January, 1989

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Accepted for publication March 7, 1988

The oxidation of Fe^{2+} with oxygen in sulfate solutions was studied in the presence of *T. ferrooxidans.* To measure the chemical activity of bacteria, and the oxidation rate of iron, the redox potentials of solutions were continuously monitored during the experiments. The redox potentials were simultaneously monitored on the platinum and pyrite indicator electrodes. The redox potential versus time curves were further used to calculate the basic kinetic parameters, such as the reaction orders, the activation energy, and the frequency factor. It was found that under atmospheric conditions, and at $Fe^{2+} < 0.001M$, $T < 25°C$, and at pH above 2.2, the oxidation of iron is governed by the following rate expression:

$$-\frac{d[Fe^{2+}]}{dt} = 1.62 \times 10^{11} C_{bact}[H^+][Fe^{2+}] p O_2 e^{-(58.77/RT)}$$

Below pH = 2.2, the oxidation rate is independent of H^+ concentration.

INTRODUCTION

Ferric ion is a powerful oxidant and as such it is effectively used in hydrometallurgy for dissolution of various minerals. However, during the reactions ferric becomes reduced to ferrous ion, a chemical form without further oxidizing capabilities. To rejuvenate the iron, it has to be reoxidized back into higher oxidation state. This is usually done with oxygen in the presence of acid:

$$2Fe^{2+} + O_2 + 2H^+ = 2Fe^{3+} + H_2O \qquad (1)$$

The oxidizing property makes the ferric iron one of the most useful reagents in hydrometallurgy. But the usefulness of ferric ion is seriously handicapped by the slow kinetics of reaction (1), and various methods have been investigated to increase the given rate of oxidation. The general conclusion, regarding the increase of ferrous ion oxidation rate, is that some kind of catalyst, such as sulfur dioxide,[1] activated carbon,[2] cupric ion,[3,4] etc., has to be present. Another method to increase the rate of oxidation of ferrous ion is to use appropriate bacteria which have to oxidize ferrous to ferric ion to satisfy the energy requirements for growth. A well known bacterium, *Thiobacillus ferrooxidans,* has such a property.

Although an enormous amount of work has been done in the application of *T. ferrooxidans* in the area of biohydrometallurgy, with the major role of reoxiding ferrous to ferric, the kinetics of oxidation of ferrous iron to ferric have not been completely studied. Actually, most of the time this bacterium was used for leaching of complex sulfide and oxide ores, where the kinetic role of bacteria for oxidation of ferrous ion to ferric was not possible to isolate. *T. ferrooxidans* have been used in pure systems, for basic studies, but with different objectives such as to study the galvanic action of various minerals on each other in the presence of this bacteria. Several authors have studied the kinetics of iron oxidation related to the growth of *T. ferrooxidans*. Major heterogenous systems were examined by Trulear and Characklis,[5] and Wichlacz and Unz.[6] The homogenous reactions were studied by Kelly et al.,[7,8] and Braddock, Loung and Brown.[9] The studies on the oxidation rates of Fe^{2+} to Fe^{3+} in the biotic systems were all performed by monitoring the concentration of ferrous ion with time, the rate of depletion of oxygen, acidification of the media, or the accumulation of bacteria.

While the oxidation of ferrous to ferric is a desirable reaction from hydrometallurgical point of view, it is a very detrimental reaction from environmental aspects. The ferric ion is responsible for release of heavy metals, by the very same hydrometallurgical leaching reactions, in mine waste waters. In those environments where *T. ferrooxidans* is present the concentration of heavy metals in mine waters will always be higher. Because the presence of heavy metals

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Biotechnology and Bioengineering, Vol. 33, Pp. 428–439 (1989)

CCC 0006-3592/89/040428–12$04.00

in waters is extremely detrimental to the aquatic life, some control of the release of the heavy metals has to be practiced. This is another reason why the understanding of the chemical mechanisms and the rates of oxidation of ferrous ion in the presence of *T. ferrooxidans* is so important.

This work represents a study on the kinetics of oxidation of ferrous ion with oxygen in the presence of *T. ferrooxidans*. Additionally, this work describes a useful approach on how to continuously measure the chemical activity of the bacteria. The technique is very simple and it can be used for measuring the chemical activity of bacteria in various systems.

From the Nernst's equation

$$E = E^0 + \frac{2.3RT}{nF} \log \frac{Fe^{3+}}{Fe^{2+}} \qquad (2)$$

it is obvious that during oxidation of ferrous ion the redox potentials of solutions, E, will become more positive. When E versus time is plotted, a graphical representation of the rate of ferrous ion oxidation is obtained. The E versus time curves can be obtained under various experimental conditions, such as pH, concentration of bacteria, temperature, etc., and the respective curves can be utilized to calculate the fundamental kinetic parameters, such as reaction orders, rate constants, activation energy etc. This work demonstrates how that can be done, and presents the obtained results.

EXPERIMENTAL APPARATUS

The Electrochemical Cell

A special electrochemical cell was designed for this and future studies on the electrochemistry of bacterial action on sulfide minerals. It consists of two 300 mL compartments, Figure 1a, for dual cell experiments, which can be also converted into a single cell for regular electrochemical experiments. Each compartment had a circular opening on one side, 12 mm diameter, which could be used to place either a porous membrane or a sulfide mineral plate between the compartments for dual electrochemical studies. When used as a single cell, this port can be either closed or used to accommodate an electrode. In this study, the side port was used to accommodate a flat pyrite indicator electrode. The complete cell was made of plexiglass, and each compartment was jacketed, with plexiglass, to maintain constant temperature conditions by circulating water from a water bath. The top of the cell had a removable cover with five ports, which were used to accommodate appropriate electrodes, gas dispersion tubes, or sampling tubes. Each port could be sealed with a polyethylene cap and an O-ring. A viton rubber gasket was used to seal the cover to the body of cell by a set of four stainless steel screws. For this study only a single compartment electrochemical cell was used, Figure 1b, which was placed on a miniature magnetic stirrer for mixing the solutions.

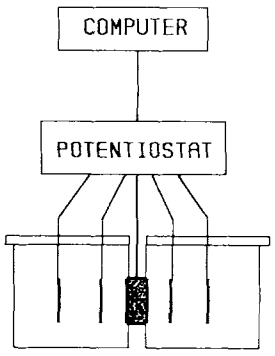

Figure 1(a). Schematic representation of a dual electrochemical cell.

