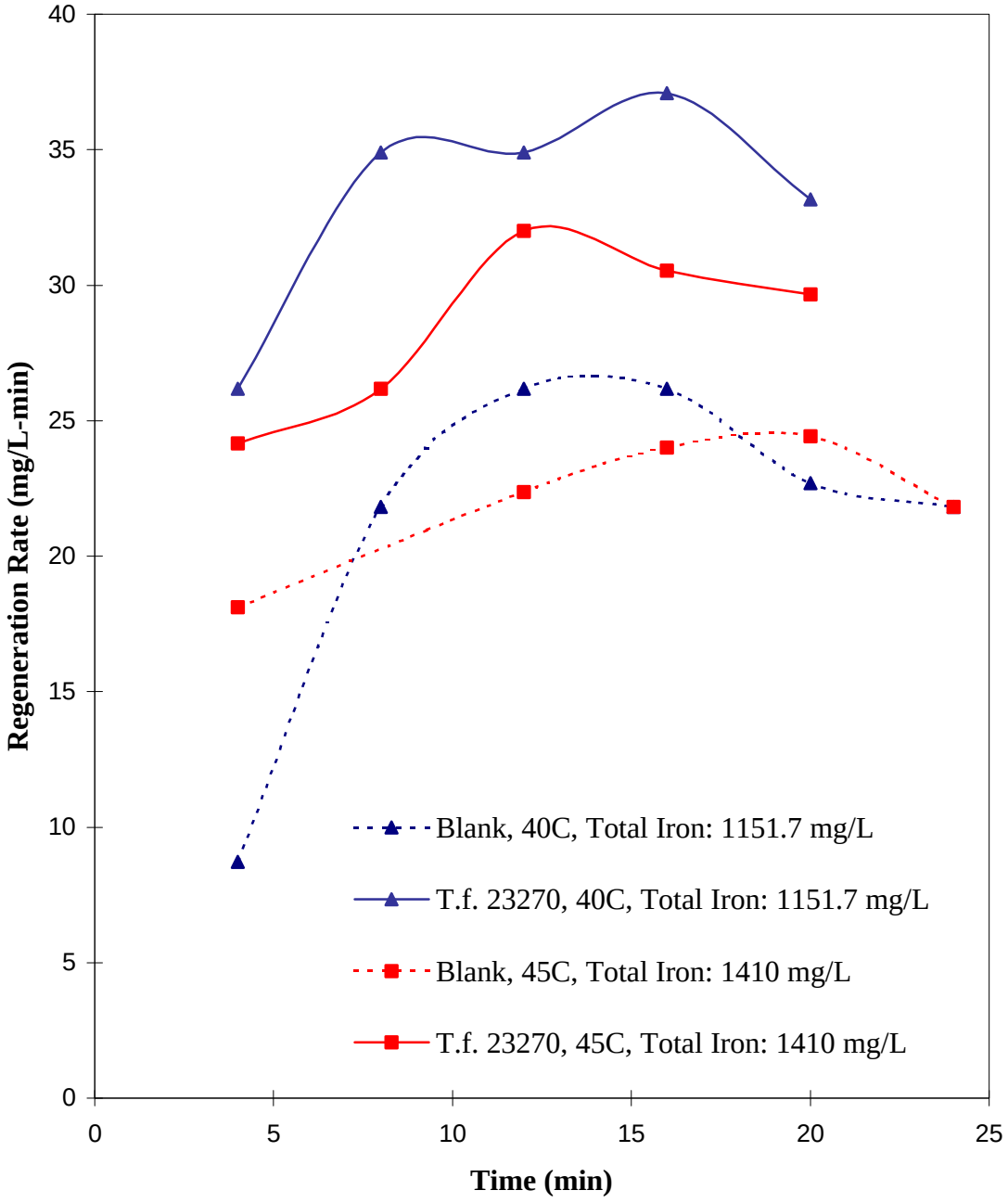
Figure 5 : Comparison of H_2S Oxidation Rates (BLANK v/s *T.f. 55720*)



pH: 8.5 Initial H_2S: 0.5%

BACTERIA HISTORY: Culture from ATCC,
Grown in High pH EDTA,
Temperature: 37C, pH: 7.5-8.0

