

The Mechanism of Bacterial Action in the Leaching of Pyrite by *Thiobacillus ferrooxidans*

An Electrochemical Study

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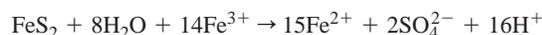
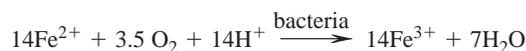
In many of the experiments reported in the literature on the leaching of pyrite by *Thiobacillus ferrooxidans*, the concentrations of ferric and ferrous ions in the presence of bacteria differ significantly from experiments conducted in their absence. In addition, these concentrations change throughout the course of the experiment. This makes it difficult to determine whether the presence of bacteria increases the rate of leaching above that for chemical leaching at the same solution conditions. We have designed an experimental apparatus to overcome this problem. This apparatus controls the redox potential in one compartment of an electrolytic cell by manipulating the current to the cell. In this manner, the concentrations of ferrous and ferric ions are maintained at their initial values for the duration of the experiment. Two types of experiments are reported in this paper. In the first, pyrite electrodes were exposed to solutions of the same bulk conditions in the presence and absence of bacteria, and their mixed potentials were determined. In the second, particulate pyrite was leached with and without bacteria to determine the effect that bacteria have on the rate of leaching. The mixed potential of bacterially dissolved pyrite decreases as microcolonies and biofilms form on the surface of pyrite electrode over a 14 day period. On the other hand, the mixed potential of chemically dissolved pyrite is constant over the same period. The results of the leaching experiments show that *Thiobacillus ferrooxidans* enhances the rate of leaching above that found in the absence of bacteria at the same conditions in solution. An electrochemical model of pyrite dissolution is derived that describes the mixed potential and the kinetics of pyrite leaching. This analysis indicates that the decrease in mixed potential and the increase in the leaching rate in the presence of bacteria are due to an increase in the pH at the surface.

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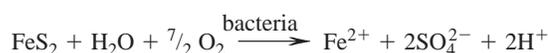
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The oxidation of pyrite, FeS₂, by bacteria is important in the extraction of metals, such as gold and cobalt, and in the formation of acid mine drainage. The bacterium *Thiobacillus ferrooxidans* was first isolated from acid mine drainage, and it is the primary microorganism associated with these processes. The rate of generation of acid from dumps and sites containing sulfide minerals is increased in the presence of iron- and sulfur-oxidizing bacteria, such as *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans*, and *Leptospirillum ferrooxidans*. These are the same bacteria that are used in the commercially successful process to extract gold from pyritic ores. The processing plant at Sansu, Ghana, treats more than 960 tons per day of pyrite concentrate with bacteria, and other processing plants have been commissioned in Australia, Brazil, and South Africa.¹ A similar processing facility will be constructed in Uganda to extract cobalt from pyritic ores.

Despite the commercial and environmental interest in the bacterial oxidation of sulfide minerals, the mechanisms of bacterial interaction with sulfide minerals remain a subject of debate. *Thiobacillus ferrooxidans* is able to oxidize ferrous ions at a rate 10⁶ times greater than the chemical oxidation of ferrous ions by dissolved oxygen.² Because ferric ions are able to oxidize sulfide minerals, the following mechanism has been proposed for the oxidation of pyrite in the presence of bacteria



The role of the bacteria in this mechanism is to regenerate the ferric ions that are consumed in the oxidation of pyrite. This mechanism has been referred to as the indirect mechanism. An alternative proposal is a mechanism by which the bacteria are able to interact directly with the mineral. It is proposed that attached bacteria oxidize the sulfide mineral in the region of attachment by a mechanism that does not involve the oxidation of the mineral by ferric ions.³⁻⁵ The direct mechanism of bacterial dissolution of pyrite can be given by the following reaction



Because bacteria attach readily to surfaces⁶ and the action of the attached bacteria may be through the ferrous-ferric ion couple, albeit in a localized area,⁷⁻¹⁰ the distinguishing feature of these mechanisms is the participation of ferric ions in the dissolution of the mineral.

Various authors have made opposing claims concerning the role these two mechanisms since Silverman and Ehrlich³ proposed the direct mechanism. For example, Boogerd *et al.*¹¹ reported that the direct mechanism dominated, whereas Espejo and Ruiz¹² claimed that the indirect mechanism dominated. Boon *et al.*¹³⁻¹⁵ found that the rate of oxygen consumption in the bacterial leaching of pyrite is similar to that for the bacterial oxidation of ferrous ions. From this result, the authors¹³⁻¹⁶ concluded that the only role of the bacteria is the oxidation of ferrous ions. However, they made no measurements of the rate of leaching, and therefore could not compare the rate of leaching in the presence and absence of bacteria.

The rate-determining step in the leaching of pyrite by either ferric ions or oxygen is a charge-transfer step,^{17,18} and the rate of bacterial oxidation of ferrous ions has been described by electrochemical kinetics.¹⁹ In spite of these findings, there have been few electrochemical studies of the bacterial leaching of pyrite. Palencia *et al.*²⁰ noted that the presence of *Thiobacillus ferrooxidans* did not influence the anodic dissolution of pyrite as studied by chronoamperometry and linear sweep voltammetry. In addition, results from their X-ray photoelectron spectroscopy (XPS) study of the pyrite surface indicated that bacteria only have a modest effect on the surface composition. Pesic and Kim²¹ noted a small difference in their cyclic voltammograms after 2 days of bacterial leaching. These differences are a measure of the changes in the concentrations of ferrous and ferric ions in solution over time. The results from both of these studies concluded that there was little difference between the bacterially treated and chemically treated pyrite electrodes.

Mustin *et al.*²² measured the mixed potential of pyrite in the presence of bacteria. They reported that that mixed potential was not equivalent to the solution redox potential after about 160 h of leaching. As with the work of Pesic and Kim,²¹ however, it is difficult to interpret these results because the concentrations of ferrous and ferric ions changed throughout the course of the experiment. Indeed, in

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most of the experiments described in the literature the concentration profiles of ferrous and ferric ions with bacteria differ significantly from those without bacteria. This makes it difficult to determine the mechanism of bacterial action in the leaching process.