Electrodes

Indicator electrodes: Two kind of electrodes were used for simultaneous measurement of redox potentials during bacterial oxidation of ferrous ion. One electrode was a combination platinum redox electrode, Orion Model 96-78. The second indicator electrode was made of pure pyrite mineral by cutting a 2 × 2-cm pyrite plate of 3-mm thickness. The original pyrite, cubical in shape, was acquired through Ward's Co. from Spain. The pyrite was cut with a Buehler Isomet low speed saw. On one edge of the pyrite plate, a copper wire was attached by conductive silver-epoxy glue, which was further covered with a regular epoxy glue to increase the mechanical strength of the bond. The electrical resistance of the pyrite electrode was checked by an ohmmeter, and was less than 4 ohms, between any place on the pyrite and the tip of the copper wire. One side of the pyrite electrode was polished with #600 metallographic paper, washed with plenty of water, rinsed with deionized water, and mounted on a side of a single compartment electrochemical cell. Rubber gaskets were placed on both sides of the pyrite electrode to keep it in place with a supporting plexiglass plate properly positioned and fastened to the body of cell with the stainless steel fasteners.

Reference electrodes: The reference electrode for the Pt redox electrode was Ag/AgCl. According to the manufacturer's specifications, the potential developed by this elec-

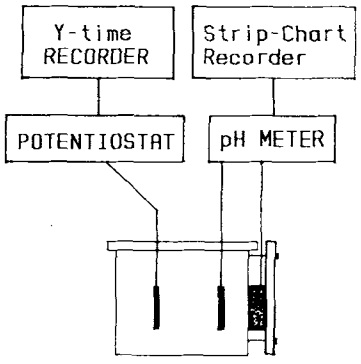

Figure 1(b). Schematic representation of a single electrochemical cell which was used in this study. The pyrite indicator electrode, (Py), is on the outside wall of the cell, fastened to the body by the o-rings, and a plexiglass plate.

trode relative to the standard hydrogen electrode, at 25°C, should be 199 mV.

The reference electrode for the pyrite indicator electrode was a saturated calomel electrode, which was placed into a specially designed water jacketed reference electrode compartment. The reference electrode compartment was extended by a plastic capillary tube to the vicinity of pyrite electrode. The end of plastic capillary was closed with a Corning Replacement Ceramic Junction (#477269). The filling solution for the reference electrode compartment was a saturated KCl solution. The potential developed by this electrode, relative to the standard hydrogen electrode, at 25°C, should be 241 mV.

Electronics

The pyrite electrode potentials were measured by a pH meter, Corning, Model 135, and recorded on a strip chart recorder. The platinum electrode potentials were measured by a potentiostat, ECO Instruments, Model 553, and recorded on a Y-t recorder.

Reagents and Analytical Procedure

The reagents used in this study were of analytical grade. The water used was deionized by Barnstead's Nanopure II deionizer.

The ferrous ion concentration was measured with *o*-phenanthroline by the method of Skoog and West.[10] The UV/VIS spectrophotometer, IBM Instruments, Model 9420, was used for that purpose.

Bacteria Preparation

Thiobacillus ferrooxidans (strain ATCC 13598) were grown on mineral salts medium containing 45 g/L $FeSO_4$. The bacteria were harvested by filtration through Whatman No. 1 filter paper and centrifugation at 12,000 xg for 15 min. The bacteria were washed twice with 10m*M* H_2SO_4 and suspended at concentration of 3 mg dry weight per milliliter. The bacteria prepared under these conditions maintained a constant metabolic activity for up to 8 h at room temperature.

Determination of Standard Potentials and Experimental Procedure

The first step in the given study was the determination of standard potentials, E^0, for the pyrite and platinum indicator electrodes under the desired experimental conditions. These were determined by varying the initial ferrous ion concentrations at constant initial ferric ion concentration. The initial ferric ion concentration was 0.001*M*. Four different initial ferrous ion concentrations were used: 0.0001*M*, 0.0003*M*, 0.001*M*, and 0.003*M*. For example, when the temperature of solution was 25°C, and the pH = 2.0, the following Nernst expressions were found, Figure 2:

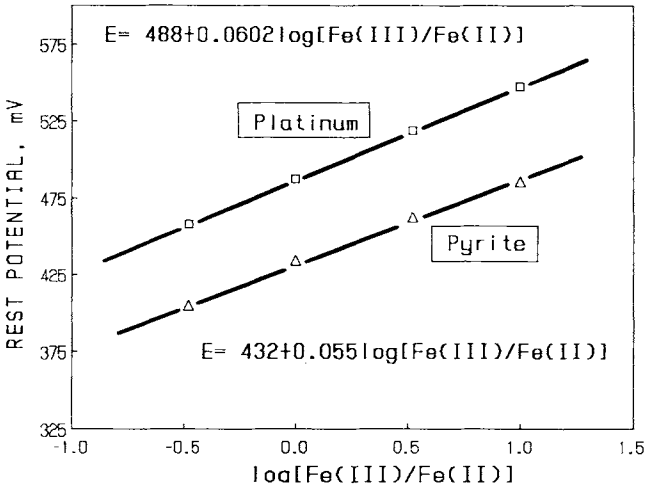

Figure 2. Determination of the standard potentials, and the *RT/F* values, in the Nernst's equation, for platinum and pyrite indicator electrodes.

$$\text{Pyrite: } E_1 = 0.432 + 0.055 \log \frac{Fe^{3+}}{Fe^{2+}} \quad (3)$$

$$\text{Platinum: } E_2 = 0.488 + 0.0602 \log \frac{Fe^{3+}}{Fe^{2+}} \quad (4)$$

When bacteria were present, the redox potential of the solution continuously increased, according to (3) or (4), due to the continuous oxidation of ferrous ion. The amount of ferrous oxidized at any time was calculated from the corresponding redox potentials and the known total iron concentrations. The calculated values for oxidized ferrous iron were also compared with the spectrophotometric analytical data, because in each oxidation experiment solution samples were withdrawn at particular time intervals for Fe^{2+} analysis.

The bacterial oxidation of ferrous ion was performed under the following standard conditions:

Table I. Standard experimental conditions.

Volume = 250 mL	Fe^{2+} = 0.001*M*
T = 25°C	Bacteria = 1 mL (3 mg dry weight)
pO_2 = 0.21	Fe^{3+} = 0.001*M*
pH = 2.0	

These parameters were maintained constant except for the parameter under study. The effects of pH, temperature, initial concentration of bacteria, and the effect of the initial concentration of ferrous ions on the oxidation rate of ferrous iron in the presence of *T. ferrooxidans* were examined. The experiments were initiated by the addition of a known amount of bacteria in the desired solution. The oxidation of ferrous ion was then followed by simultaneous monitoring of the redox potentials on the platinum and pyrite indicator electrodes. As mentioned above, the solution samples were also withdrawn at proper time intervals for Fe^{2+} analysis. All experiments were performed in sulfate medium, which was made of sulfuric acid and ferrous and ferric sul-

fates. Oxygen for oxidation was provided by purging the air through the system.