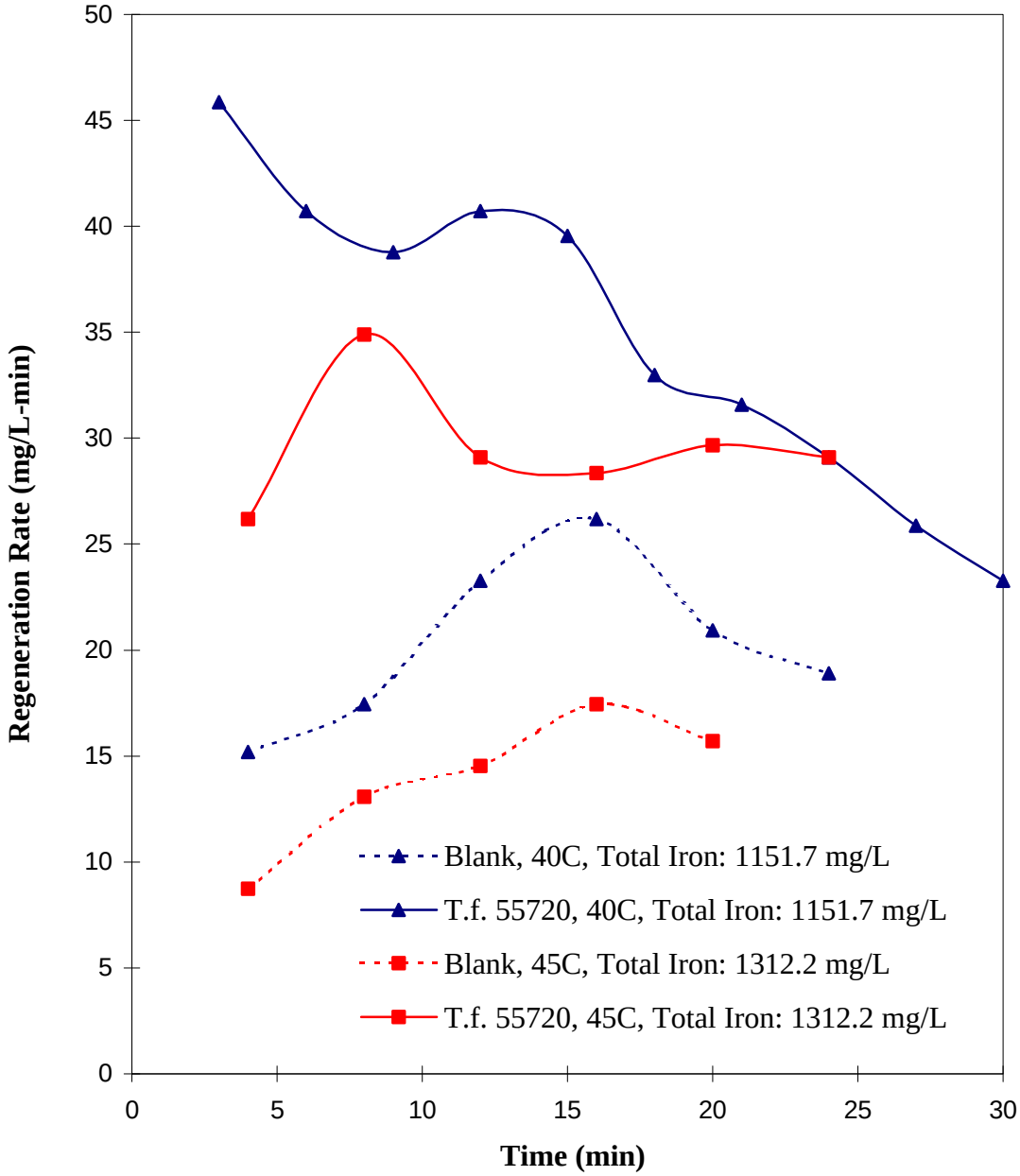
Figure 6 : Comparison of Ferric ion Regeneration Rate (BLANK v/s *T.f. 23270*)



pH: 8.5 Initial H_2S: 0.5%

BACTERIA HISTORY: Culture from ATCC,
Grown in High pH EDTA,
Temperature: 37C, pH: 7.5-8.0