Because the mixed potential is influenced by the half-reactions occurring at the surface, a comparison of the mixed potentials of pyrite samples that are subjected to chemical and bacterial leaching may provide valuable information on the mechanism of bacteria action if the concentrations of species in solution are maintained at a constant value. We report here the results of such a study. The conditions in the bulk solution concentrations are controlled at a constant value in a specially designed electrochemical cell.^{23,24} In addition, we report results of leaching experiments in the same apparatus^{23,24} which support our electrochemical measurements. We propose a novel mechanism that accounts for these results.

Experimental

Description of reagents.—Single crystals of pyrite were obtained from Wards Natural Science Establishment, Inc., Rochester, NY, for use as electrodes. A sample of ground pyrite was obtained from the Bureau de Recherches Géologiques et Minières (BRGM), Orleans, France. This ground pyrite contained 40.3% iron and 1.4% cobalt. The cobalt was uniformly disseminated throughout the pyrite, so that the amount of iron dissolved was directly proportional to the amount of cobalt dissolved.²⁵ Ore in the size fraction $-53 +45 \mu\text{m}$ was used. Analytical-grade reagents were used in the experimental investigation. Doubly distilled water was used to prepare all solutions. Ultrahigh purity nitrogen was used during the electrochemical characterization of pyrite oxidation.

The bacterial sample, a pure strain (FC1) of *Thiobacillus ferrooxidans*, was obtained from D. Rawlings (University of Cape Town). A stock culture of the bacteria was maintained in a solution which consisted of 133 g/L ground pyrite, 50 g/L $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 1.5 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.5 g/L K_2HPO_4 , and 0.5 g/L MgSO_4 .²⁶ The value of the pH was adjusted to 1.6 by the addition of sulfuric acid. The temperature was maintained at 35°C. Air was sparged to the vessel, and the solution was agitated by a Teflon-coated impeller driven by an overhead motor. The bacteria in this stock culture were maintained in an active state by removing 85% of the solution and replacing it with fresh solution and pyrite every 48 h.

Apparatus.—The apparatus was the controlled redox potential reactor described in previous publications.^{23,24} In this apparatus, an experiment is conducted in one compartment (working compartment) of a two-compartment electrolysis cell. The redox potential in the working compartment is maintained at a constant value by manipulating the electrolysis current. Therefore, the bulk solution conditions are maintained at their initial values for the duration of the experiment. This allowed the preparation of pyrite samples and the leaching of pyrite in the presence and absence of bacteria in solutions that were the same for the duration of the experiment. The apparatus is illustrated in Fig. 1.

The controlled redox potential reactor consisted of an electrolysis cell, partitioned into two compartments by an anion-exchange membrane, with platinum electrodes in each compartment. The working compartment, which had a volume of either 3.5 or 2.0 L, was stirred by a Teflon-coated stainless steel impeller driven by an overhead motor. The electrolysis cell was placed in a constant-temperature bath and the temperature was maintained at $35 \pm 0.1^\circ\text{C}$.

The redox potential of the working compartment was measured using a platinum electrode and a saturated calomel electrode (SCE). This measurement was galvanically isolated and amplified, and its value was recorded by an analog/digital control card in a personal computer. The electrolysis cell was connected in series to a 25 V, 40 A power supply and a variable resistor. A computer program determined the adjustment of the variable resistor (and hence the current) by the implementation of a feedback control mechanism.

Pyrite electrodes were cut from single crystals and electrical contact was made by attaching a brass strip to the back of the pyrite electrode with silver-impregnated glue. The pyrite electrodes were mount-

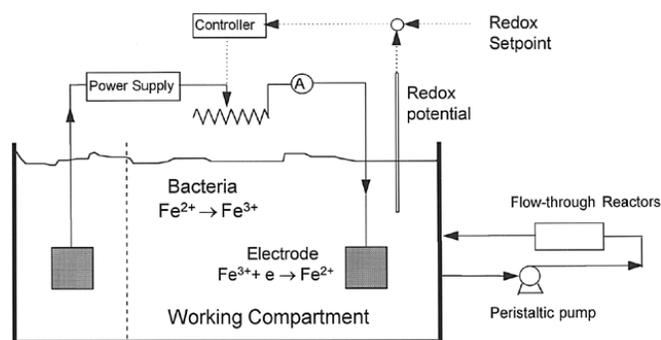


Figure 1. A schematic diagram of the experimental apparatus, showing the controlled redox reactor, and the flow-through reactors. The bacteria were grown in the working compartment, and the redox potential is controlled by the manipulation of the current. Solution from the working compartment was pumped to the flow-through reactors. The same apparatus, without the flow-through reactors, was used for the leaching studies.

ed in araldite resin, then polished and rinsed in acetone and distilled water. The bulk resistance of the pyrite electrodes was less than 1 Ω .

The pyrite electrodes were placed in flow-through reactors. A glass cover slip on one side formed an observation window. The dimensions of the chamber once the pyrite electrode was in place were $40 \times 12 \times 1.0 \text{ mm}$. Solution was pumped from the working compartment of the controlled redox reactor to the flow-through reactors, arranged in parallel, using a peristaltic pump. A flow-through reactor is illustrated in Fig. 2.

The mixed-potential measurements were conducted in a jacketed vessel. The solution was agitated by a magnetic stirrer, and the temperature was controlled at 35°C. The mixed potential of the pyrite electrodes and the redox potential of the solution were measured with respect to SCE using a high-impedance voltmeter.

Scanning electron microscopic (SEM) observations were conducted on a JEOL 840 scanning electron microscope. The bacterially leached samples were fixed by placing the pyrite samples in a 3% glutaraldehyde solution for 12 h. The pyrite samples were dried for 10–15 min in a series of ethanol-aqueous solutions ranging from 20 to 100% ethanol. The pyrite samples were then dried at the critical point and coated with gold and palladium. X-ray photoelectron spectroscopy (XPS) was conducted on samples using a Physical Electronics Quantum 2000 scanning electron spectroscopy for chemical analysis microprobe spectrometer. The C 1s peak was monitored for shifts in binding energy. The samples were also analyzed by Raman spectroscopy using a Jobin-Yvon T64000 spectrometer.

The solution samples from the leaching experiments were analyzed for cobalt and iron (total) using an atomic absorption spectrophotometer. The concentration of ferrous ions in solution was determined by titration with potassium dichromate using sodium diphenylamine as the indicator.

