

Rate Equations and Kinetic Parameters of the Reactions Involved in Pyrite Oxidation by *Thiobacillus ferrooxidans*

HECTOR M. LIZAMA AND ISAMU SUZUKI*

Department of Microbiology, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2

Received 1 June 1989/Accepted 23 August 1989

Rate equations and kinetic parameters were obtained for various reactions involved in the bacterial oxidation of pyrite. The rate constants were $3.5 \mu\text{M Fe}^{2+}$ per min per FeS_2 percent pulp density for the spontaneous pyrite dissolution, $10 \mu\text{M Fe}^{2+}$ per min per mM Fe^{3+} for the indirect leaching with Fe^{3+} , $90 \mu\text{M O}_2$ per min per mg of wet cells per ml for the *Thiobacillus ferrooxidans* oxidation of washed pyrite, and $250 \mu\text{M O}_2$ per min per mg of wet cells per ml for the *T. ferrooxidans* oxidation of unwashed pyrite. The K_m values for pyrite concentration were similar and were 1.9, 2.5, and 2.75% pulp density for indirect leaching, washed pyrite oxidation by *T. ferrooxidans*, and unwashed pyrite oxidation by *T. ferrooxidans*, respectively. The last reaction was competitively inhibited by increasing concentrations of cells, with a K_i value of 0.13 mg of wet cells per ml. *T. ferrooxidans* cells also increased the rate of Fe^{2+} production from Fe^{3+} plus pyrite.

Bacterial leaching of sulfide ores is a complex process potentially involving many different reactions, such as direct action by bacteria, indirect action by ferric iron, and electrochemical interaction of minerals (3, 4, 9, 17). These biological, chemical, and electrochemical reactions are interrelated, and their favorable interaction should lead to a successful bacterial leaching.

We have selected a simple sulfide, pyrite (FeS_2), as a test mineral and have attempted to estimate the rates of some of these reactions and to compare them with the overall bacterial leaching rates. In this work we have studied the spontaneous solubilization of pyrite, the indirect leaching of pyrite with Fe^{3+} , and the bacterial leaching of pyrites (washed and unwashed) and have tried to interrelate the rates of these reactions.

MATERIALS AND METHODS

Pyrite. The pyrite sample used was provided to us by the Mines Branch of Manitoba Energy and Mines. It was ground to -140 mesh (100- μm diameter by microscopic observation) and contained 45.9% Fe, 45.1% S, and 9% impurities.

Media. The standard reaction medium used throughout this study was the HP medium (7, 10) consisting of 0.1 g of K_2HPO_4 , 0.4 g of $(\text{NH}_4)_2\text{SO}_4$, and 0.4 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ per liter, adjusted to pH 2.3 with H_2SO_4 . The growth medium for *Thiobacillus ferrooxidans* was the HP medium plus 33.3 g of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ per liter (7, 10) and that for *Thiobacillus thiooxidans* was the HP medium with unsterilized powdered sulfur, 50 g/liter, as substrate (15).

Organisms. *T. ferrooxidans* SM-4 and *T. thiooxidans* SM-6 were isolated from a sulfide ore mine site (7). *T. ferrooxidans* SM-4 was grown as described previously (16), and the harvested cells were washed and suspended in the HP medium to a concentration of 50 mg of wet cells per ml. *T. thiooxidans* was grown in 2.7-liter Fernbach flasks containing 1 liter of the HP medium. After inoculation with a 2.5% culture, 50 g of powdered elemental sulfur (sulfur precipitated; BDH Chemicals, Toronto, Canada) was spread evenly on the liquid surface. The flasks were incubated at 28°C for 5 days under stationary conditions. Sulfur was removed by filtration through a Whatman no. 1 filter paper

under suction, and the cells were collected by centrifugation at $12,000 \times g$ for 10 min. The collected cells were washed and suspended to a concentration of 50 mg of wet cells per ml in the HP medium. Both cell suspensions were stored at 4°C and were used within 2 to 3 days.

Pyrite concentration. The concentration of pyrite used was expressed as percent pulp density (% PD), which is defined as $[\text{weight of solid (g)}]/[\text{volume of liquid (ml)}] \times 100$.

Spontaneous pyrite dissolution. The steady-state rate of spontaneous pyrite dissolution was determined by measuring the rate of Fe^{2+} release with *o*-phenanthroline (1), as modified below. Pyrite was washed in the HP medium (6.4 ml) in centrifuge tubes for 2 to 3 h with reciprocal shaking at 30°C. The liquid was then removed by centrifugation at $13,000 \times g$ for 10 min, and the washed pyrite was resuspended in the fresh HP medium (6.4 ml). The washed pyrite slurries were then shaken again at 30°C, and 50- to 100- μl samples were taken at time intervals for 1 h for Fe^{2+} analyses. Samples were diluted to 1.5 ml with the HP medium, and after centrifugation 1 ml of supernatant was mixed with 1 ml of 0.1% *o*-phenanthroline. The red color which developed after 5 min was measured at 500 nm in a Diode Array Spectrophotometer 8452A (Hewlett-Packard Co., Palo Alto, Calif.) to obtain the Fe^{2+} concentration. The reaction was linear with time, and the rate was expressed as micromolar Fe^{2+} per minute.