Figure 7 : Comparison of Ferric ion Regeneration Rates (BLANK v/s *T.f. 55720*)



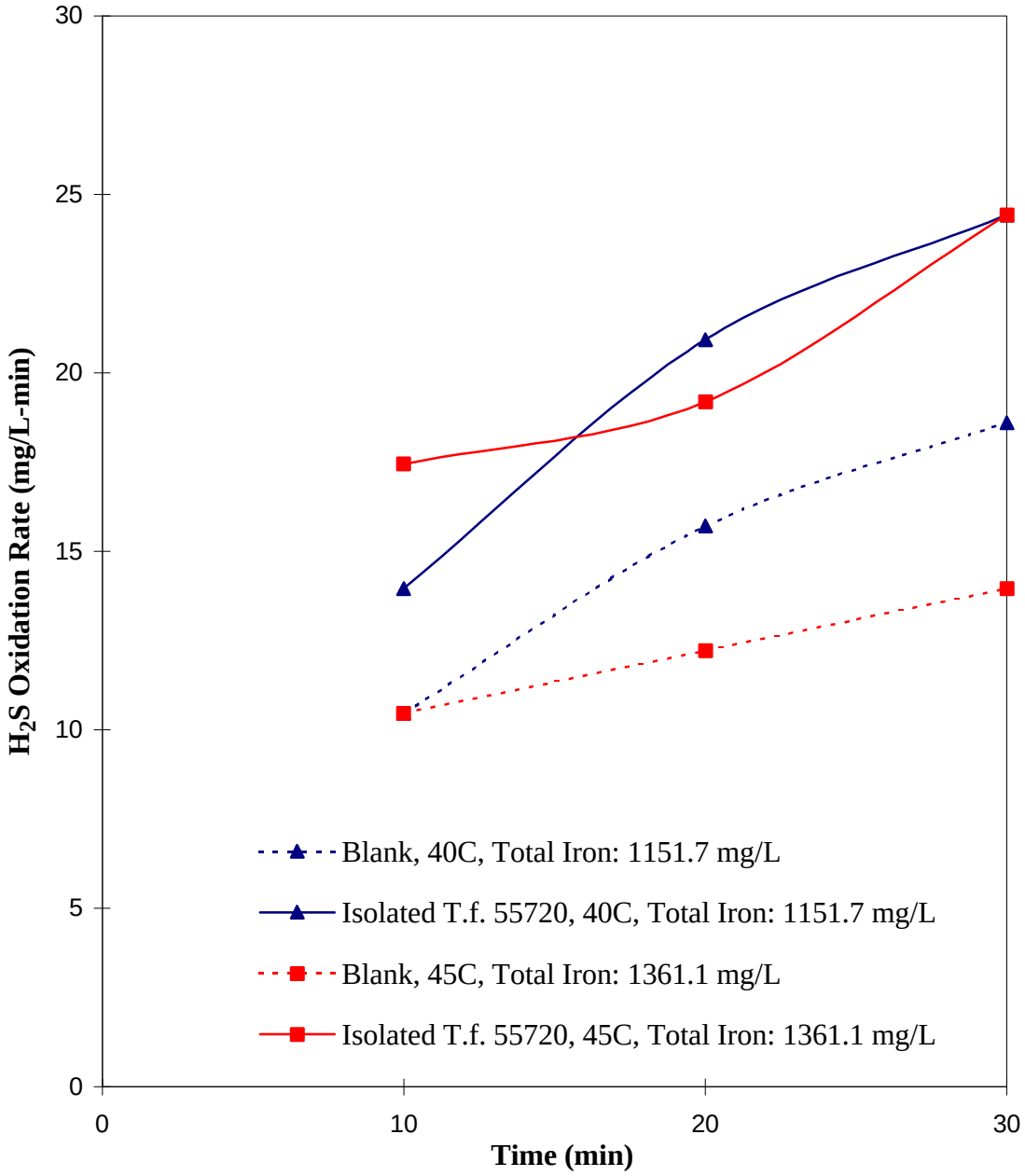
pH: 8.5 Initial H_2S: 0.5%

BACTERIA HISTORY : Culture from ATCC,
Grown in High pH EDTA,
Temperature: 37C, pH: 7.5-8.0

