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Iron Oxidation by *Thiobacillus ferrooxidans*

Scientific Note

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INTRODUCTION

Several investigators have shown that microorganisms are involved in many naturally occurring oxidation processes. At present, microbial leaching, which is the solubilization of metals catalyzed by microorganisms, is widely used commercially to produce copper, and to a lesser extent uranium, from low-grade mining wastes (*1,2*). Microbial leaching can also be used as a pretreatment step in the mining of precious metals, such as gold and silver (*3*). In this application, the solubilization of pyrite makes the precious metals more accessible for cyanide leaching.

The bacteria most frequently used in microbial leaching processes are *Thiobacilli,* which thrive at pH 2–3. *Thiobacillus ferrooxidans* (*Tf*) derives energy for growth from the oxidation of ferrous iron:

$$Fe^{2+} + O_2 + 4H^+ \xrightarrow{Tf} Fe^{3+} + 2H_2O \qquad (1)$$

The specific growth rate of *T. ferrooxidans* depends strongly on temperature, pH, and ferrous iron concentration (i.e., composition of medium). The optimum conditions for cell growth occur at a temperature of approx 31°C and a pH of about 2.5 in 9K medium, an ideal *T. ferrooxidans* medium developed by Silverman and Lundgren (*4*), containing basal salts and ferrous sulfate.

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Pyrite (FeS_2) oxidation in the presence of *T. ferrooxidans* has been studied by many researchers (*5–8*). Microbial leaching experiments in a batch system have shown that the oxidation of a metal sulfide, such as pyrite, is not usually complete and that several weeks are usually required to obtain the maximum soluble metal concentration (*5*).

The microbial oxidation of ferrous iron has also been studied with regard to the physical, chemical, and biological factors affecting the rate of iron oxidation. Much of this research has dealt with ferrous iron oxidation as a form of ferrous sulfate (*9–11*). *T. ferrooxidans* is able to oxidize ferrous iron at a rate about 500,000 times as fast as would occur in its absence (*9*). Although the kinetic studies of ferrous iron oxidation have been done in the presence of *T. ferrooxidans* using the Monod equation, no attempt has been made to develop a simple and quick procedure for predicting the effectiveness of a given strain of *T. ferrooxidans* in microbial leaching operations. Because ferrous iron oxidation by *T. ferrooxidans* is such an important reaction in microbial leaching operations, e.g., it is the rate-limiting step in pyrite oxidation (*11*), this study was undertaken to examine several factors affecting the rate of ferrous iron oxidation in the presence of *T. ferrooxidans*.

EXPERIMENTAL METHODS

Bacterial Growth

T. ferrooxidans strain ATCC 13661 was selected as the primary microorganism for this study because it has been used in many previous microbial leaching investigations (*7,12*). The *T. ferrooxidans* used as an inoculum in each experiment was grown for about 1 wk in 9K medium at an initial pH of 2–3. A shaker (Lab Line Environ-Shaker Model 3527) at 28°C and 120 rpm was utilized to obtain a maximum cell number of approx 10^8 cells/mL.

Ferrous Iron Oxidation

General Experimental Procedure

Iron oxidation kinetic-rate experiments were also conducted at a temperature of 28°C. Duplicate experiments were run simultaneously to check the reproducibility of the various experiments. In each experiment, sterile 9K medium in 250-mL Erlenmeyer flasks was inoculated with bacterial culture in measured aliquots. Following inoculation, the flasks were placed on an orbital shaker set at 120 rpm. The pH in each flask was measured about every 6 h with a Beckman Model 21 pH meter. Samples were taken initially, and with each pH measurement after the pH began to rise, by inserting a sterile pipet into each flask and removing 1-mL liquid samples. Each liquid sample was filtered through a 0.45-μm filter to separate bacteria from the liquid sample.

Effect of Initial pH

The pH of the 9K medium in each flask was adjusted to values between 1.93 and 2.70 by the addition of concentrated sulfuric acid so that the effect of initial pH on ferrous iron oxidation by *T. ferrooxidans* could be investigated.

Effect of Initial Ferrous Iron Concentration

To examine the effect of initial ferrous iron concentration on iron oxidation by *T. ferrooxidans*, various amounts of a filtered solution containing 31 g/L of $FeSO_4$ were mixed with 70 mL of heat-sterilized 9K basal-salts medium. Next, distilled water was added to bring the total volume in each flask up to 100 mL. Using 5, 10, 20, and 30 mL of the ferrous iron solution resulted in solutions containing 1500, 3000, 6000, and 9000 ppm, respectively, of ferrous iron.