Procedure.—*Mixed-potential measurements.*—The mixed-potential experiments with bacteria were conducted in the following man-

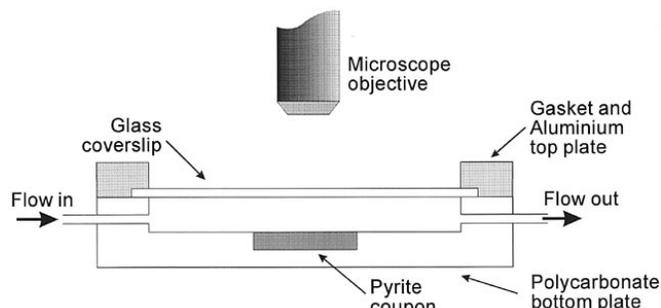


Figure 2. A detailed diagram of a flow-through reactor, indicating the positioning of the pyrite electrode.

ner. The working compartment of the electrolysis cell was filled with 3 L of solution from the bacterial stock culture containing 9 g/L total iron and nutrients. Once the desired redox potential was achieved, the computer program, which controlled the redox potential at the setpoint value, was initiated. Air was sparged into the working compartment in the bacterial experiment.

The redox potential, pH, dissolved oxygen concentration, and bacterial number were measured daily. The pH was controlled to within 0.05 pH unit by the addition of sulfuric acid, and the redox potential was controlled to within 5 mV of the setpoint value over 14 days. The oxygen concentration in the bacterial experiment ranged between 4.9 and 5.7 mg/L. The bacterial numbers typically increased from 7×10^8 to 3×10^9 cells/mL.

The same procedure was followed in the mixed-potential experiments without bacteria, except that nitrogen was sparged into the working compartment to maintain sterile conditions. The removal of air from the solution did not affect the redox potential or the mixed potential of pyrite. Solution samples from the chemical experiments were examined under a light microscope on a daily basis. No bacteria contaminated the chemical experiments.

After 14 days the pyrite electrodes were removed from the flow-through reactors, and immediately placed in a sterile solution of pH 1.45. The mixed potential was measured at constant concentration of ferrous ions (0.001 M) at various concentrations of ferric ions (0.001 to 0.2 M). The concentration of ferric ions was increased by progressively adding ferric sulfate to the solution. The values of the mixed potentials were recorded when the reading stabilized. The pH changed from 1.45 at 0.001 M Fe^{3+} to 1.31 at 0.2 M Fe^{3+} . This change in the pH had little effect on the value of the mixed potential in this concentration and pH range. A similar set of measurements was performed to determine the effect of ferrous sulfate on the mixed potential.

Leaching studies.—Leaching experiments with the ground pyrite ore were conducted using the controlled redox-potential apparatus to maintain the concentrations of ferrous and ferric ions at their initial values throughout the experiment. The working compartment was filled with 2 L of solution containing the ferrous and ferric sulfate and the medium. The experiment was initiated by adding 20 g of ground pyrite into the working compartment. This amount of pyrite was added so that the iron leached changed the total concentration of iron in the working compartment by less than 5%. The redox potential was controlled to within 1 mV of the initial value. Typical results indicated that the concentration of ferrous ions differed from the initial value by less than 2%. The pH was adjusted by the manual addition of sulfuric acid or sodium hydroxide to within 0.05 pH units of the initial value. Samples were withdrawn at regular intervals and analyzed for cobalt, total iron, and ferrous ions. Because there was a direct correspondence between the amount of iron and the amount of cobalt released, the cobalt analysis allowed the conversion of pyrite to be determined. Between successive experiments the electrolysis cell was soaked in a hydrochloric acid solution, cleaned with an ammonia-based solution (pH 10), and finally rinsed in distilled water.

Results

The effect of leaching time on the mixed potential of pyrite.—The effect of bacterial action on the mixed potential of pyrite is shown in Fig. 3. Pyrite electrodes were leached chemically and bacterially in the flow-through reactors for a period of 7 days. The solutions for both chemical and bacterial experiments were the same: the total iron concentration was 9 g/L, the redox potential was 650 mV vs. SCE, and the pH was 1.6. The pyrite electrodes were removed from the flow-through reactors each day, placed immediately in a sterile solution of the same composition, and the mixed potential was measured. The results, presented in Fig. 3, show that the mixed potential of the chemically treated pyrite was constant with time exposed to the leaching solution. In contrast, the mixed potential of the bacterially treated pyrite decreased with time exposed to the leaching solution. Notice also that the value of the mixed potential of the chemically and bacterially treated electrodes differs significantly from the redox potential.

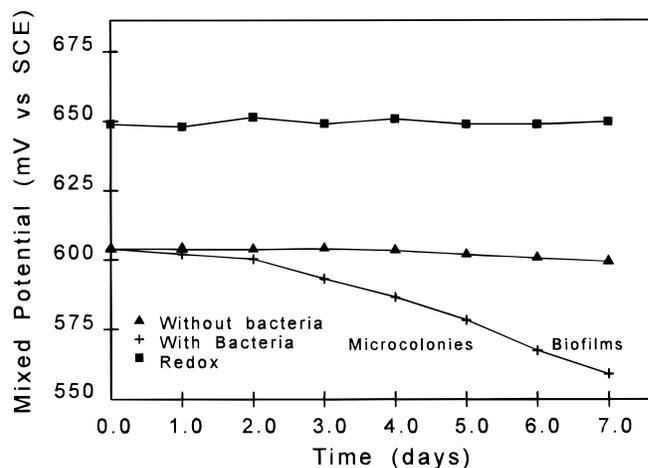


Figure 3. The mixed potentials of the pyrite samples leached in the presence and absence of bacteria. The solution conditions for both tests were 9 g/L total iron, redox potential 650 mV (vs. SCE), pH 1.6, and 35°C.