RESULTS

The Effect of Ferrous Iron Concentration

The effect of the initial ferrous ion concentration on the redox potential of the solutions during bacterial oxidation of ferrous ion is presented in Figure 3. Potentials were monitored with the pyrite (open symbols) and the platinum (closed symbols) electrodes. The initial potentials, for pyrite (versus S.C.E.) and platinum (versus Ag/AgCl) electrodes respectively, before bacteria addition were: 405 mV and 459 mV for 0.003*M* initial Fe^{2+}, 435 mV and 488 mV for 0.001*M* initial Fe^{2+}, and 462 mV and 520 mV for 0.0003*M* initial Fe^{2+}. The initial ferric ion concentration was 0.001*M*, as indicated above for standard experimental conditions. The other experimental conditions are given in the same figure. Notice that the redox potential increased with time, indicating the chemical activity of bacteria during the oxidation reactions. The amount of ferrous ion oxidized was calculated by using the redox potential values from Figure 3 and the Nerst equation for the corresponding electrode. These results are presented in Figure 4. The calculated

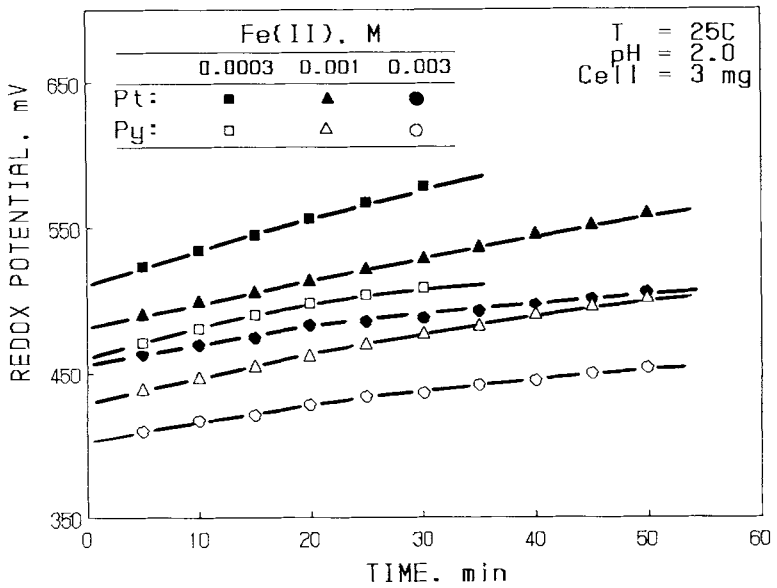

Figure 3. Redox potentials of solutions as a function of initial ferrous ion concentration, measured simultaneously on the platinum and pyrite electrodes. The experimental lines also represent the biological activity of bacteria.

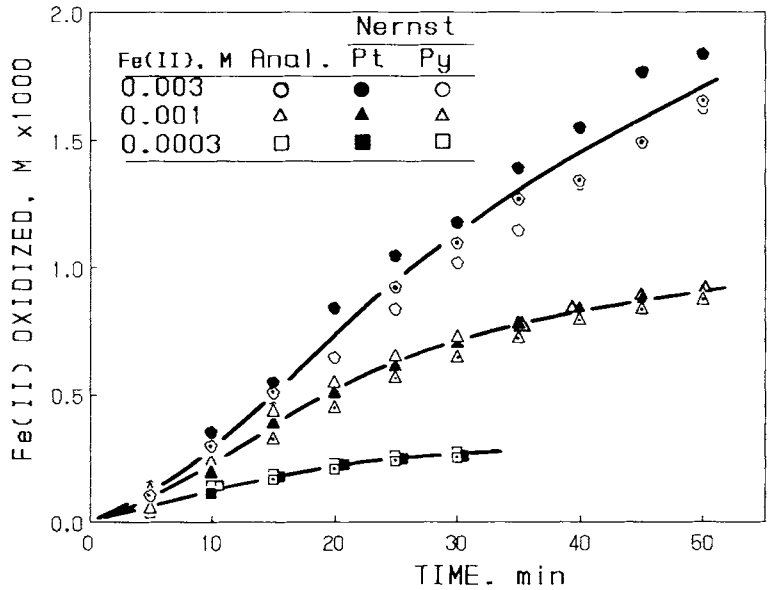

Figure 4. The results on the amount of ferrous ion oxidized, during bacterial oxidation, according to the analysis, and the calculations performed by using the redox potential values from Figure 3 in the Nernst's equation.

amounts of ferrous ion oxidized are also compared with the analyzed values, open symbols in Figure 4, for each initial ferrous ion concentration. Notice a very good agreement between the calculated values, for both indicator electrodes, and the analyzed values. This is a very important property of the system from both practical and fundamental points of view, because it enables an implementation of a relatively simple technique, which requires only a redox indicator electrode and a pH meter, for example, to continuously monitor the chemical activity of bacteria either in a practical application or in a fundamental study. In other words, the solution samples do not need to be withdrawn and analyzed for iron, as is usually done to ascertain the chemical activity of the bacteria. Also, if the potential-time plots are recorded on a plotter, as in this case, an experimenter obtains a visual interpretation of the bacterial activity from the slopes of the lines and also the indication when the reaction of oxidation is completed when the curves level off.

Effect of Initial Amount of Bacteria

The effect of concentration of *T. ferrooxidans* on the oxidation rate of ferrous ion was studied by adding three different volumes 0.5 mL, 1.0 mL, and 2.0 mL of prepared bacteria. These volumes are equivalent to the weights of dry cells of 1.5 mg, 3.0 mg, and 6.0 mg, respectively. The change of redox potentials, measured by both electrodes, with time as a function of initial bacteria concentration indicated that the potential increase was much faster when higher initial concentrations of bacteria were present in the solutions, reflecting the faster oxidation rates of ferrous ion. The amounts of iron oxidized were calculated by using the above Nerst equations and the values compared once more with the results from the analysis of the solutions. The results are given in Figure 5. Again, notice excellent agreement between the results for each case.

pH Effect

Three different initial sulfuric acid concentrations were used to study the effect of pH on bacterial activity during oxidation of ferrous ion, Figure 6. There was a pronounced effect of pH above 2.2. However, the rates of iron oxidation were almost identical at pH = 2.2 and pH = 1.6.

The Temperature Effect

The effect of temperature on the rate of iron oxidation by *T. ferrooxidans* is presented in Figure 7. Only four different temperatures were studied, 10°C, 20°C, 25°C and 30°C. Notice that there was no difference in the rates of oxidation at temperatures 25°C and 30°C.