Effect of Initial Cell Number

To determine the effect of initial cell number of ferrous iron oxidation, bacterial cultures of *T. ferrooxidans* in 1-, 2-, and 4-mL aliquots were inoculated into 9K medium. In this manner, we could study the effect of doubling and quadrupling the initial cell number without actually making bacterial counts.

Analytical Procedure

Investigations of all parameters were carried out following the general experimental procedure outlined above. The 1-mL samples collected approximately every 6 h were analyzed by a standard volumetric-titration method with $KMnO_4$ to obtain ferrous iron concentration profiles as a function of time.

RESULTS

Sigmoidal Model for Ferrous Iron Oxidation

The observed ferrous iron concentration profiles indicate that the conversion of ferrous iron to ferric iron exhibits a sigmoidal behavior, i.e., it follows an S-shaped curve (*see* Fig. 1). Therefore, a sigmoidal model equivalent to the Michaelis-Menten equation including the Hill coefficient (*13*) can be used to represent the behavior of the oxidation of ferrous iron to ferric iron:

$$X = (X_{max}\, t^m) / (K + t^m),\ m > 1 \qquad (2)$$

where X is the conversion of ferrous iron to ferric iron, X_{max} is the maximum conversion, t is the time, and K and m are experimentally determined constants. All the ferrous iron that is present initially has the

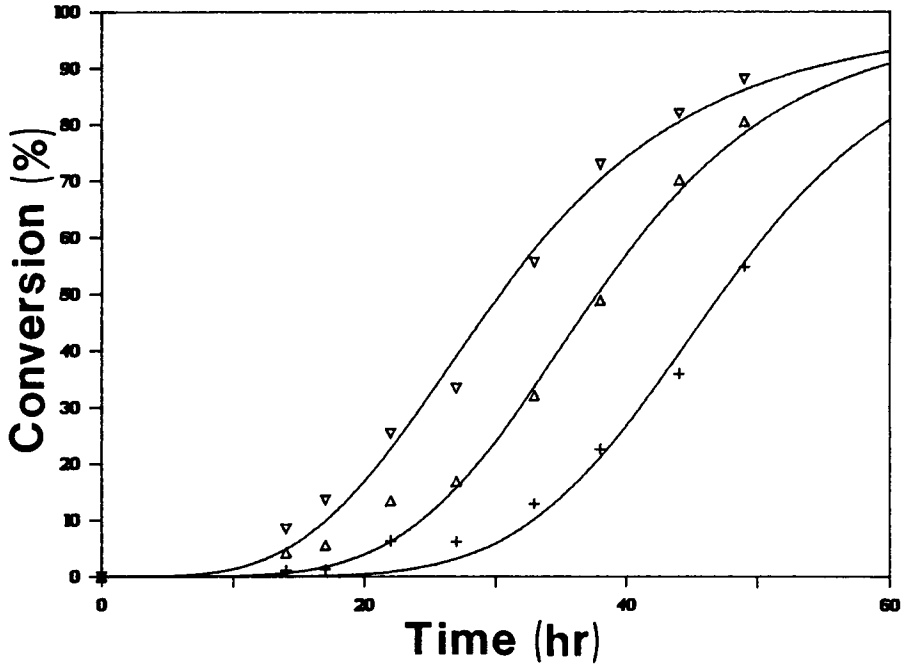

Fig. 1. Representation of ferrous iron oxidation by the sigmoidal model (——) for various inoculum sizes (1 mL, +; 2 mL, △; 4 mL, ▽) at an initial pH of 1.9.

potential to be oxidized to ferric iron; therefore, the maximum conversion in Eq. 2 is 100%.

Equation 2 can be linearized to obtain

$$\log [X/(X_{max} - X)] = m \log t - \log K \quad (3)$$

Thus, a log–log plot of $X/(X_{max}-X)$ as a function of time gives a straight line with slope m and y-intercept K. For example, Fig. 2 illustrates that values of log $K=7.87$ and $m=4.99$ were obtained for ferrous iron oxidation by *T. ferrooxidans* at 28°C with 9K medium at an initial pH of 1.9, an initial ferrous iron concentration of 9000 ppm, and an inoculum concentration of 2% (i.e., 2 mL of inoculum/100 mL of solution).