Pyrite electrodes that had been exposed to the bacteria solution were examined using SEM. This analysis showed that the colonization of the surface by bacteria occurs through the attachment of single cells, the development of microcolonies, and the formation of biofilms, in a manner described previously.^{8,27} The periods illustrated in Fig. 3 depict the times at which microcolonies and biofilms were first observed on the pyrite surface. During the period of attachment of single cells, the mixed potential of the chemically and bacterially treated pyrite electrodes differed by a few millivolts. As the surface became more colonized by bacteria, the mixed potentials of the chemically and bacterially leached pyrite deviated significantly. Precipitates, which were identified by SEM, XPS, and Raman spectroscopy as mixtures of jarosite, iron oxides, and hydrated ferric sulfate species, were observed with the microcolonies and the biofilm. Precipitates were not found on the surface of chemically treated pyrite. It should also be noted that the SEM study showed the presence of only rod-shaped bacteria; no contamination by the spiral-shaped *Leptospirillum ferrooxidans* was observed.

Previous authors have argued that bacteria initiate corrosion pits,^{9,28,29} whereas others¹⁰ claimed that the secretion of phospholipids is central to the leaching reaction in the presence of bacteria. Corrosion pits were observed on the surface of the pyrite under SEM during the initial stages of bacterial dissolution. The bacterial biofilm was removed by ultrasonic cleaning from the surface of the pyrite that had been bacterially leached for 14 days, so that a comparison of the underlying surface could be made with the surface of the chemically dissolved pyrite. The corrosion features of these two surfaces were similar,³⁰ which suggests that the dissolution mechanism is the same in both cases.

The surfaces of chemically treated and bacterially treated pyrites were examined by Raman spectroscopy. The Raman spectrum of the chemically treated pyrite had large peaks at 341 and 378 cm^{-1} , and a very weak broad peak at about 465 cm^{-1} . The peaks at 341 and 378 cm^{-1} are associated with the pyrite, whereas the peak at 465 cm^{-1} is associated with polysulfides on the pyrite surface. No peaks associated with elemental sulfur were detected. The Raman and XPS spectra for bacterially treated pyrite indicated the presence of ferric hydroxide precipitates in the biofilm. After the biofilm was removed by ultrasonic cleaning, the Raman spectrum had a small peak associated with sulfur at 219.4 cm^{-1} , large peaks associated with pyrite at 342.2 and 378.8 cm^{-1} , and small peaks associated with polysulfides 451.9 and 468.8 cm^{-1} . These results indicated that there was no difference in the surface composition of the chemically treated pyrite and the bacterially treated pyrite (after ultrasonic cleaning), except for the small amount of sulfur detected on the bacterially treated pyrite.

The effect of the concentration of ferric ions on the mixed potential of pyrite.—The effect of the concentration of ferric ions on the mixed potentials of chemically and bacterially treated pyrite after 14 days is shown in Fig. 4 for pyrite leached in a solution with a redox potential of 650 mV vs. SCE. A similar set of results is shown in Fig. 5 for pyrite leached in a solution with a redox potential of 600 mV vs. SCE.

At low concentrations of ferric ions, the mixed potential of the bacterially dissolved pyrite sample is independent of the concentration of ferric ions. At higher concentrations, the mixed potential increases with increasing concentration of ferric ions. The mixed potential of the chemically dissolved pyrite increases over the entire range. The mixed potential of the chemically treated pyrite is similar to the solution redox potential in this concentration range.

It is significant that the mixed potentials of the bacterially dissolved pyrite are less than those of the chemically dissolved pyrite. The biofilm was removed from this electrode by ultrasonic treatment, and the mixed potential was again measured over this range of concentration of ferric ions. These results are shown in Fig. 6. The mixed potentials after ultrasonic cleaning assume values that are closer to those measured for the chemically dissolved pyrite. This confirms

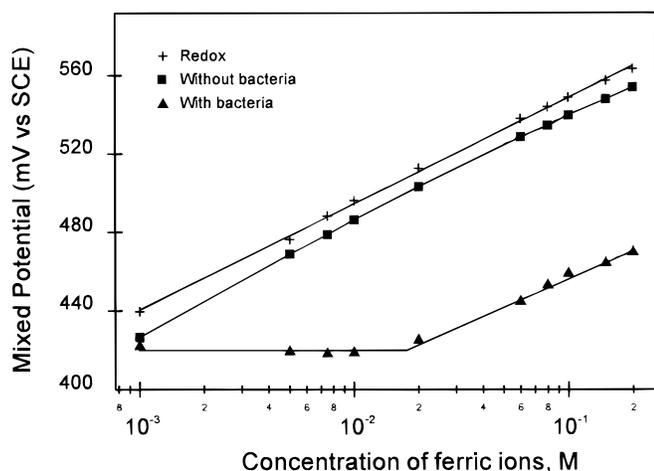


Figure 4. The effect of the concentration of ferric ions on the mixed potentials of pyrite leached at 650 mV (vs. SCE) with a pH of 1.6 in the presence and absence of *Thiobacillus ferrooxidans*. Conditions of mixed-potential measurements: 0.001 M Fe²⁺, pH 1.45, and 35°C.

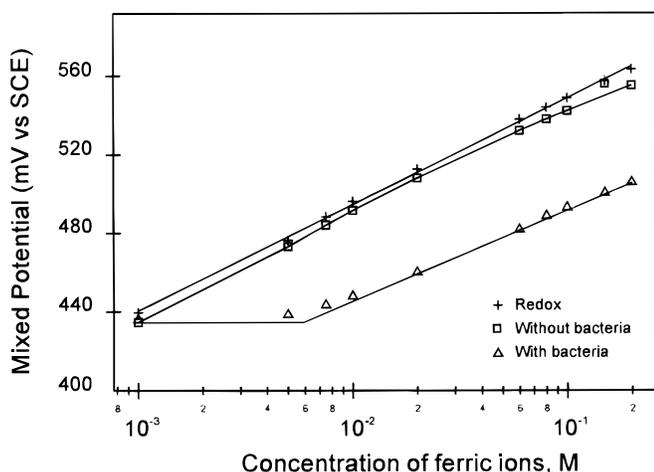


Figure 5. The effect of the concentration of ferric ions on the mixed potentials of pyrite leached at 600 mV (vs. SCE) with a pH of 1.5 in the presence and absence of *Thiobacillus ferrooxidans*. Conditions of mixed-potential measurements: 0.001 M Fe²⁺, pH 1.45, and 35°C.