Indirect leaching. The Fe^{3+} -mediated production of Fe^{2+} from pyrite was also determined by using the *o*-phenanthroline method. The washing of pyrite was the same as described above. The washed pyrite was suspended in the fresh HP medium (6.4 ml) containing various concentrations of Fe^{3+} . The incubation and the color determination were the same as those described above. The reaction was linear with time.

Oxygen consumption. The rate of O_2 consumption was determined by the standard manometric technique (18) by using a Warburg apparatus with 16-ml double-sidearm flasks (Bronwill Scientific Inc., Rochester, N.Y.). The total reaction mixture consisted of 3.2 ml of HP medium with specified amounts of cells and pyrite. The reaction was started by tipping the cells from a side arm after 20 min of preincubation. The reaction was carried out at 30°C, and the oxygen

* Corresponding author.

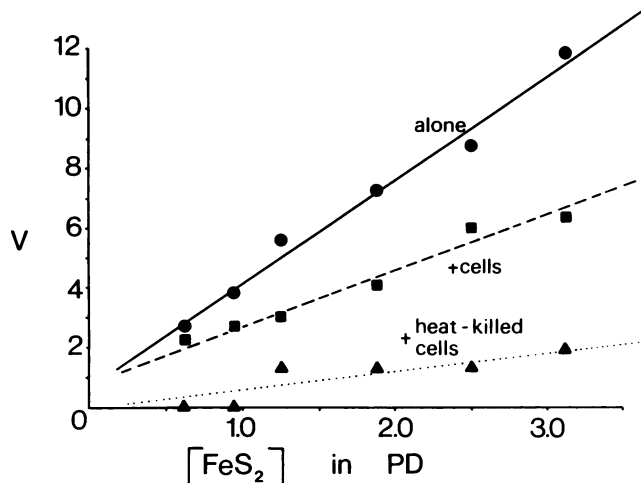


FIG. 1. Effect of pyrite concentration on the rate of spontaneous dissolution of Fe^{2+} . The rate (v) was determined as micromolar Fe^{2+} per minute at $30^{\circ}C$ in the HP medium. Where indicated, intact or heat-inactivated (5 min at $100^{\circ}C$) cells of *T. thiooxidans* SM-6 were present at 1.56 mg of wet cells per ml.

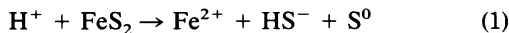
consumption rate was expressed as micromolar O_2 per minute during the linear steady-state reaction.

Washed pyrite sample used for some experiments was prepared as follows. A 10% pyrite slurry in the HP medium was stirred for 4 h, filtered through a Whatman no. 1 filter paper, and dried for 2 days in a desiccator over $CaCl_2$.

Anaerobic production of Fe^{2+} from pyrite and Fe^{3+} . The rate of Fe^{2+} production from washed pyrite plus Fe^{3+} by *T. ferrooxidans* cells was determined in Warburg flasks under O_2 -free N_2 gas at $30^{\circ}C$. The reaction volume was 3.2 ml in the HP medium. The reaction was started by tipping cells and Fe^{3+} from the side arms. Samples of 25 μ l were removed with microsyringes at time intervals through serum bottle stoppers capped on the side arms of flasks for Fe^{2+} determination as described above.

RESULTS

Spontaneous solubilization of pyrite. Pyrite (FeS_2) crystal is not very soluble in water, and the solubility product of the reaction



is supposed to be 2×10^{-19} for $[Fe^{2+}][HS^-]$ at pH 2.3 (11), but most pyrite samples contain some components which are more soluble due to grinding treatments or contaminating minerals with lower sulfur content.

The steady-state rate of Fe^{2+} solubilization from pyrite at various concentrations is shown in Fig. 1. The reaction was the first order with respect to pyrite (without cells), with a slope of $3.5 \mu M Fe^{2+}$ per min per $FeS_2\%$ PD. Figure 2 shows the rate of O_2 consumption of pyrite without cells ($0.36 \mu M O_2$ per min per $FeS_2\%$ PD), which is much smaller than the value expected even for the oxidation of HS^- to S^0 ($HS^- + 1/2O_2 + H^+ \rightarrow S^0 + H_2O$).