The rate of conversion of ferrous iron to ferric iron is simply the derivative of Eq. 2:

$$\dot{X} = dX / dt = (X_{max} K m t^{m-1}) / (K + t^m)^2 \quad (4)$$

The maximum rate of conversion of ferrous iron to ferric iron can be found by taking the derivative of Eq. 4, setting it equal to zero, solving for the critical value of t (t_{lag}), and substituting t_{lag} back into Eq. 4. The critical value of t is lag time, defined in this paper as the time needed to reach the maximum conversion rate:

$$t_{lag} = [K (m - 1) / (m + 1)]^{1/m} \quad (5)$$

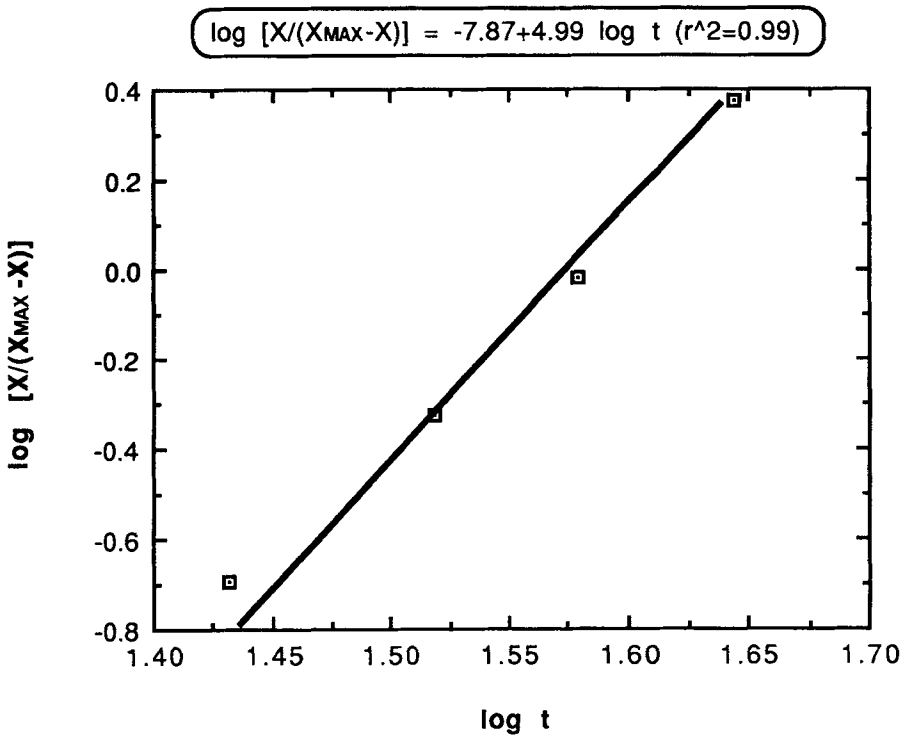

Fig. 2. Determination of the values of K and m during ferrous iron oxidation by *T. ferrooxidans* strain ATCC 13661 in 9K medium with an inoculum concentration of 2 mL/100 mL of solution at 28°C.

Note that our definition of lag time is different than the conventional definition used by microbiologists to indicate the beginning of exponential growth. That is, our lag time is a precisely calculable value for a given set of data, whereas, according to the conventional definition, the beginning of exponential growth is determined by a scientist "eyeing" the data.

Factors Affecting Ferrous Iron Oxidation

Effect of Initial pH

Comparisons between maximum conversion rate and lag time are illustrated in Fig. 3 as a function of initial pH. In the initial pH range of 1.9–2.7, the maximum conversion rate increases linearly with the initial pH; in contrast, lag time is essentially unaffected by initial pH. When the bacterium in its iron-containing growth medium was mixed with a fresh solution of 9K basal salts, precipitation occurred at an initial pH above 2.0. Furthermore, precipitation occurred at pHs above 2.4 when ferrous sulfate was mixed with a solution of the 9K basal salts. The precipitate is believed to be iron (both ferrous and ferric) phosphate. Because redissolving precipitates may affect the rate of iron oxidation by keeping the solu-