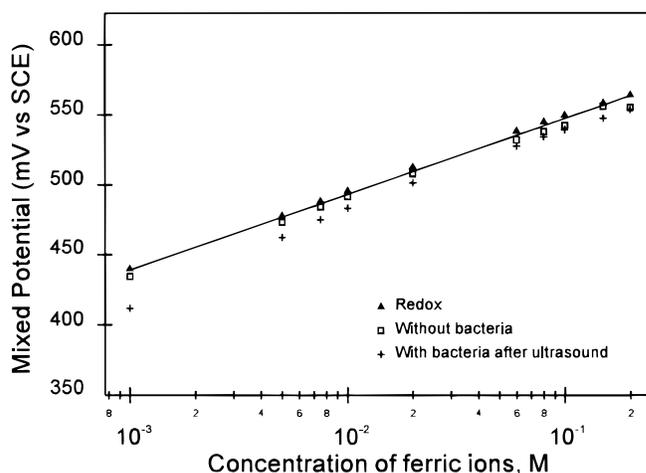


Figure 6. The effect of removing the attached bacteria and biofilm from the pyrite surface by ultrasonic cleaning on the mixed potential. Conditions of leaching: redox potential 600 mV vs. SCE, pH 1.5, and 35°C. Conditions of mixed-potential measurements: 0.001 M Fe²⁺, pH 1.45, and 35°C.

that the processes occurring within the biofilm are those responsible for the observed difference between the two electrodes. The small difference between the chemically treated pyrite and the bacterially leached sample after ultrasonic treatment suggests that singularly attached bacteria or exopolymeric substances (polysaccharides, sugars, organic acids, etc.) may persist on the pyrite surface.

The effect of the concentration of ferrous ions on the mixed potential of pyrite.—The effects of the ferrous concentrations on the mixed potentials of chemically and bacterially dissolved pyrite are shown in Fig. 7 and 8. The mixed potential of the bacterially dissolved pyrite is less than that of the chemically dissolved pyrite at low concentrations of ferrous ions. The mixed potential is independent of the concentration of ferrous ions between approximately 0.0001 and 0.01 M. When the concentration of ferrous ions in the bulk solution is higher than approximately 0.04 M, the mixed potentials of the chemically and bacterially dissolved pyrite assume the same values.

Leaching of pyrite at controlled solution conditions.—The results of the experiments using the controlled redox reactor on the leaching of ground pyrite in the presence and absence of *Thiobacillus ferrooxidans* are shown in Fig. 9. These results indicate that the

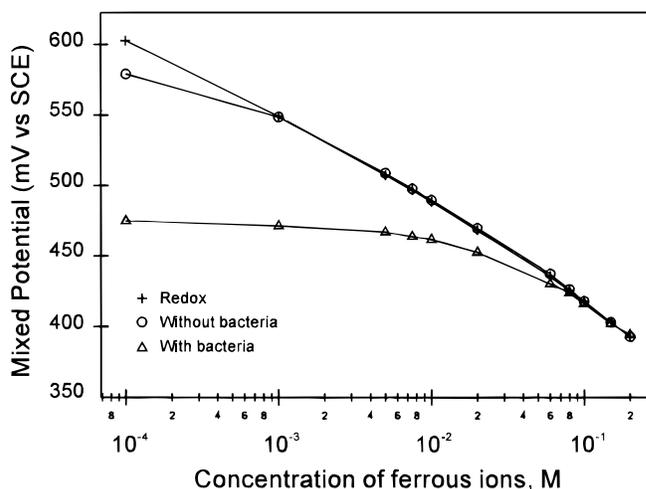


Figure 7. The effect of the concentrations of ferrous ions on the mixed potentials of pyrite leached at 650 mV (vs. SCE) with a pH of 1.6 in the presence and absence of *Thiobacillus ferrooxidans*. Conditions of mixed-potential measurements: 0.1 M Fe³⁺, pH 1.45, and 35°C.

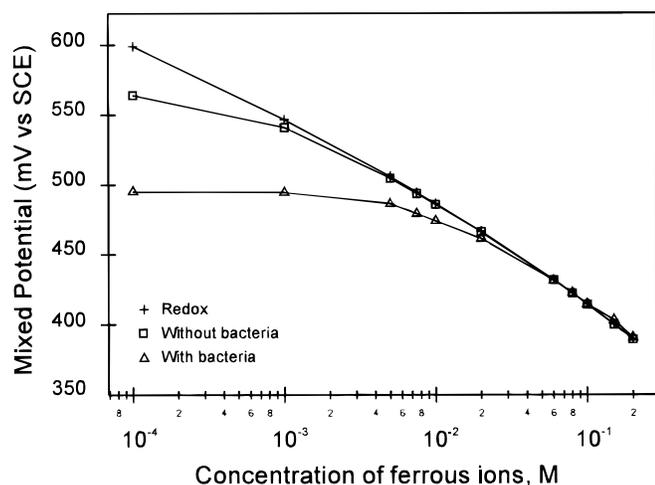


Figure 8. The effect of the concentrations of ferrous ions on the mixed potentials of pyrite leached at 600 mV (vs. SCE) with a pH of 1.5 in the presence and absence of *Thiobacillus ferrooxidans* for 14 days. Conditions of mixed-potential measurements: 0.1 M Fe^{3+} , pH 1.45, and 35°C.

presence of bacteria increases the rate of leaching of pyrite by a factor of between 1.33 and 1.9. Twenty sets of chemical and bacterial leaching experiments have been conducted at different conditions in which the concentrations were maintained at their initial values. All experiments yielded the same result: that leaching of pyrite in the presence of bacteria occurs at a higher rate than in the absence of bacteria. These results contrast with our results on the leaching of zinc sulfide using the constant redox reactor, in which we found that there was no increase in the rate of leaching in the presence of *Thiobacillus ferrooxidans* at high concentrations of ferric ions.²⁴ In addition, these results are contrary to much of the current thinking in the field.^{13-16,31,32}

The effects of the concentration of ferric ions and the pH on the rate of leaching were investigated. The order of reaction with respect to ferric ions is 0.50 with bacteria and 0.51 without bacteria. The order of reaction with respect to H^+ is -0.40 with bacteria and -0.50 without bacteria. Changes in the concentration of ferric ions did not affect the degree to which the bacteria increased the rate, whereas changes in the pH did affect the bacterial enhancement of the rate.