T. ferrooxidans and *T. thiooxidans* cells are known to adsorb on solid surfaces even when they are killed by heat (19). The heat-killed *T. thiooxidans* cells virtually stopped the Fe^{2+} release from pyrite (Fig. 1), presumably by covering the surface of pyrite particles. Intact cells also inhibited the Fe^{2+} release, but less drastically (Fig. 1). The O_2

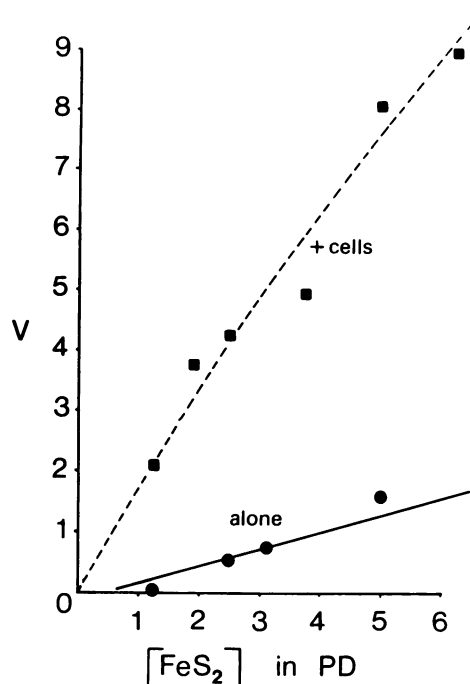
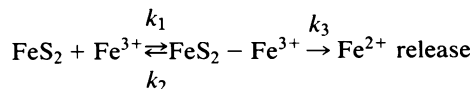


FIG. 2. Effect of pyrite concentration on the rate of oxygen consumption with and without *T. thiooxidans* cells. The rate (v) was determined as micromolar O_2 per minute at $30^{\circ}C$. The pyrite sample used was unwashed. When indicated, the intact *T. thiooxidans* cells were present at 1.56 mg of wet cells per ml.

consumption in Fig. 2 indicates that *T. thiooxidans* cells were oxidizing the sulfide or sulfur portion of pyrite, since they are not capable of oxidizing Fe^{2+} . The rate of O_2 consumption, $1.7 \mu M O_2$ per min per $FeS_2\%$ PD, was almost twice what is expected of the oxidation of HS^- (generated in reaction 1 (assuming the same rate as Fe^{2+} release) to S^0).

Indirect leaching of pyrite by ferric iron. The oxidation of pyrite by ferric iron, known as indirect action in bacterial leaching (4, 9, 17), has been extensively studied (8). The complete oxidation of pyrite by ferric iron ($FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+$) requires harsh conditions, and under the mild conditions of bacterial leaching, a more probable reaction will be $FeS_2 + 2Fe^{3+} \rightarrow 3Fe^{2+} + 2S^0$ (4, 9).

The rate of Fe^{2+} release from pyrite was determined at various concentrations of Fe^{3+} and at fixed concentrations of pyrite (Fig. 3). The rate of Fe^{2+} release was linear (first order) with respect to Fe^{3+} concentration. The effect of pyrite concentration at two fixed concentrations of Fe^{3+} is shown in the Fig. 4 insert, after correction for spontaneous reaction 1 (no Fe^{3+} addition). The rate of Fe^{2+} formation followed a typical saturation kinetics at either 10 or 20 mM Fe^{3+} . The double-reciprocal plots (6) shown in Fig. 4 were linear, intersecting on the x axis at the same point, with a K_m value of 1.9% PD for pyrite. The results agree with the concept of pyrite as reactant and ferric iron as catalyst (although it is also a reactant), with the formation of the FeS_2-Fe^{3+} complex as the rate-limiting step:



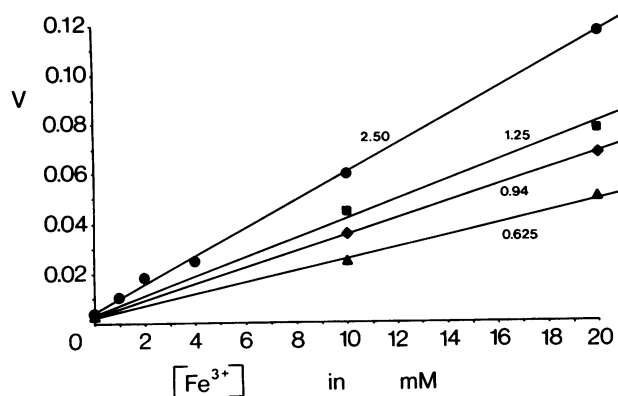


FIG. 3. Effect of Fe^{3+} concentration on the indirect leaching of pyrite. The rate of Fe^{2+} dissolution was determined at various Fe^{3+} concentrations and fixed pyrite concentrations (0.625, 0.94, 1.25, and 2.5% PD), as indicated. The rate (v) was expressed as micromolar Fe^{2+} per minute at 30°C .

Under the steady-state conditions, the rate of Fe^{2+} release (v) can be derived by following the standard enzyme kinetics equations:

$$v = \frac{k_3[\text{Fe}^{3+}][\text{FeS}_2]}{K_m + [\text{FeS}_2]} \quad (2)$$

where $K_m = (k_2 + k_3)/k_1$. In the double-reciprocal form, the equation becomes

$$\frac{1}{v} = \frac{1}{k_3[\text{Fe}^{3+}]} + \frac{K_m}{k_3[\text{Fe}^{3+}]} \left(\frac{1}{[\text{FeS}_2]} \right) \quad (3)$$

The rate constant, k_3 , is obtained from the y axis intercept in Fig. 4, $1/k_3[\text{Fe}^{3+}]$ ($1/V_{\text{max}}$, where V_{max} is the maximal velocity or rate), as 0.01 mM Fe^{2+} per min per mM Fe^{3+} .