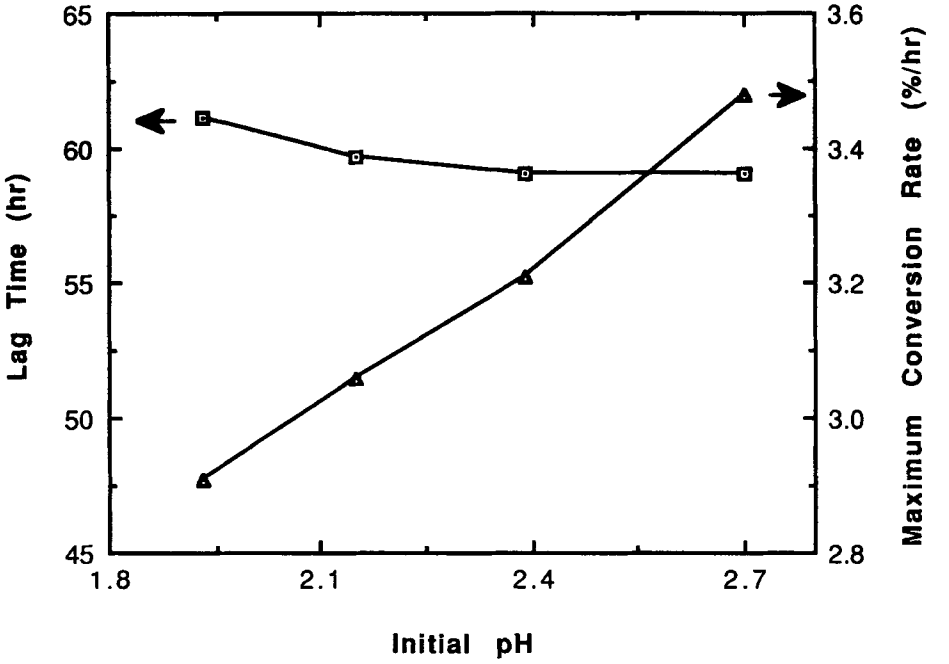

Fig. 3. Effect of initial pH on ferrous iron oxidation.

tion saturated, an initial pH below 2.0 should be used to compare iron-oxidation rates of given strains of *T. ferrooxidans.*

Effect of Initial Ferrous Concentration

Initial ferrous iron concentrations of 1500, 3000, 6000, and 9000 ppm were prepared and oxidized in the presence of *T. ferrooxidans.* The tests were initiated at a pH of about 2.15 and with bacterial cultures of 1 mL/100 mL of medium solution. Comparisons of maximum conversion rate and lag time during ferrous iron oxidation by *T. ferrooxidans* at different initial ferrous iron concentrations are illustrated in Fig. 4. The media with lower initial ferrous iron concentrations resulted in slightly higher maximum conversion rates and smaller lag times. Note, however, that when the initial ferrous concentration is quadrupled, the maximum conversion rate drops only 20%. This indicates that the total amount of iron, on a mass basis, increases with initial ferrous iron concentration. Nonetheless, considering that the desire is to minimize the time required to compare iron-oxidation rates of given strains of *T. ferrooxidans,* 9K medium with a low ferrous iron concentration, which reduces lag time, should be used.

Effect of Initial Cell Number

A comparison of maximum conversion rate and lag time at three different inoculum sizes of the same *T. ferrooxidans* cultures is given in Fig. 5. A larger inoculum size gives a smaller lag time; however, the maximum conversion rate is essentially independent of inoculum size. The maximum

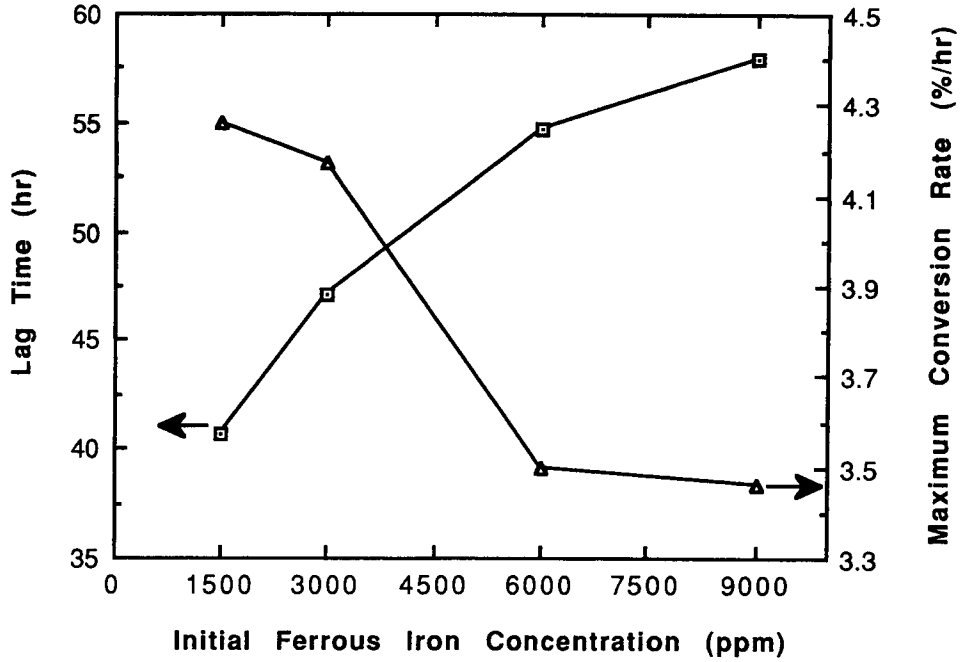

Fig. 4. Effect of initial ferrous iron concentration on ferrous iron oxidation.