Discussion

Development of a model of the mixed potential of pyrite.—The mixed potential of pyrite is a result of the anodic and cathodic half-

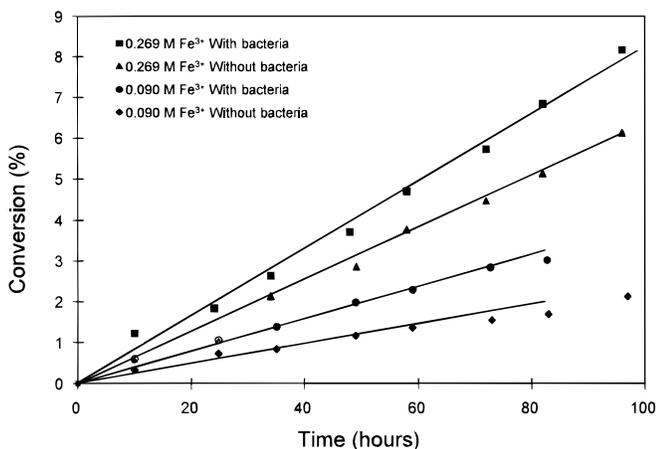


Figure 9. The leaching of pyrite in the presence and absence of *Thiobacillus ferrooxidans*. Conditions: 0.0179 M Fe^{2+} , pH 1.3, and 35°C.

reactions that occur simultaneously on the surface. These half-reactions, in the absence of bacteria, are the oxidation of pyrite to ferrous and sulfate ions and the reduction of ferrous ions at the pyrite surface, given by



The anodic oxidation of pyrite, given by Eq. 1, has been studied in detail.^{17,30,33,34-38} Both sulfur and sulfate are formed as reaction products, and the sulfate yield increases as the potential increases.^{30,37} The current density, i , is a function of potential, E , and the concentration of H^+ , and is given by^{17,30,33-38}

$$i_{\text{FeS}_2} = k_{\text{FeS}_2} [\text{H}^+]^{-n} \exp\left(\frac{\alpha_{\text{FeS}_2} FE}{RT}\right) \quad [3]$$

where k_{FeS_2} is the rate constant, n is the H^+ reaction order, α_{FeS_2} is the transfer coefficient, F is the Faraday constant, R is the gas constant, and T is the temperature. The value of n has been found in electrochemical studies to be about 0.5, and the value of α_{FeS_2} falls between 0.6 and 0.7.^{17,30,33-38}

The oxidation and reduction of dissolved iron at the pyrite surface, given by Eq. 2, is described by the Butler-Volmer equation³⁰

$$i_{\text{Fe}} = k_{\text{Fe}^{2+}} [\text{Fe}^{2+}] \exp\left(\frac{\alpha_{\text{Fe}} FE}{RT}\right) - k_{\text{Fe}^{3+}} [\text{Fe}^{3+}] \exp\left(\frac{-(1 - \alpha_{\text{Fe}}) FE}{RT}\right) \quad [4]$$

where the value of α_{Fe} has been found to be close to 0.5 for the reduction of ferric ions on pyrite.³⁰

Because there is no net current during leaching and the surface area for both half-reactions is the same, $i_{\text{FeS}_2} = -i_{\text{Fe}}$ at the mixed potential E_m . Equating the two expressions given in Eq. 3 and 4 gives an implicit expression for the mixed potential, E_m

$$k_{\text{FeS}_2} [\text{H}^+]^{-1/2} \exp\left(\frac{\alpha_{\text{FeS}_2} FE_m}{RT}\right) + k_{\text{Fe}^{2+}} [\text{Fe}^{2+}] \exp\left(\frac{\alpha_{\text{Fe}} FE_m}{RT}\right) = k_{\text{Fe}^{3+}} [\text{Fe}^{3+}] \exp\left(\frac{-(1 - \alpha_{\text{Fe}}) FE_m}{RT}\right) \quad [5]$$

If the approximation is made that $\alpha_{\text{FeS}_2} \approx \alpha_{\text{Fe}} = \alpha$, we may simplify this expression to obtain the following explicit expression for the mixed potential

$$E_m = \frac{RT}{2\alpha F} \ln\left(\frac{k_{\text{Fe}^{3+}} [\text{Fe}^{3+}]}{k_{\text{FeS}_2} [\text{H}^+]^{-1/2} + k_{\text{Fe}^{2+}} [\text{Fe}^{2+}]}\right) \quad [6]$$

Equation 6 indicates that if $k_{\text{FeS}_2} [\text{H}^+]^{-1/2} \gg k_{\text{Fe}^{2+}} [\text{Fe}^{2+}]$, for example at low concentrations of ferrous ions, the mixed potential is independent of the concentration of ferrous ions. If $k_{\text{FeS}_2} [\text{H}^+]^{-1/2} \ll k_{\text{Fe}^{2+}} [\text{Fe}^{2+}]$, then the mixed potential is determined by the concentrations of the ferrous and ferric ions in solution.

Previous workers have erroneously equated the mixed potential with the redox potential.^{16,39} The redox potential, E_{redox} , is given by

$$E_{\text{redox}} = E_{\text{redox}}^0 + \frac{RT}{F} \ln \frac{a(\text{Fe}^{3+})}{a(\text{Fe}^{2+})} \quad [7]$$

where $a(\text{Fe}^{3+})$ and $a(\text{Fe}^{2+})$ are the activities of the ferric and ferrous species, respectively. It is clear from Eq. 6 and 7 that the mixed potential of pyrite is not the same as the redox potential in ferric sulfate solutions. The redox potential is a thermodynamic measurement; the mixed potential is a kinetic measurement. The term $k_{\text{FeS}_2} [\text{H}^+]^{-1/2}$, associated with the dissolution of pyrite, appears in the denominator of Eq. 6, but does not appear in the Nernst equation for the redox potential, given by Eq. 7. The difference between the

two potentials is largest at the conditions found in bacterial leaching reactors where the concentration of ferrous ions is low. This is shown clearly in Fig. 3, where the redox potential is approximately 650 mV and the mixed potential of the chemically dissolved pyrite is about 604 mV.