Oxidation of pyrite by *T. ferrooxidans*. The rate of pyrite oxidation by *T. ferrooxidans* cells was determined by measuring the steady-state rate of O_2 consumption (micromolar O_2 per minute) at various concentrations of cells and pyrite. The values were corrected for the slow spontaneous rates

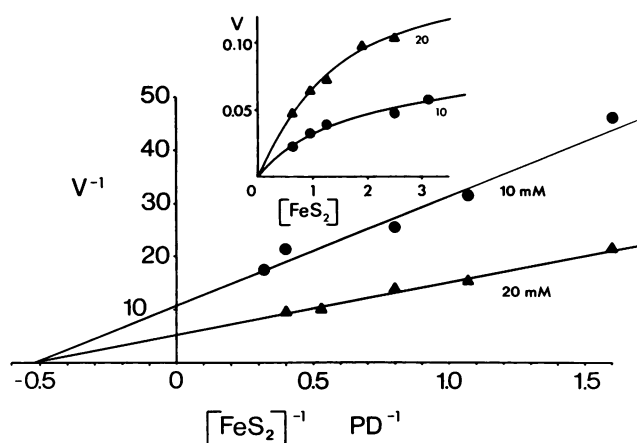


FIG. 4. Effect of pyrite concentration on the indirect leaching of pyrite. Conditions were the same as in Fig. 3, except that the rate (v) was plotted against the pyrite concentrations (% PD) in a double-reciprocal manner at two fixed Fe^{3+} concentrations (10 and 20 mM), as indicated.

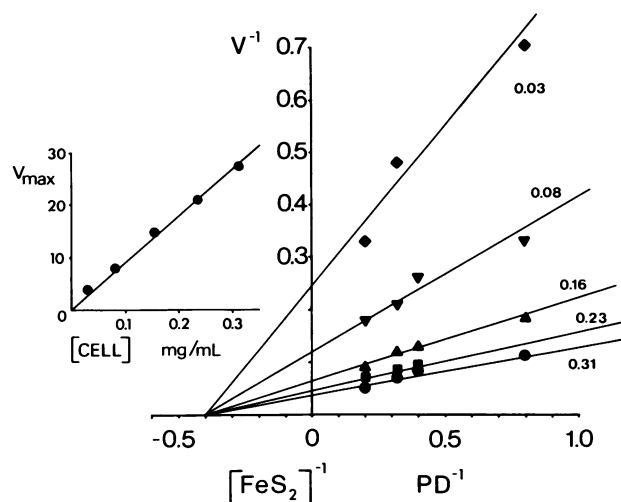


FIG. 5. Effect of pyrite concentration on the rate of oxygen consumption by *T. ferrooxidans* SM-4 cells. The rate (v) was determined as micromolar O_2 per minute at 30°C by using various amounts of washed pyrite and fixed concentrations of cells (0.03, 0.08, 0.16, 0.23, and 0.31 mg of wet cells per ml), as indicated.

(without cells), which were only 0 to 5% of the bacterial rates. Two samples of pyrite, washed and unwashed, were used.

Figure 5 shows the results with washed pyrite in a double-reciprocal rate (v)-pyrite concentration plot. The results indicate that the rate of oxidation follows the standard Michaelis-Menten kinetics of $v = k[\text{cell}][\text{FeS}_2]/(K_m + [\text{FeS}_2])$, where k is the rate constant for O_2 consumption by cells with pyrite. The linear lines at different cell concentrations intersect the x axis at the same point, with a K_m value of 2.5% FeS_2 PD. The insert shows that the maximal rate for O_2 consumption, $k[\text{cell}](V_{\text{max}})$, obtained from the y intercept has a linear relationship with the cell concentration used, with a slope (k) of $90 \mu\text{M O}_2$ per min per mg of wet cells per ml.

The results with unwashed pyrite gave an entirely different pattern (Fig. 6). Here the rate was much higher, and the K_m values increased with increasing cell concentrations. When the O_2 uptake rate was calculated as the specific rate (v_{sp}), micromolar O_2 per minute per milligram of wet cells per milliliter, and replotted in the double-reciprocal manner (Fig. 7), all the lines now intersected the y axis at the same point, characteristic of a competitive inhibition. Thus, *T. ferrooxidans* SM-4 cells acted as competitive inhibitor as well as pyrite oxidizing agent. We have previously demonstrated (16) the competitive inhibition of *T. ferrooxidans* cells on the oxidation of Fe^{2+} and derived the equations:

$$v = \frac{k_3[C][S]}{[S] + K_m(1 + [C]/K_i)} \quad (4)$$

where $[C]$ and $[S]$ are the concentrations of cell and substrate and K_i is the inhibition constant and

$$\frac{1}{v_{\text{sp}}} = \frac{1}{k_3} + \frac{K_m}{k_3} \left(1 + \frac{[C]}{K_i} \right) \left(\frac{1}{[S]} \right) \quad (5)$$