DISCUSSION

The results from Figures 2–7 clearly demonstrate that the measurement of redox potentials with either platinum or pyrite indicator electrodes can be used as a diagnostic criteria for determining the chemical activity of *T. ferrooxidans* during oxidation of ferrous iron. Whenever measuring the redox potentials of solutions the indicator electrodes have to satisfy one main requirement, to be inert. While the platinum electrode satisfies that requirement, this may not be exactly true for the pyrite electrode. Pyrite can react with ferric ion, oxygen, and acid, the reagents present in the studied solutions, and this property could disqualify it from being a satisfactory indicator electrode. However, the rate of reaction of pyrite was too small compared with the rates of oxidation of ferrous ion in the presence of *T. ferro-*

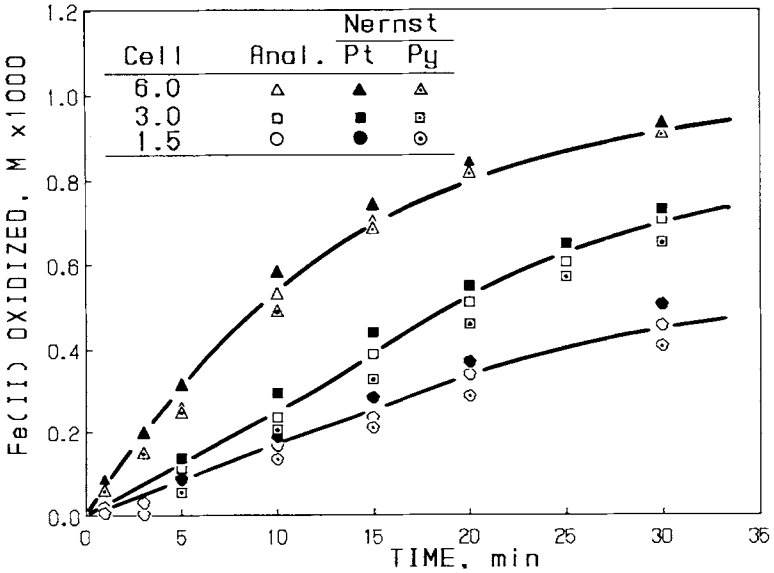

Figure 5. The comparison of the results on the oxidation rates of ferrous ion, which are obtained by the analysis, and the calculations performed by using the redox potentials.

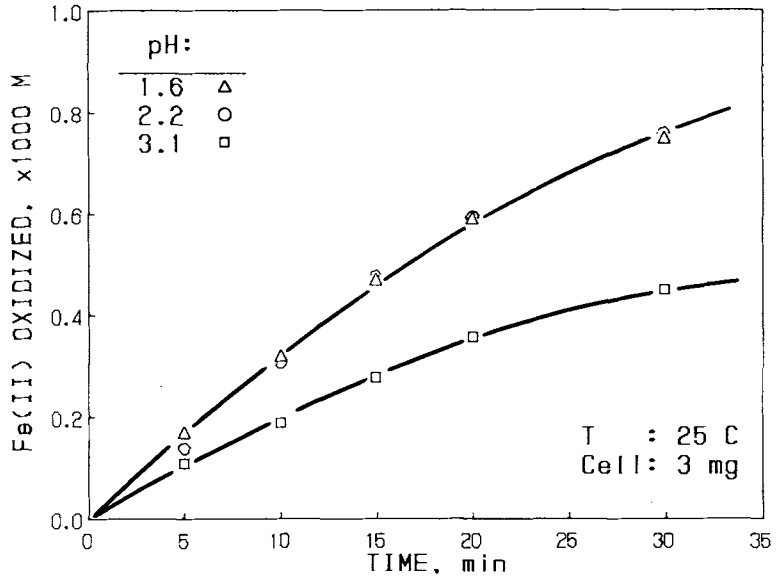

Figure 6. The rate of ferrous ion oxidation as a function of pH.

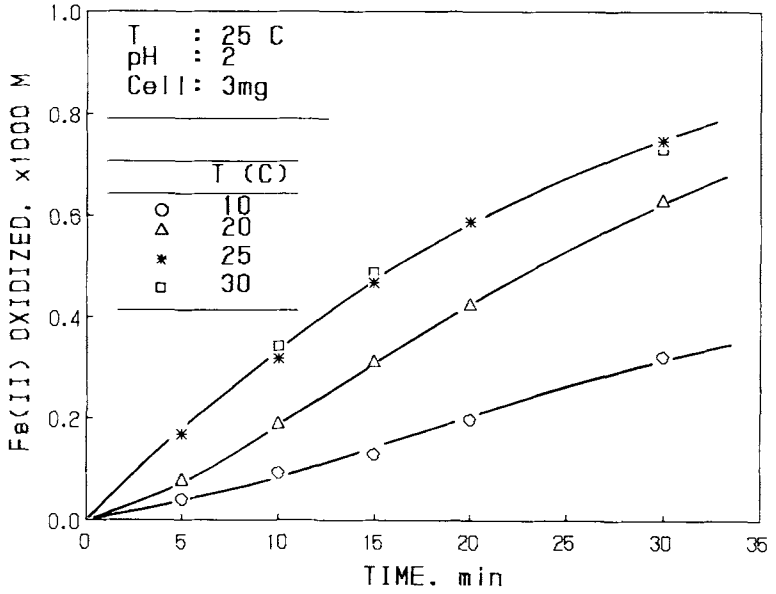

Figure 7. The rate of ferrous ion oxidation as a function of temperature. Notice no further increase in the rate of oxidation between 25°C and 30°C.

oxidans thus making the pyrite appear inert. The use of pyrite as an indicator electrode, on the basis of its slow oxidation rate, has also been recognized by others, Singer and Stumm.[11]

Another feature of the given system was the high reproducibility of the redox potentials. Platinum and pyrite electrodes were cleaned by polishing only at the beginning of each daily experiment, and not in between the experiments. The reproducibility of the potentials measured proved that this procedure was satisfactory. Even when electrodes were left unused for a prolonged time, reproducible potentials, within 3–4 mV, were obtained when their use was resumed. Platinum electrodes are known to suffer from poisoning side effects, and whenever working with this electrode, one has to bear this inherent property in mind, and frequently check the electrode's characteristics. The frequent check of the platinum electrode used in this study, the standard potentials derived by the ZoBell[12] solution, indicated that there were no poisoning side effects present in this system. For further information on the use of platinum electrodes in hydrometallurgical systems, the reader should examine the work by Natarajan and Iwasaki.[13–17]

The excellent performance of pyrite as an indicator electrode was rather surprising. It was expected that various

products formed from the reactions with pyrite would alter the properties of the electrochemical double layer with time and render it useless for this kind of work. However, the pyrite electrode did not exhibit any problems. The potentials were very stable, and no potential drifts were observed, although the electrode was never polished between the uses, just rinsed.