An expression for the rate of leaching, r_{FeS_2} , may be obtained by substituting the expression for E_m given by Eq. 6 into Eq. 3, and noting that r_{FeS_2} and i_{FeS_2} are related by Faradays law. Therefore, the rate of leaching is given by

$$r_{\text{FeS}_2} = \frac{k_{\text{FeS}_2} [\text{H}^+]^{-1/2}}{14F} \left(\frac{k_{\text{Fe}^{3+}} [\text{Fe}^{3+}]}{k_{\text{FeS}_2} [\text{H}^+]^{-1/2} + k_{\text{Fe}^{2+}} [\text{Fe}^{2+}]} \right)^{1/2} \quad [8]$$

An examination of this analysis indicates how the concentrations in solution affect the rate of leaching and the mixed potential. An increase in the concentration of ferric ions results in an increase in the mixed potential and in the rate of dissolution. In addition, an increase in the mixed potential indicates an increase in the rate of leaching if $k_{\text{FeS}_2} [\text{H}^+]^{-1/2} \ll k_{\text{Fe}^{2+}} [\text{Fe}^{2+}]$. However, if $k_{\text{FeS}_2} [\text{H}^+]^{-1/2} \gg k_{\text{Fe}^{2+}} [\text{Fe}^{2+}]$, a decrease in the mixed potential may indicate an increase in the rate of leaching. Therefore, an increase in the mixed potential does not always indicate an increase in the rate of leaching.

Under the conditions shown in Fig 3, the concentration of ferrous ions is low, because the value of the redox potential is high. Under these conditions $k_{\text{FeS}_2} [\text{H}^+]^{-1/2} \gg k_{\text{Fe}^{2+}} [\text{Fe}^{2+}]$, which means that the decrease in mixed potential in the presence of bacteria indicates an increase in the rate of leaching, as shown in Fig. 9. The role of bacteria is discussed in detail later.

Equation 8 is in agreement with the empirical rate expressions obtained from the literature on the chemical leaching of pyrite. An expression of the same form of Eq. 8 has not been proposed previously for pyrite, and therefore such an expression has not been fitted to the leaching data. McKibben and Barnes⁴⁰ found that the order of reaction was 0.5 with respect to Fe^{3+} and -0.5 with respect to H^+ , whereas Williamson and Rimstidt⁴¹ found that the reaction order was 0.3 with respect to Fe^{3+} , -0.32 with respect to H^+ , and -0.47 with respect to Fe^{2+} . The reactions orders obtained in this work for the chemical leaching of pyrite are in agreement with those of McKibben and Barnes.⁴⁰ The reaction orders with respect to ferric and ferrous ions are broadly in agreement with Eq. 8. An examination of the two limiting forms of Eq. 8 can explain the difference in the reaction order with respect to H^+ found by McKibben and Barnes⁴⁰ and that found by Williamson and Rimstidt.⁴¹ If $k_{\text{FeS}_2} [\text{H}^+]^{-1/2} \ll k_{\text{Fe}^{2+}} [\text{Fe}^{2+}]$, the reaction order with respect to H^+ is -0.5 , in agreement with McKibben and Barnes⁴⁰ and this work; on the other hand, if $k_{\text{FeS}_2} [\text{H}^+]^{-1/2} \gg k_{\text{Fe}^{2+}} [\text{Fe}^{2+}]$, the reaction order with respect to H^+ is -0.25 , in agreement with Williamson and Rimstidt.⁴¹

Mixed potential of chemically leached pyrite.—The results given in Fig. 4, 5, 7, and 8 for the leaching of pyrite without bacteria are in general agreement with Eq. 6. The deviation from a straight line at low concentrations of ferrous ion (at 0.001 M) shown in Fig. 5 and 8 indicates that $k_{\text{FeS}_2} [\text{H}^+]^{-1/2} \ll k_{\text{Fe}^{2+}} [\text{Fe}^{2+}]$ in the chemical leaching of pyrite when the concentration of ferrous ions is greater than 0.001 M and the pH is less than 1.45.

Equation 6 indicates that the slope of the mixed potential with respect to $\ln([\text{Fe}^{3+}])$ and $\ln([\text{Fe}^{2+}])$ should be RT/F and $-RT/F$, respectively, if it is assumed that α has a value close to 0.5. This is also the value expected for the slope of the redox potential, if it is assumed that the activities of ferric and ferrous ions are approximately equal to their concentrations (see Eq. 7). At 35°C the value of RT/F is 26.5 mV. The values of slopes of the mixed potential and the redox potential given in Fig. 4 and 7 are 23 and -30 mV, respectively. The redox potential is a thermodynamic measurement that is dependent on the activities of the species in solution. As the slopes for the redox potential are not equal to RT/F , the deviation of the slopes of the mixed potential from RT/F is not a feature of the pyrite half-reaction, but is a result of conditions in solution such as the for-

mation of iron sulfate complexes in solution. The concentrations and activities of the ferric sulfate and ferric hydroxide species were calculated by a computer program using the method and values suggested by Filippou *et al.*,⁴² and a theoretical value for the redox potential was calculated. The values for the slopes of the redox potential obtained from this simulation were 24.6 mV with respect to ferric ions and -28.2 mV with respect to ferrous ions.³⁰

The mixed potential of chemically leached pyrite was measured as a function of pH at 0.025 M Fe^{3+} and 0.0001 M Fe^{2+} and 25°C. Under these conditions, the mixed potential differs significantly from the redox potential. The mixed potential was linear with pH in the range 1.6 to 2.0, with a slope of -0.029 mV/pH unit. The expected dependence of the mixed potential on the pH given by Eq. 6 is -30.6 mV/pH unit. At pH values below 1.6, the mixed potential was almost independent of the pH. The dependence of the mixed potential on the pH corresponds closely with that given by Eq. 6.

It is concluded from this discussion that the electrochemical (or mixed-potential) model developed here describes the results for the mixed potentials, and gives the correct rate expression for the chemical leaching of pyrite reported previously in the literature.

Mixed potential of bacterially leached pyrite.—The data given in Fig. 3 and 9 may be a result of the bacteria affecting the chemical conditions at the pyrite surface, or it may be an indication that the bacteria dissolve the pyrite by a direct mechanism.