Performance of *T.f. Isolates* : Iron Oxidizing and Heterotrophic Components

Experiments were also conducted in the presence of the *T.f. Isolates* under the same set of conditions using Omni Culture Bioreactor. The iron oxidizing component from *T.f. # 55720* was isolated by growing it in Fe·EDTA medium and heterotrophic component by growing it in nutrient broth. The *Isolated T.f. 55720* component that was grown in Fe·EDTA medium behaved in a similar fashion as *T.f. 55720*. Experiments were carried out at a pH of 8.5 and temperature range from 40^{o} to 45^{o}C. The H_2S oxidation rate enhanced by 32.4% and 66.7% with Iron Oxidizing Isolate at 40^{o}C at 45^{o}C respectively as compared to the baseline (blank) experiments. Figure 8 illustrates the H_2S oxidation rate improvement. The ferric ion regeneration rates were found to improve by 34% and 86.3% respectively with iron oxidizing Isolate. Figure 9 illustrates this effect. The *Isolated T.f. 55720* - Heterotroph that was grown in a nutrient broth did not affect the ferric ion regeneration rates. However, the H_2S oxidation rates at a pH of 8.5 and in the temperature range of 40 to 45^{o}C showed slight enhancement. The results using T.f. 55720 - Heterotroph Isolate are illustrated in Figures 10 and 11.

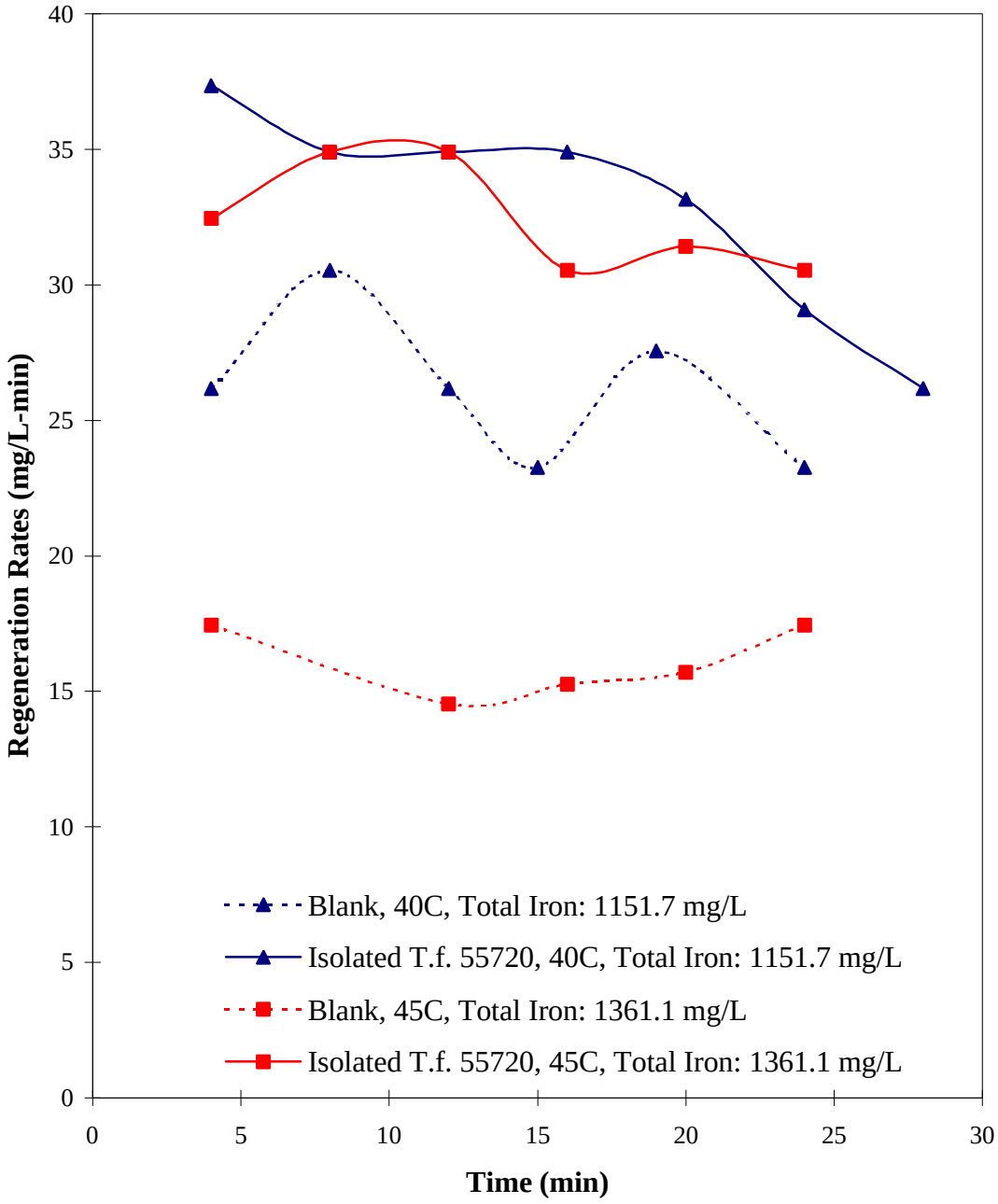
Figure 8 : Comparison of H_2S Oxidation Rates
(BLANK v/s *T.f. 55720* - Iron Oxidizing Isolate)