The standard potential of the Fe^{3+}/Fe^{2+} couple measured by the pyrite electrode was $E_h = 0.673$ V, eq. (2), which was much lower than the theoretical potential of $E_h = 0.771$ V. The slope, $RT/F = 0.0561$, for the pyrite electrode, was also lower than the theoretical value of 0.0591 at 25°C. The obtained value of $E_h = 0.673$ V was much closer to the rest potential of the pyrite electrode than to the standard potential of Fe^{3+}/Fe^{2+} couple. The rest potentials of pyrite were measured and reported by Masuko and Hisamatsu,[18] $E_h = 0.63$ V in $1M$ H_2SO_4, by Peters and Majima,[19] $E_h = 0.62$ V in $1M$ $HClO_4$, by Rachenberg,[20] $E_h = 0.66$ V at pH = 4.

The standard potential of the Fe^{3+}/Fe^{2+} couple, on the Pt electrode, $E_h = 0.687$ V, was also lower than the theoretical potential, $E_h = 0.771$ V, under the standard experimental conditions. The RT/F value, 0.0602, for this electrode, was much closer to the theoretical value. The observed differences between the measured and the theoretical standard redox potentials for the platinum and pyrite electrodes can be explained by using the mixed potentials theory, and the reader should refer to the work by Natarajan and Iwasaki[17] for discussion on this subject.

The discrepancy between the theoretical and measured values for the standard potentials of the Fe^{3+}/Fe^{2+} couple on the platinum or pyrite electrodes does not diminish the usefulness of these electrodes for measuring the chemical activity of *T. ferrooxidans* during the oxidation of ferrous to ferric. As a matter of fact, the theoretical values for the standard potentials and RT/F are not necessary for calculating the amount of Fe^{2+} oxidized with time in the presence of bacteria, and the apparent redox potentials are sufficient for the intended purpose. The eq. (2), actually, can be regarded as an ordinary calibration line for each electrode used, which can be used to calculate the unknown amount of Fe^{2+} at any particular time, as it was proved in Figures 4 and 5.

The Rate Expression

The analysis of the above results, the concentration of ferrous ions with respect to time, showed that the oxidation of ferrous ion with oxygen in the presence of *T. ferrooxidans*

$$Fe^{2+} + 1/2O_2 + 2H^+ \xrightarrow{\text{bacteria}} Fe^{3+} + H_2O \qquad (5)$$

followed the first order reaction rate expression with respect to Fe^{2+}, i.e., the rate of Fe^{2+} oxidation was proportional to the existing Fe^{2+} concentration:

$$\text{Rate} = -\frac{d[Fe^{2+}]}{dt} = k[Fe^{2+}] \qquad (6)$$

where, $[Fe^{2+}]$ is the concentration of Fe^{2+} at any time t, and $k(1/\text{min})$ is the specific reaction rate constant. The eq. (6) can be transformed by integration into:

$$-\ln \frac{[Fe^{2+}]}{[Fe^{2+}]_0} = kt \qquad (7)$$

where, the $[Fe^{2+}]_0$ is the initial Fe^{2+} concentration.

If the reaction (5) is of the first order, then the plots of $\ln[Fe^{2+}]/[Fe^{2+}]_0$ versus time should produce a straight line with the slope corresponding to the specific reaction rate constant k. Another method to prove that the reaction is of the first order is to study the given reaction rates for several different initial Fe^{2+} concentrations, and to calculate the reaction rate constants k for each concentration. If little change of k with respect to concentration was found, the reaction would be of the first order with respect to the studied species. The oxidation of Fe^{2+} with oxygen in the presence of *T. ferrooxidans* was examined for three different initial Fe^{2+} concentrations, $0.0001M$, $0.0003M$, and $0.001M$. In all three cases, the relationship $\ln[Fe^{2+}]/[Fe^{2+}]_0$ versus time was linear, with very little difference between the slopes for each line. This kind of relationship proved that oxidation of ferrous ion with oxygen in the presence of *T. ferrooxidans* was of the first order with respect to Fe^{2+}.

If the oxidation of Fe^{2+} was of the first order then the reaction rate constant k in the eqs. (6) and (7) shouldn't be a function of any variable except temperature. However, that was not the case in the system studied. For example, when the oxidation rate of Fe^{2+} was measured as a function of the initial *T. ferrooxidans* concentration, Figure 4, an excellent linear relationship between $\ln[Fe^{2+}]/[Fe^{2+}]_0$ versus time was obtained, Figure 8, which also confirmed the first order type of reaction. However, the different slopes in Figure 8 clearly indicate that the given oxidation reaction is not truly of the first order but rather the pseudo-first order, because of its dependence on the bacteria concentration.

This finding, the dependence of the reaction rate on the bacteria concentration, requires the modification of eq. (7) with respect to k. This constant has to be redefined into the pseudo-first order reaction rate constant, which contains at least one concentration term, the concentration of *T. ferrooxidans*. When the apparent reaction rate constants, evaluated from the slopes in Figure 8, were plotted versus the initial *T. ferrooxidans* concentration, a straight line with a slope equal to one was obtained, Figure 9. This plot confirmed the first order oxidation reaction of Fe^{2+} with respect to *T. ferrooxidans*.

The next parameter, the initial pH of the solution, Figure 6, revealed that the oxidation rate of Fe^{2+} was also a function of the initial H^+ concentration, Figure 10. Hydrogen ion had an effect on the biological activity of *T. ferrooxidans*, with the oxidation rate of Fe^{2+} directly proportional to the concentration of H^+ in the range pH = 3.1 to pH = 2.2, and independent of H^+ concentrations corresponding to the values below pH = 2.2 (Fig. 11). The observed decline of the rates of ferrous ion oxidation above pH = 2 could be due to the possible precipitation of ferric hydroxides, or to the decreased activity of bacteria.

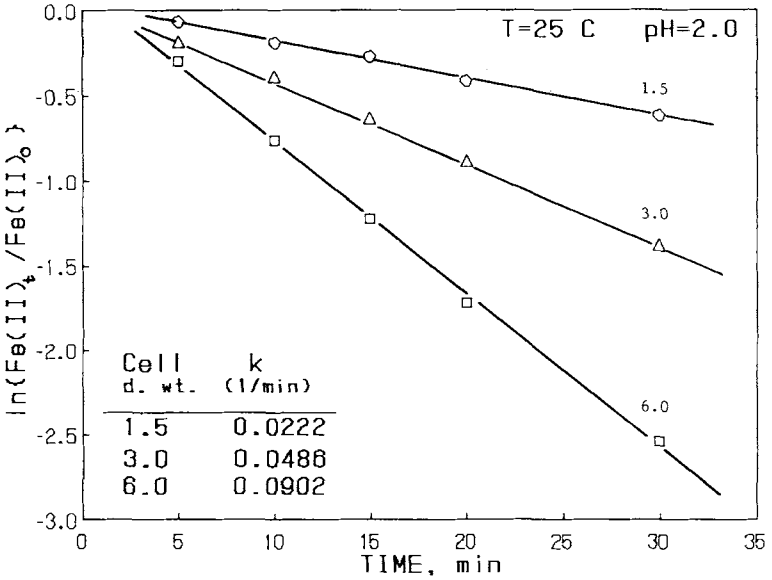

Figure 8. The first order reaction plot as a function of the initial concentration of bacteria. Data for this plot were taken from Figure 5.