Although the reaction pathways for the direct mechanism of bacterial leaching are unclear, it is possible to explain their effect on the mixed potential of pyrite. The direct mechanism is that in which ferric sulfate does not play a role in the dissolution reaction. In order for dissolution to occur by such a mechanism, the bacteria must replace ferric sulfate with another oxidant, presumably of greater oxidation potential than that of ferric sulfate. This means that the direct bacterial mechanism contributes to the enhancement of the reduction half-reaction in the dissolution of pyrite by substituting ferric sulfate with a more powerful oxidant. For example, the bacteria may secrete a substance that oxidizes the surface. A further possibility is that the electrons produced in the anodic dissolution reaction, Eq. 1, are captured directly by enzymes of the bacterial cell wall and are transported through the respiratory pathway to the terminal oxidase. In this way, the enzymes of the cell wall act as the oxidant, rather than ferric ions.

The mixed potential analysis leading to the derivation of Eq. 6 is general and can describe the dissolution of minerals using different oxidants.¹⁸ This analysis indicates that the replacement of ferric sulfate by any biochemical oxidant results in the replacement of the term $k_{\text{Fe}^{3+}} [\text{Fe}^{3+}]$ in Eq. 6 and 8 by a similar term, *e.g.*, $k_B [\text{Ox}]$, where $[\text{Ox}]$ is the concentration of the biochemical oxidant. Biochemical enhancement or catalysis of the reduction half-reaction means that the value of $k_B [\text{Ox}]$ is higher than $k_{\text{Fe}^{3+}} [\text{Fe}^{3+}]$, which should result in an increase in the mixed potential of the pyrite and an increase in the rate of reaction. Therefore, if the direct mechanism increased the rate of leaching, there should be a corresponding increase in the mixed potential. However, the data clearly show that the mixed potential decreases and the rate of reaction increases in the presence of *Thiobacillus ferrooxidans*. Additional oxidants or the catalysis of the reduction half-reaction cannot account for the results shown in Fig. 3 and 9. The results shown in Fig. 3 and 9 exclude biological oxidation of the pyrite as a possible cause for the enhanced rate of dissolution.

Figures 7 and 8 indicate that the mixed potential of the bacterially leached electrode is the same as that for the chemically leached electrode at high concentrations of ferrous ions. At low concentrations of ferrous ions, the mixed potential is lower than that of the chemically leached sample, and it is independent of the concentration of ferrous ions. Examination of Eq. 6 indicates that the first term in the denominator of Eq. 6 is more prominent in the case of the bacterially leached electrode than in that of the chemically leached electrode. This can arise if there is either an increase in the pH or a decrease in the ferrous ion at the pyrite surface. However, a decrease in the concentration of ferrous ions results in an increase in the mixed potential, so that the potential of the bacterially treated electrode

would be more positive than that of the chemically treated electrode. As Fig. 3 indicates that this is not the case, it is concluded that the reason for the difference in the mixed potential of the bacterially and chemically treated pyrite is that the bacteria cause the pH at the pyrite surface to be higher than it is in the absence of bacteria. This results in the condition that $k_{\text{FeS}_2}[\text{H}^+]^{-1/2} \gg k_{\text{Fe}^{2+}}[\text{Fe}^{2+}]$, which is consistent with the data of Fig. 3 and 9.

The conclusion that the pH at the surface is higher in the presence of bacteria is supported by the observation of jarosite and ferric hydroxide precipitates within the biofilm. These precipitates are not found in the bulk solution, nor are they found on the pyrite surface in the corresponding chemical experiment.

The mixed potential of the bacterially treated pyrite shown in Fig. 4 and 5 is independent of the concentration of ferric ions at values below about 0.01 M, probably due to the ferric ions bound as organic complexes in the biofilm.

If the effect of the bacteria on the leaching of pyrite is to increase the pH at the surface of the pyrite, then Eq. 8 indicates that the order of reaction with respect to H^+ should be affected. The leaching results showed that this was the case. The reaction order with respect to H^+ without bacteria is -0.5 , whereas with bacteria it is -0.40 . This change in reaction order is consistent with the explanation of the mixed potential data. In addition, this model suggests that the reaction order with respect to the ferric ions should be unchanged, which is the case. The reaction order with respect to ferric ions with bacteria is -0.50 and without bacteria is -0.51 .

An investigation of the leaching of zinc sulfide (sphalerite) in the presence of *Thiobacillus ferrooxidans* using the same leaching apparatus as this study did not find enhanced rates of leaching in spite of evidence of bacterial attachment to the particles of zinc sulfide.²⁴ However, the rate of leaching of zinc sulfide is not affected by the pH in this range.^{43,44} Therefore, changes in the pH at the mineral surface by attached bacteria are not expected to affect the rate of leaching of zinc sulfide. The result found with zinc sulfide therefore serves as support for the proposed mechanism.

The results from the mixed potential and leaching experiments are in agreement: attached bacteria affect the pyrite surface significantly by increasing the pH at the surface. Possible mechanisms by which the bacteria raise the pH at the surface are the consumption of acid in the oxidation of ferrous ions by the bacteria, and the buffering action of the exopolymers secreted on the surface. The increase in the pH at the surface increases rate of leaching and decreases the mixed potential of pyrite.

Conclusions

Pyrite samples were chemically and bacterially dissolved under the same bulk solution conditions for 14 days, and their mixed potentials were measured. The formation of microcolonies of *Thiobacillus ferrooxidans* resulted in the mixed potential of the bacterially dissolved pyrite becoming less than the mixed potential of the chemically dissolved pyrite. The etching patterns of the pyrite surface below the biofilm and those of the surface of the chemically leached sample were similar, suggesting a similar mechanism of leaching.

Ground pyrite was leached in the presence and absence of *Thiobacillus ferrooxidans* under the same bulk conditions. The results of these leaching experiments indicate that the rate of leaching with bacteria is higher than leaching without bacteria at the same conditions in the bulk solution.

The results were analyzed using the mixed-potential theory of leaching.¹⁸ It is argued that the only consistent explanation is that attached bacteria increase the pH at the pyrite surface. An increase in the pH at the pyrite surface accounts for the decreased mixed potentials and the increased rates of leaching.

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