pH: 8.5 Initial H_2S: 0.5%

BACTERIA HISTORY: Isolated from *T.f. 55720*,
Grown in High pH EDTA,
Temperature: 37C, pH: 7.5-8.0

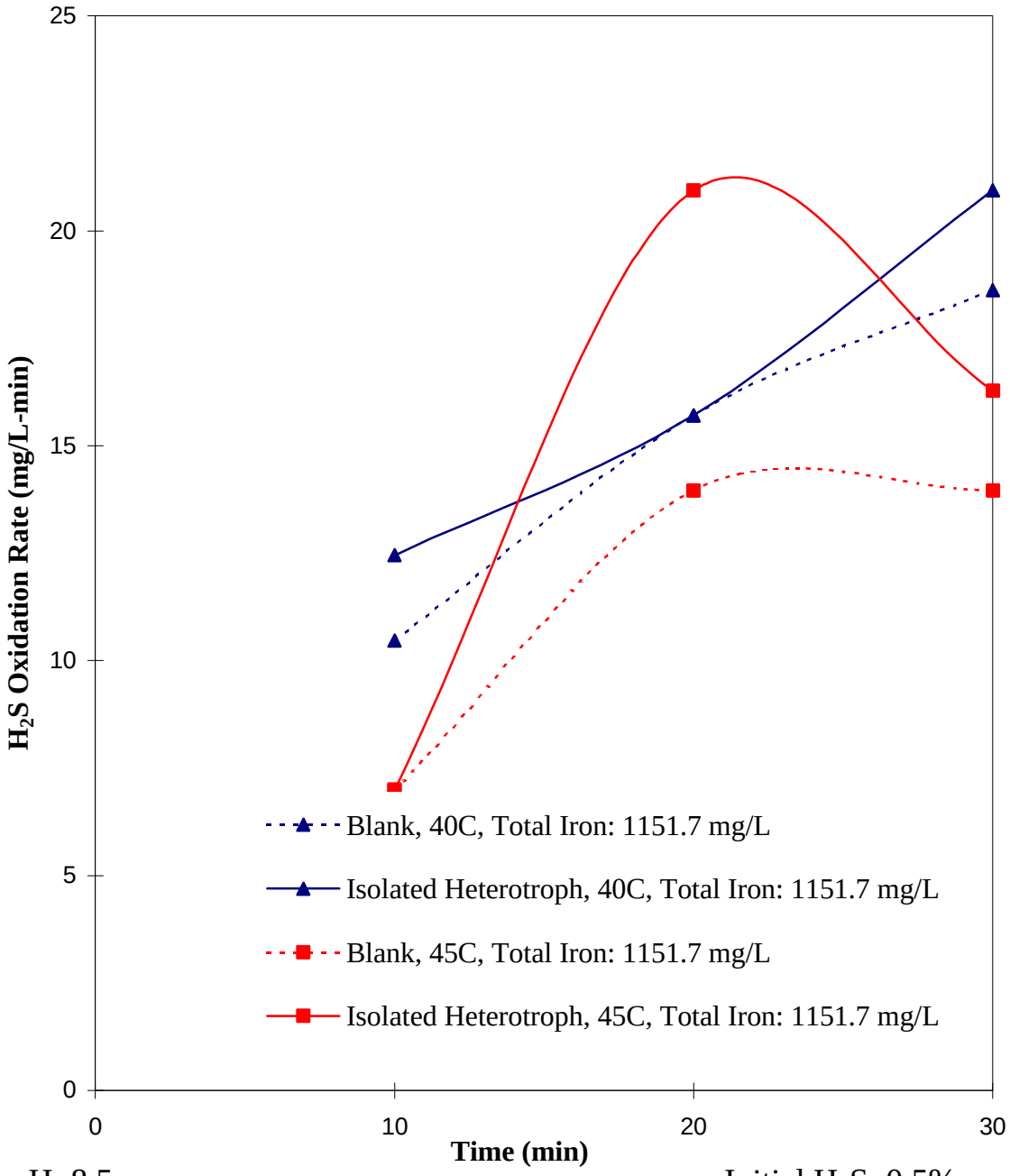
Figure 9 : Comparison of Ferric ion Regeneration Rates (BLANK v/s *T.f. 55720* - Iron Oxidizing Isolate)