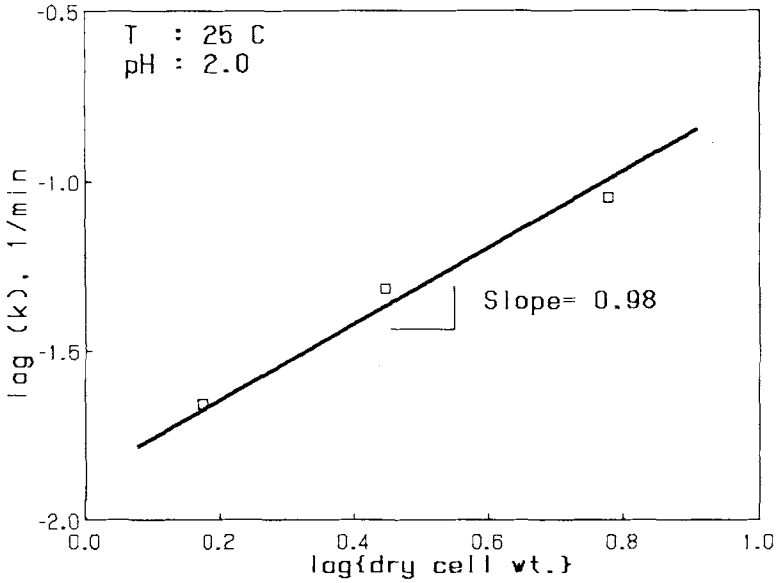

Figure 9. Determination of the reaction order with respect to bacteria.

The observed influence of the concentration of *T. ferrooxidans*, H^+, and Fe^{2+} requires further modification of eq. (7), which has to be rewritten in the following form to represent the rate of oxidation of Fe^{2+} with O_2 in the presence of *T. ferrooxidans*:

$$\text{Rate} = -\frac{d[Fe^{2+}]}{dt} = k[Fe^{2+}] = k'C_{bact}[H^+][Fe^{2+}]p_{O_2} \quad (8)$$

where, $k = k'C_{bact}[H^+]p_{O_2}$, is the pseudo-first order reaction rate constant, C_{bact} is the concentration of bacteria, H^+ is the concentration of hydrogen ion, and p_{O_2} is the partial pressure of oxygen. Although the effect of p_{O_2} was not examined in this study, it is included in the above equation on the basis of generally accepted knowledge of the importance of this parameter on oxidation rate of Fe^{2+} in biological and abiotic systems. The eq. (8) represents the pseudo-first order reaction rate model which can explain the effect of the studied parameters during oxidation of Fe^{2+} with oxygen in the presence of *T. ferrooxidans*. The k' constant, in the pseudo-first order reaction rate constant, is a function of temperature, and to include the effect of temperature on the oxidation rate, the activation energy and the frequency factor have to be determined. The pseudo-first order reaction rate constants, which are necessary to calculate the activation energy, were calculated from the slopes of the lines on a $\ln[Fe^{2+}]/[Fe^{2+}]_0$ versus time plot, Figure 12, which was obtained by using the data in Figure 7. The values of the reaction rate constants, evaluated from Figure 12, were used to construct the Arrhenius plot, Figure 13,

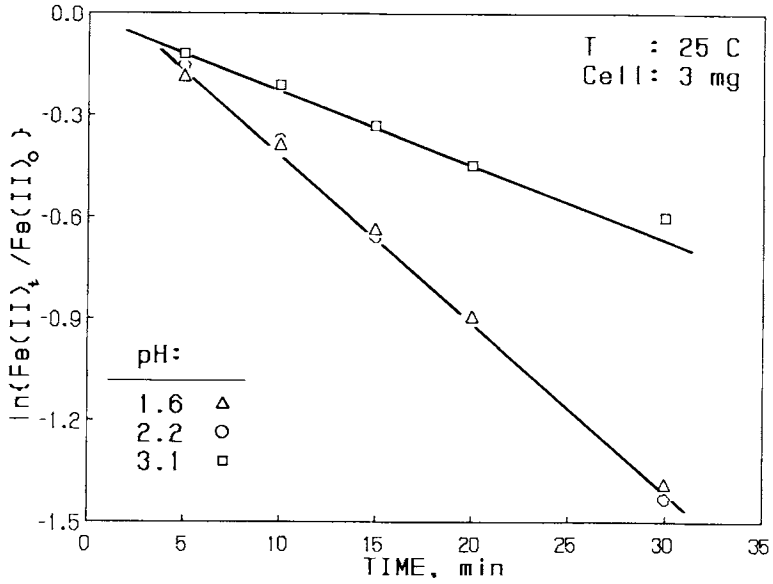

Figure 10. The first order reaction plot as a function of the initial concentration of hydrogen ion. Data for this plot were taken from Figure 6.

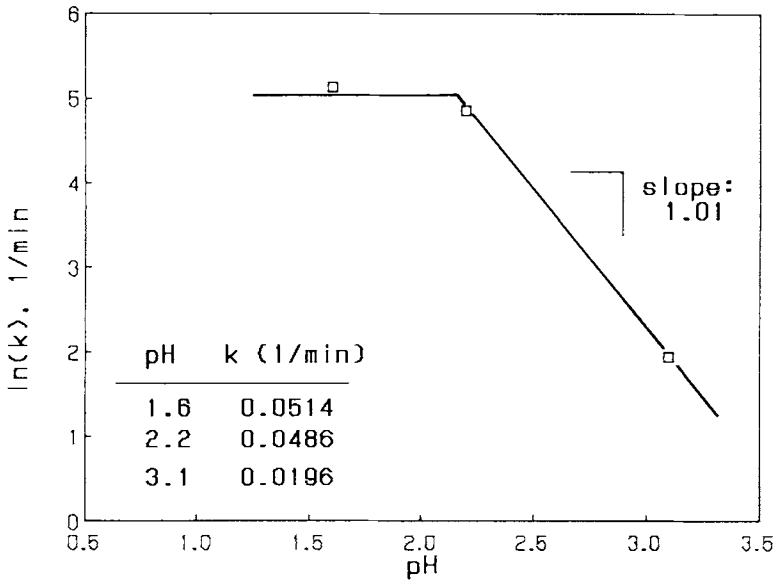

Figure 11. Determination of the reaction order with respect to hydrogen ion.