Figure 7 follows the pattern expected of equation 5 when $[S]$ is the concentration of pyrite and k_3 is obtained from the intercept on the y axis as $250 \mu\text{M O}_2$ per min per mg of wet

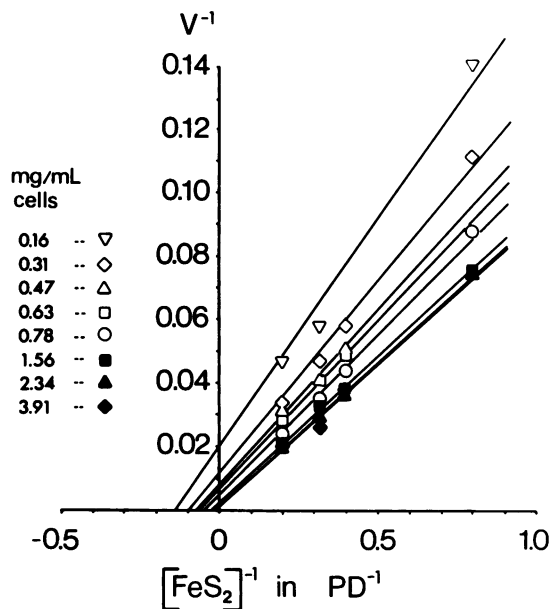


FIG. 6. Effect of unwashed pyrite concentrations on the rate of oxygen consumption by *T. ferrooxidans* SM-4 cells. The conditions were the same as in Fig. 5, except that pyrite was used without washing and the cell concentrations were fixed as indicated.

cells per ml. Figure 8 is the replot of the slope, $K_m/k_3 + (K_m/K_i k_3) [C]$, versus the cell concentration, $[C]$. The K_m value of 2.75% PD pyrite and the K_i value of 0.13 mg of wet cells per ml are obtained from the figure.

Since the rate of O_2 consumption was faster during the initial 0.5- to 1-h period before the linear steady state was

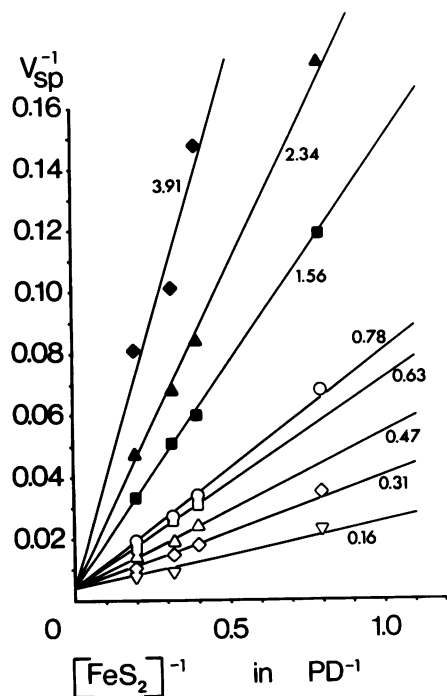


FIG. 7. Competitive inhibition of unwashed pyrite oxidation by *T. ferrooxidans* by increasing concentrations of cells. The specific rate or velocity (v_{sp}) was calculated from the data in Fig. 6 as micromolar O_2 per minute per milligram of wet cells per milliliter.

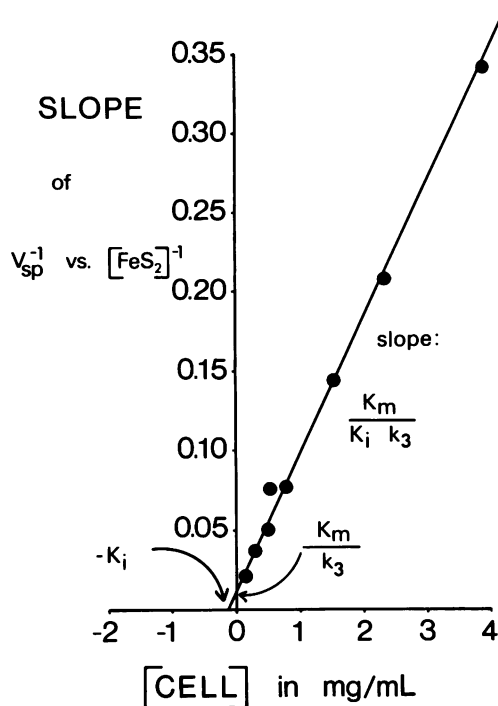


FIG. 8. Replot of slope from Fig. 7 against the concentration of cells. The line has a slope corresponding to $K_m/(K_i k_3)$ and x and y intercepts at $(-K_i)$ and K_m/k_3 , respectively.

achieved, it was suspected that there was an initial rapid release of Fe^{2+} from the unwashed sample of pyrite during the 20-min preincubation period. In separate experiments, it was determined that Fe^{2+} release during the preincubation period was 2 mM Fe^{2+} per FeS_2 % PD. At this high concentration, Fe^{2+} would contribute not only to the initial rapid rate of O_2 consumption but also to the indirect leaching by the Fe^{3+} formed by the bacterial oxidation.