pH : 8.5 Initial H_2S : 0.5 %

BACTERIA HISTORY: Isolated from *T.f. 55720*,
Grown in High pH EDTA,
Temperature: 37C, pH: 7.5-8.0

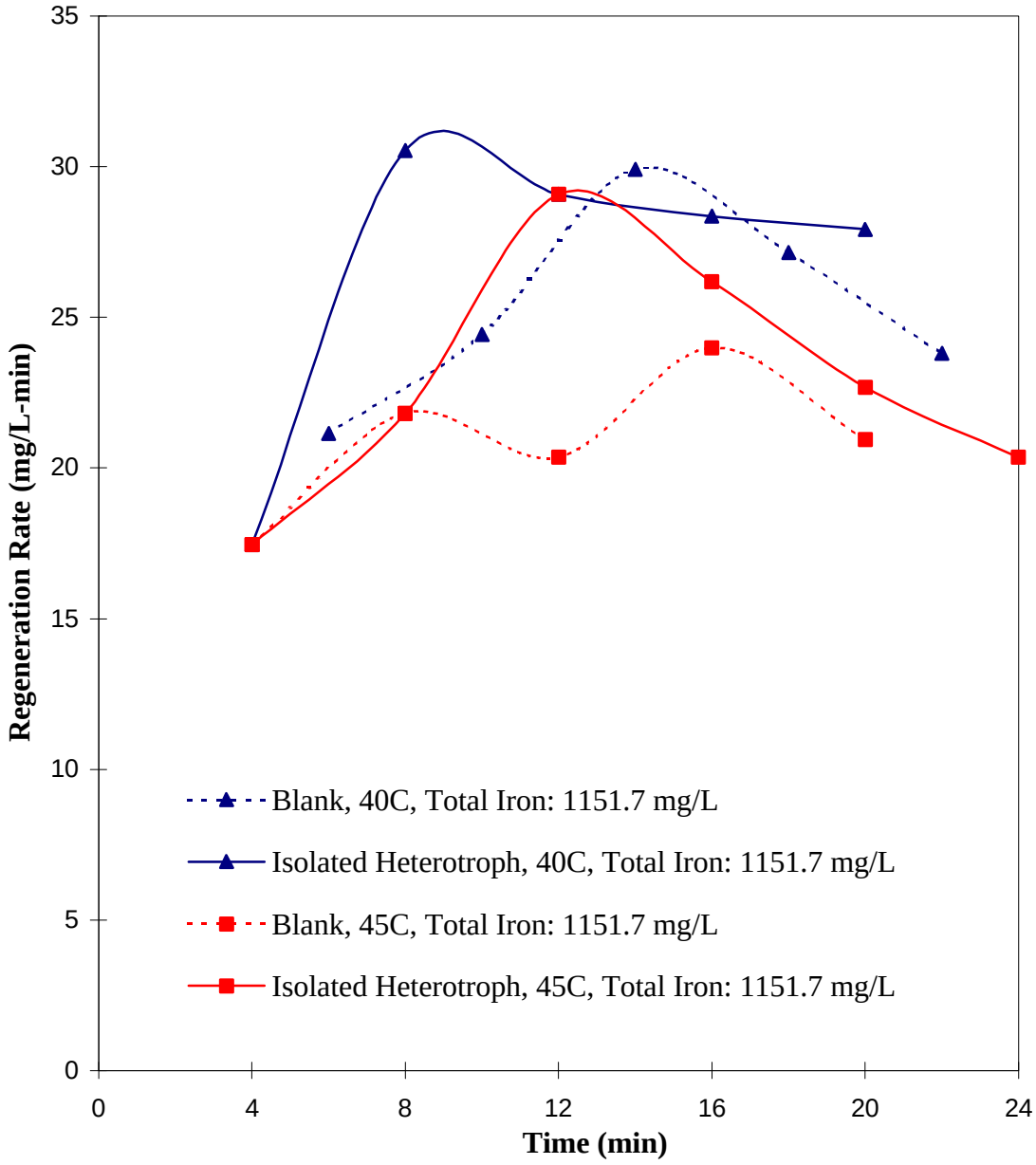
Figure 10 : Comparison of H_2S Oxidation Rates
(BLANK v/s *T.f. 55720* - Heterotroph Isolate)



pH: 8.5 Initial H_2S: 0.5%

BACTERIA HISTORY: Isolated from *T.f. 55720*,
Grown in nutrient broth,
Temperature: 37C, pH: 7.5-8.0

Figure 11 : Comparison of Ferric ion Regeneration Rates (BLANK v/s *T.f. 55720* - Heterotroph Isolate)



pH: 8.5 Initial H_2S: 0.5%

BACTERIA HISTORY: Isolated from *T.f. 55720* ,
Grown in nutrient broth,
Temperature: 37C, pH: 7.5-8.0

CONCLUSION

The vigorous growth rate of *T.f.* culture in high pH media can be attributed to the presence of a *T.f. heterotroph* that grows along with *T.f. # 23270* and helps in the oxidation of iron. Two cultures, *T.f. 23270* and *T.f. 55720*, were studied for their influence on the performance of the commercially available liquid redox catalyst, ARI 310 for the oxidation of H_2S to elemental sulfur. The study conclusively shows that in the presence of these cultures, the H_2S oxidation rates and ferric ion regeneration rates were improved considerably. During this study a contaminant was found to grow along with *T.f.* It was isolated and grown in nutrient broth and labeled as *T.f. # 55720 Isolate heterotroph*. Experiments conducted in the presence of this heterotroph indicate that it neither does influence the H_2S oxidation rates nor the ferric ion regeneration rates. Another isolate identified as *T.f. 55720 Isolate* -iron oxidizing was grown in Fe·EDTA medium and exhibited the same iron oxidizing capability as that of *T.f. # 55720.* It increased the hydrogen sulfide oxidation rates by 30 to 67 % and ferric ion regeneration rates by 34 to 86 %.

The use of bacteria in the sweetening of sour natural gas has shown a lot of promise. The laboratory work will be tested in a pilot plant under the sponsorship of The Gas Research Institute (GRI) and The U.S. Department of Energy. The use of bacteria could result in lower air pumping costs, smaller reactor sizes and thus lowering processing costs.

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