and to determine the activation energy. The calculated activation energy was Ea = 58.77 kJ/mol (14,062 kcal/mol). This value for activation energy was also used to calculate the frequency factor in the Arrhenius expression for the rate constant. The frequency factor found was 1.62×10^{11}. Finally, the rate expression which describes the oxidation of Fe^{2+} with oxygen in the presence of *T. ferrooxidans* can be written:

$$-\frac{d[Fe^{2+}]}{dt} = 1.62 \times 10^{11} C_{bact}[H^+][Fe^{2+}]p_{O_2}e^{-(58.77/RT)} \quad (9)$$

This rate expression applies in the pH region above 2.2. Below pH = 2.2, the rate of oxidation of Fe^{2+}, in the presence of *T. ferrooxidans*, is pH independent, eliminating the need for concentration of hydrogen term in (9):

$$-\frac{d[Fe^{2+}]}{dt} = 1.62 \times 10^{11} C_{bact}[Fe^{2+}]p_{O_2}e^{-(58.77/RT)} \quad (10)$$

The literature review revealed that there has not been a similar study performed, and consequently the above rate expressions could not be contested against the rate models from other researchers. In addition, the biologists use a different approach to analyze the reaction kinetics in the biotic systems. The Michaelis–Menten equation is the most widely used expression for interpretation of the reaction rates with respect to ferrous ion concentration. The concentration of ferrous ion needed to give half-maximal

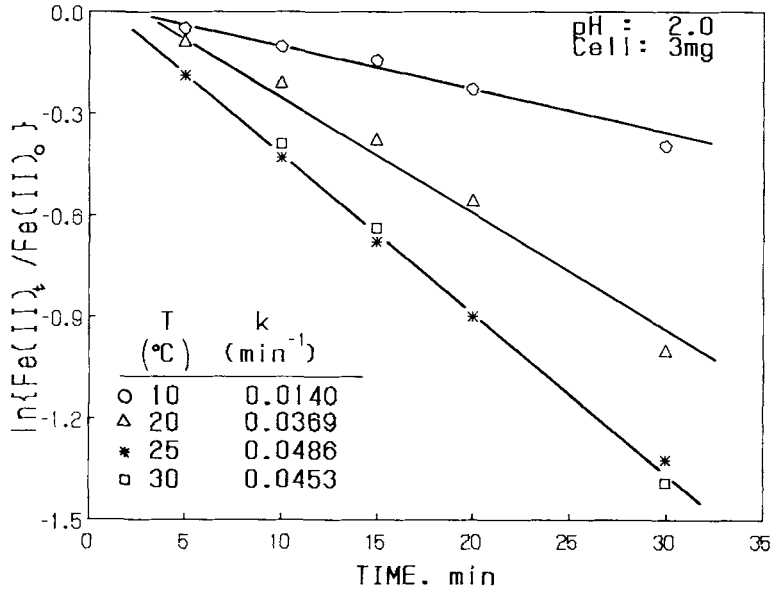

Figure 12. The first order reaction plot as a function of temperature.

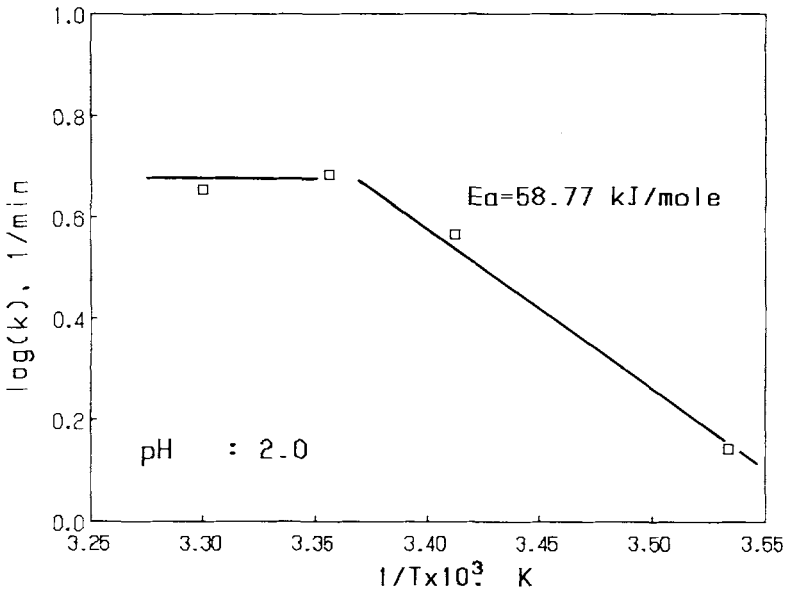

Figure 13. The Arrhenius plot for oxidation of ferrous ion with oxygen in the presence of *T. ferrooxidans*.

rates of oxidation (K_m) by *T. ferrooxidans* is usually reported to be between 1 and 10m*M*.[7-9] The much lower ferrous concentrations used in our experiments resulted in the pseudo-first order reactions observed.

On the other hand, there has been a considerable amount of work done in the area of the abiotic oxidation of Fe^{2+} with oxygen. In general, the oxidation of Fe^{2+} ion can be categorized according to the solution medium (sulfate, chloride, etc.), pH (low, neutral as in natural waters, or high), or according to the pressures, atmospheric or high pressures, at which the oxidation occurred. The oxidation of Fe^{2+} in sulfate medium was studied by Mathews and Robins,[3] and Huffman and Davidson[21] and the oxidation in chloride medium by Colborn and Nicol.[22] The oxidation of Fe^{2+} in waters in the natural pH range was studied by Stumm and Lee,[23] Sung and Morgan,[24] and Minegishi, Higuchi and Kondo.[25] The kinetic study of ferrous oxidation under pressure in sulfate medium was done by Chmielewski and Charewicz,[4] and in chloride medium by Swannathan, Subramanian and Rao.[26] Another notable category of ferrous ion oxidation is related to the catalytic action of various agents. The catalytic action of SO_2 on oxidation rate of ferrous ion was reported by Tiwari, Kolbe, and Hayden,[1] the effect of Cu^{2+} by Mathews and Robins,[3] and Chmielewski and Charewicz,[4] and the action of activated carbon was examined by Hayden.[2]

By reviewing all of the rate expressions found in the various systems by the above authors it was observed that

the oxidation rates of Fe^{2+} with oxygen can be classified into two major types of rate expressions:

Category I: Oxidation of Fe^{2+} in acidic medium (sulfate or chloride), under atmospheric or elevated pressures:

$$-\frac{d[Fe^{2+}]}{dt} = k\frac{[Fe^{2+}]^2}{[H^+]^{1/4}}p_{O_2} \quad (11)$$

Category II: Oxidation of Fe^{2+} at neutral or higher pH values (natural waters and basic medium):

$$-\frac{d[Fe^{2+}]}{dt} = k[Fe^{2+}][OH^-]^2 p_{O_2} \quad (12)$$

It can be observed that the reaction mechanism of Fe^{2+} oxidation is different in the acidic medium from the one in neutral or basic medium. For example, the reaction with respect to Fe^{2+} is second order at low pH values, and first order at higher pH values. With respect to hydrogen ion, the reaction of iron oxidation is of ¼ order in acidic solutions, and of the second order in the neutral or more basic solutions. Notice also that the hydrogen ion has a negative effect on oxidation rate of Fe^{2+} in acidic solutions.