Figure 9 shows the effect of 6.25 mM Fe^{2+} (20 μ mol of Fe^{2+} per 3.2 ml) on the bacterial oxidation of washed pyrite (100 mg/3.2 ml) to simulate the experiments with unwashed pyrite. There was an initial rapid O_2 consumption, as ex-

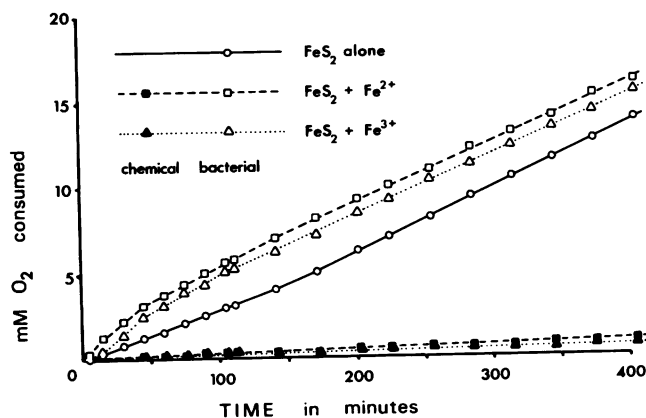


FIG. 9. Time course of oxygen consumption in the oxidation of washed pyrite with and without Fe^{2+} or Fe^{3+} by *T. ferrooxidans* SM-4. The concentrations were as follows: washed pyrite, 3.125% PD; *T. ferrooxidans* SM-4, 1 mg of wet cells per ml; Fe^{2+} , 6.25 mM; Fe^{3+} , 13.5 mM.

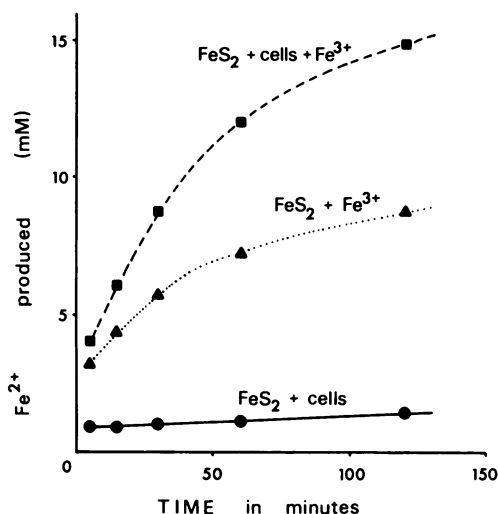


FIG. 10. Time course of anaerobic Fe^{2+} production from pyrite plus Fe^{3+} by *T. ferrooxidans* SM-4 cells. The concentrations were as follows: washed pyrite, 3.125% PD; *T. ferrooxidans* SM-4, 1 mg of wet cells per ml; Fe^{3+} , 13.5 mM.

pected, presumably due to the oxidation of Fe^{2+} by cells (1 mg/ml), followed by a linear, slower rate approximately equal to that of the control experiment (without Fe^{2+} addition). Surprisingly, however, the addition of Fe^{3+} (13.5 mM) had an effect very similar to that of Fe^{2+} , with only a slight delay in the initial rapid O_2 consumption. This initial phase was too fast to be accounted for by the indirect leaching followed by the oxidation of Fe^{2+} generated.

The formation of Fe^{2+} from washed pyrite was studied under anaerobic conditions to see whether cells can accelerate the indirect leaching with Fe^{3+} . Figure 10 shows that this is, in fact, true and explains why Fe^{3+} had an effect similar to Fe^{2+} in the O_2 consumption experiments of Fig. 9.

DISCUSSION

The bacterial oxidation of pyrite is a complex phenomenon, and in this study we have attempted to define various possible reaction parameters contributing to the oxidation.

The results are summarized in Table 1. The Fe^{2+} oxidation rate equation was from our earlier paper (16), and the bacterial reduction of Fe^{3+} to Fe^{2+} with FeS_2 (Fig. 10) is not listed in Table 1, since this reaction has not been studied in detail.

The rate of steady-state spontaneous solubilization, 3.5 μM Fe^{2+} per min per $\text{FeS}_2\%$ PD, corresponds to the 0.875 μM O_2 per min per $\text{FeS}_2\%$ PD, if Fe^{2+} released is oxidized to Fe^{3+} ($4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}^+ \rightarrow 4\text{Fe}^{3+} + 2\text{H}_2\text{O}$) by bacterial cells. This value is less than 10% of the rate of bacterial oxidation (1 mg of wet cells per ml) of either washed or unwashed pyrite.

The indirect leaching rate can be considerably faster, especially if $[\text{Fe}^{3+}]$ can be maintained at a high level, for example, 72.5 μM Fe^{2+} per min with 10 mM Fe^{3+} and 5% PD FeS_2 . The results in Fig. 10 show that the Fe^{2+} production from Fe^{3+} plus pyrite is faster in the presence of bacterial cells. This could be due to the Fe^{3+} -sulfide (sulfide) oxidoreductase discovered recently in *T. ferrooxidans* (13, 14), which oxidizes sulfur or sulfide to sulfite by using Fe^{3+} ($\text{S} + 4\text{Fe}^{3+} + 3\text{H}_2\text{O} \rightarrow \text{H}_2\text{SO}_3 + 4\text{Fe}^{2+} + 4\text{H}^+$). This interpretation implies that the bacteria can oxidize the pyrite sulfur or its dissociated form with Fe^{3+} . A more detailed study is required in order to establish the concept.

The bacterial oxidation of washed pyrite followed a standard Michaelis-Menten kinetics (Fig. 5), with cells as enzymes and pyrite as substrate, at least in the concentration range of pyrite used. Addition of Fe^{2+} or Fe^{3+} affected only the initial O_2 consumption rate, and the steady-state rate was relatively constant (Fig. 9). The steady-state rate was still much faster than that expected from the indirect leaching followed by Fe^{2+} oxidation. In fact, if the pyrite oxidation were simply a combination of the indirect leaching ($\text{Fe}^{3+} \rightarrow \text{Fe}^{2+}$ with pyrite, including bacterial action) and the Fe^{2+} oxidation to Fe^{3+} by bacteria, the initial rapid rate with Fe^{2+} or Fe^{3+} in Fig. 9 should have continued without slowing down to the level of the control experiment. The steady-state rate must be governed also by other parameters which were not studied in this work, for example, the fate of HS^- or S^0 after release from FeS_2 , and their effect on the oxidation of FeS_2 . The rate of HS^- or S^0 oxidation might affect the steady-state pyrite oxidation rate. The results in Fig. 2 with *T. thiooxidans* cells which cannot oxidize Fe^{2+} suggest that

TABLE 1. Summary of various reaction rates^a

Reaction no. and type	Rate equation	Constants
(1) Spontaneous	$v = k[\text{FeS}_2]$	$k = 3.5 \mu\text{M Fe}^{2+}/\text{min per FeS}_2\% \text{ PD}$
(2) Indirect leaching	$v = \frac{k_3[\text{Fe}^{3+}][\text{FeS}_2]}{K_m + [\text{FeS}_2]}$	$k_3 = 10 \mu\text{M Fe}^{2+}/\text{min per mM Fe}^{3+}$ $K_m = 1.9\% \text{ PD FeS}_2$
(3) <i>T. ferrooxidans</i> washed FeS_2	$v = \frac{k[\text{cell}][\text{FeS}_2]}{K_m + [\text{FeS}_2]}$	$k = 90 \mu\text{M O}_2/\text{min per mg of cells per ml}$ $K_m = 2.5\% \text{ PD FeS}_2$
(4) <i>T. ferrooxidans</i> unwashed FeS_2	$v = \frac{k_3[\text{cell}][\text{FeS}_2]}{K_m(1 + [\text{cell}]/K_i) + [\text{FeS}_2]}$	$k_3 = 250 \mu\text{M O}_2/\text{min per mg of cells per ml}$ $K_m = 2.75\% \text{ PD FeS}_2$ $K_i = 0.13 \text{ mg of cells/ml}$
(5) <i>T. ferrooxidans</i> Fe^{2+} oxidation ^b	$v = \frac{k_3'[\text{cell}][\text{Fe}^{2+}]}{K_m(1 + [\text{cell}]/K_i') + [\text{Fe}^{2+}]}$	$k_3' = 125 \mu\text{M O}_2/\text{min per mg of cells per ml}$ $K_m = 110 \mu\text{M Fe}^{2+}$ $K_i' = 0.33 \text{ mg of cells/ml}$

^a $[\text{FeS}_2]$, Pyrite concentration in % PD; $[\text{Fe}^{3+}]$, Fe^{3+} concentration (millimolar); $[\text{cell}]$, cell concentration in milligrams of wet cells per milliliter; $[\text{Fe}^{2+}]$, Fe^{2+} concentration (micromolar).

^b The equation and constants are from our previous paper (16).

the rate of oxidation of sulfur moieties of FeS₂ with O₂ is slow (around 1 μM O₂ per min per mg of cells per ml per FeS₂% PD). *T. ferrooxidans* cells can, in addition, oxidize sulfur compounds with Fe³⁺ (13, 14), as mentioned above, but the rate (about twice the chemical indirect leaching rate in Fig. 10) still cannot approach the rapid rate of Fe²⁺ oxidation or pyrite oxidation.