It is therefore clear that both of the above rate expressions, eqs. (11) and (12), are different from the rate expressions found in this study, which corresponds to the oxidation of Fe^{2+} in the presence of *T. ferrooxidans*, eqs. (9) and (10).

To obtain some kind of a physical feeling for the kinetic importance of *T. ferrooxidans* during oxidation of ferrous iron with oxygen, it would be necessary to compare the appropriate reaction rates, in abiotic and biotic systems. The comparison of related reaction rate constants would not provide the desired effect, because of the different units involved, therefore another method of comparison should be used. The half-times, the time required to achieve 50% conversion of initially present reactant, would be more appropriate. The half-time for the first order, or the pseudo-first order reactions, can be calculated by dividing 0.693 with the related rate constant. For this type of reactions, the half-time is not dependent upon the initial concentration of the reactant. For the second order reactions, which can be calculated by dividing $1/[Fe^{2+}]_0$ with the apparent reaction rate constant, the half-time is dependent upon the initial concentration of the respective reactant, and that should be recognized when comparing the half-times of the reactions governed by different mechanisms. For that reason, one has to calculate the half-times, from the rate expressions (9), (11), and (12), for the same initial ferrous ion concentration, for example $[Fe^{2+}]_0 = 0.001M$. In the abiotic systems, above pH = 3.5, the rate of Fe^{2+} oxidation is extremely pH sensitive, and the rate of oxidation is governed by eq. (11), for which Stumm and Lee[23] have calculated the reaction rate constant, $k = 8 \times 10^{13}$ L^2/mol^2 atm min at 25°C. Thus, at pH = 4.5, it will take $0.693/8 \times 10^{13} \times (10^{-9.5})^2 \times 0.21 = 286$ d to oxidize 50% of ferrous into ferric iron. If the same oxidation reaction was performed at pH = 5.5, it would take 2.86 d to achieve the same oxidation. In more acidic solution, the oxidation of ferrous ion will follow the rate expression (11), derived by Mathews and Robins.[3] For example, to oxidize 50% of $0.001M$ ferrous ion initially present, at pH = 1.45 (0.0355 mol/L), T = 30°C, and $p_{O_2} = 0.21$ (or 2.34×10^{-4} mol/L), it will take 3740 d. It is more than obvious, therefore, that the oxidation of ferrous ion with molecular oxygen is an extremely slow reaction in the acidic solutions. The slow oxidation rate of Fe^{2+} can be substantially increased by the catalytic action of various additives. For example, when $0.001M$ Cu^{2+} is added to the same acidic solution of $0.001M$ ferrous ion, the half-time will be only 6.6 d, calculated according to the data from Mathews and Robins.[3] The further enhancement of Fe^{2+} oxidation rate can be achieved by increasing the temperature, because of the high activation energies involved, and the partial pressure of oxygen.

On the other hand, in the biotic systems, with the *T. ferrooxidans*, the oxidation of ferrous to ferric ion, with molecular oxygen, is much faster, even under acidic conditions. For example, when the data from Figure 11 are used, it can be calculated that it takes only 31 min ($0.693/0.0222 min^{-1}$) to react 50% of initially present Fe^{2+}, when 1.5 mg (dry weight) of *T. ferrooxidans* is present. When the concentration of bacteria is higher, such as 6.0 mg dry weight, the half time is only 7.8 min. The above numbers clearly demonstrate the significance of *T. ferrooxidans* during oxidation of ferrous ion.

CONCLUSIONS

The measurement of the redox potentials of solutions during oxidation of Fe^{2+} to Fe^{3+} in the presence of *T. ferrooxidans* represents a useful technique to ascertain the chemical activity of this bacteria. In addition, this technique provides an analytical tool to calculate the yield of reaction with time, which can be further used to calculate basic kinetic parameters.

Two types of indicator electrodes were used, a metal, Pt electrode, and a sulfide mineral, pyrite electrode. It was shown that each electrode can be successfully used to measure the rates of oxidation on the basis of redox potentials. When calculating the amount of Fe^{2+} oxidized, from the Nernst equation, it was found that the results of the calculation are very sensitive on the intercept, E^0, and fairly insensitive on the RT/F values. For a successful application, the E^0 values should be determined within 5 mV accuracy, for each system.

The kinetic parameters reported in this study are based on calculations from the Nernst equation. However, the calculated values for each experiment were also checked against the analyzed values, as a safeguard for the reliability of the method.

The oxidation rate of Fe^{2+} to Fe^{3+} with oxygen in the presence of *T. ferrooxidans*,

$$Fe^{2+} + 1/2O_2 + H^+ \xrightarrow{\text{bacteria}} Fe^{3+} + H_2O$$

is directly proportional to the concentration of bacteria, ferrous ion, oxygen, and hydrogen ion, above pH = 2.2:

$$-\frac{d[Fe^{2+}]}{dt} = 1.62 \times 10^{11} C_{bact}[H^+][Fe^{2+}]p_{O_2}e^{-(58.77/RT)}$$

Below pH = 2.2, the oxidation rate is independent of pH:

$$-\frac{d[Fe^{2+}]}{dt} = 1.62 \times 10^{11} C_{bact}[Fe^{2+}]p_{O_2}e^{-(58.77/RT)}$$

The upper limit of pH independence was not determined, and it will be studied in the future.

The derived rate expressions, especially the frequency factor, should be taken cautiously, however, because the studied oxidation rates are directly dependent upon the biological performance of bacteria, a property which is very difficult to predict or control experimentally.

One of the future goals will be to extend the electrochemical technique discussed in this article into the study for development of an enumeration technique, to measure the concentration of *T. ferrooxidans* continuously, which will be primarily based on the possibility to instantaneously measure the chemical activity of this bacteria.

The work described in this article was supported by the Interior Department's Bureau of Mines under Contract No. J0134035 through Department of Energy Contract No. DE-AC07-76ID01570. The appreciation is also extended to Dr. Ximeng Zhu, who helped in performing some of the experiments.

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