The bacterial oxidation of unwashed pyrite showed a higher rate constant (250 compared with 90 for washed pyrite) but surprisingly was inhibited by increasing concentrations of cells, similar to the Fe²⁺ oxidation (16). The oxidation of pyrite is a more complex process than the Fe²⁺ oxidation, but since the inhibition was not observed with the washed pyrite, it was probably related to the initial rapid solubilization of unwashed pyrite. The release of 2 mM Fe²⁺ per FeS₂% PD corresponds to more than 2% of pyrite iron. Presumably, a similar quantity of sulfur and sulfide must have been released. Since the attempted simulation experiments with Fe²⁺ in Fig. 9 did not affect the steady-state O₂ consumption rate with washed pyrite, perhaps the presence of these dissociated sulfur moieties is important in the interpretation of unwashed pyrite oxidation. We have previously concluded that *T. ferrooxidans* and *T. thiooxidans* solubilize sulfide minerals by different mechanisms (7). We are currently studying the pyrite oxidation by *T. thiooxidans* to clarify the fate of sulfur moieties of pyrite. Recent studies on the direct bacterial attack of synthetic pyrite crystals (12) and ultrathin films (2) also suggest the importance of sulfide or sulfur oxidation.

ACKNOWLEDGMENTS

We thank the Natural Sciences and Engineering Research Council of Canada for grants to I.S. and a Postgraduate Fellowship to H.M.L. and CANMET for ongoing interest and support.

LITERATURE CITED

- Ballentine, R., and D. D. Burford. 1957. Determination of metals. *Methods Enzymol* 3:1002-1035.
- Bärtels, C. C., G. Chatzitheodorou, M. Rodriguez-Leiva, and H. Tributsch. 1989. Novel technique for investigation and quantification of bacterial leaching by *Thiobacillus ferrooxidans*. *Biotechnol. Bioeng.* 33:1196-1204.
- Berry, V. K., and L. E. Murr. 1978. Direct observation of bacteria and quantitative studies of their catalytic role in the leaching of low-grade, copper bearing waste, p. 103-136. *In* L. E. Murr, A. E. Torma, and J. A. Brierley (ed.), *Metallurgical applications of bacterial leaching and related microbiological phenomena*. Academic Press, Inc., New York.
- Ehrlich, H. L. 1981. *Geomicrobiology*. Marcel Dekker, Inc., New York.
- Karavaiko, G. I. 1985. *In* A. E. Torma (ed.), *Microbiological processes for the leaching of metals from ores. State-of-the-art review*. United Nations Environment Program, USSR Commission for UNEP. Center of International Projects, USSR State Committee for Science and Technology. All-Union Institute for Scientific and Technological Information, Moscow.
- Lineweaver, H., and D. Burk. 1934. The determination of enzyme dissociation constants. *J. Am. Chem. Soc.* 58:658-666.
- Lizama, H. M., and I. Suzuki. 1988. Bacterial leaching of a sulfide ore by *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*. I. Shake flask studies. *Biotechnol. Bioeng.* 32:110-116.
- Lowson, R. T. 1982. Aqueous oxidation of pyrite by molecular oxygen. *Chem. Rev.* 82:461-497.
- Lundgren, D. G., and M. Silver. 1980. Ore leaching by bacteria. *Annu. Rev. Microbiol.* 34:263-283.
- McCready, R. G. L., D. Wadden, and A. Marchbank. 1986. Nutrient requirements for the in-place leaching of uranium by *Thiobacillus ferrooxidans*. *Hydrometallurgy* 17:61-71.
- Morse, J. W., F. J. Millero, J. C. Cornwell, and D. Rickard. 1987. The chemistry of the hydrogen sulfide and iron sulfide systems in natural waters. *Earth-Sci. Rev.* 24:1-42.
- Rodriguez-Leiva, M., and H. Tributsch. 1988. Morphology of bacterial leaching patterns by *Thiobacillus ferrooxidans* on synthetic pyrite. *Arch. Microbiol.* 149:401-405.
- Sugio, T., T. Katagiri, K. Inagaki, and T. Tano. 1989. Actual substrate for elemental sulfur oxidation by sulfur:ferric ion oxidoreductase purified from *Thiobacillus ferrooxidans*. *Biochim. Biophys. Acta* 973:250-256.
- Sugio, T., W. Mizunashi, K. Inagaki, and T. Tano. 1987. Purification and some properties of sulfur:ferric ion oxidoreductase from *Thiobacillus ferrooxidans*. *J. Bacteriol.* 169:4916-4922.
- Suzuki, I. 1965. Oxidation of elemental sulfur by an enzyme system of *Thiobacillus thiooxidans*. *Biochim. Biophys. Acta* 104:359-371.
- Suzuki, I., H. M. Lizama, and P. D. Tackaberry. 1989. Competitive inhibition of ferrous iron oxidation by *Thiobacillus ferrooxidans* by increasing concentrations of cells. *Appl. Environ. Microbiol.* 55:1117-1121.
- Torma, A. E. 1977. The role of *Thiobacillus ferrooxidans* in hydrometallurgical processes. *Adv. Biochem. Eng.* 6:1-37.
- Umbreit, W. W., R. H. Burris, and J. F. Stauffer. 1949. *Manometric techniques and tissue metabolism*, 2nd ed. Burgess Publishing Co., Minneapolis.
- Wakao, N., M. Mishina, Y. Sakurai, and H. Shiota. 1984. Bacterial pyrite oxidation. III. Adsorption of *Thiobacillus ferrooxidans* cells on solid surfaces and its effect on iron release from pyrite. *J. Gen. Appl. Microbiol.* 30:63-77.