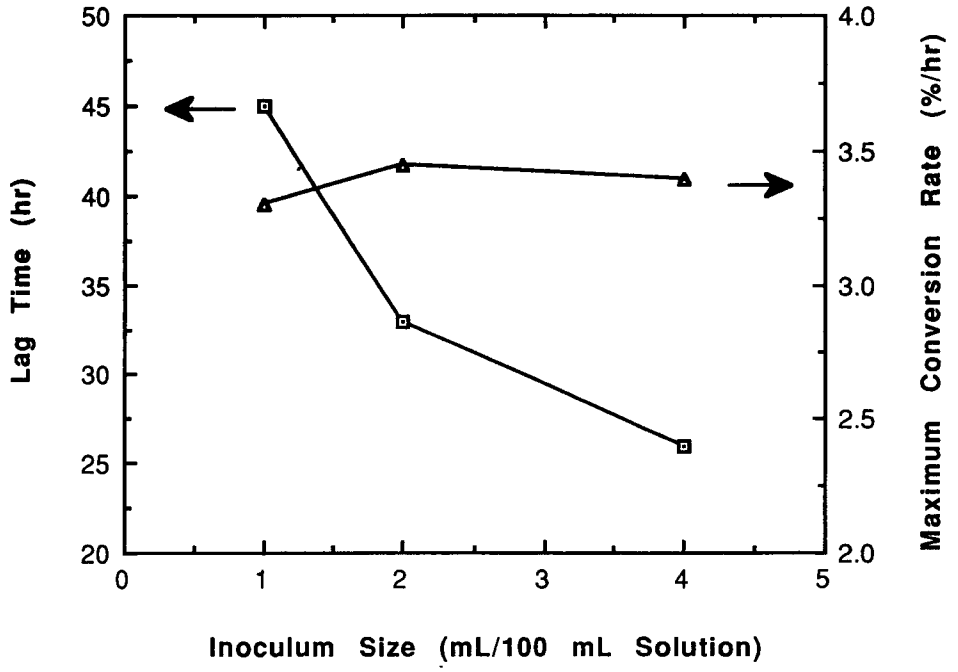

Fig. 5. Effect of inoculum size on ferrous iron oxidation.

conversion rate varied only from 3.30 to 3.44%/h when the cell number was quadrupled. The main conclusion to be drawn from this study is that a larger inoculum size, which leads to a reduced lag time, should be used for comparing iron-oxidation rates of given strains of *T. ferrooxidans*.

DISCUSSION

The experiments represented by the left-most points in Figs. 3 and 5 were run under similar conditions, i.e., an initial pH of 1.9, an inoculum vol of 1 mL, and an initial ferrous iron concentration of 9000 ppm. However, they were carried out at different times with different batches of *T. ferrooxidans*. As a result, the cell number and initial ferric iron concentration in the inoculum were undoubtedly different. As Fig. 5 illustrates, cell number (inoculum size) affects lag time, which explains the difference between the observed lag times of 61.1 and 44.7 h for the first data points in Figs. 3 and 5, respectively.

In each experiment, duplicate runs were carried out and then averaged to obtain the maximum conversion rate and corresponding lag time. The duplicates varied by less than ±1%, which means that for a given data set, the computed maximum conversion rate is also quite accurate. Therefore, the observed differences of 2.9 and 3.3%/h in Figs. 3 and 5, respectively, cannot be explained by experimental error. This significant difference must be attributable to other factors, e.g., probably the difference in initial ferric iron concentration. According to a previous study (*15*), ferric iron inhibits cell growth and ferrous iron oxidation by *T. ferrooxidans*.

The pH of the bacterial growth media was initially not strictly adjusted, i.e., it was somewhere between 2 and 3. The higher the initial pH, the more ferric iron precipitated out during cell growth. As a result, the amount of ferric iron transferred with the inoculum was lower, causing a higher maximum conversion rate. Each of the three studies (effect of initial pH, effect of initial ferrous iron concentration, and effect of cell number) was conducted with a different batch of *T. ferrooxidans*, which means that comparisons can be made within a data set (*see* Figs. 3, 4, and 5), but not among data sets.

CONCLUSIONS

Results of the parametric study of factors affecting ferrous iron oxidation show that the maximum conversion rate, which corresponds to the relative effectiveness of a given strain of *T. ferrooxidans* on iron oxidation, is a function of initial pH, initial ferrous iron concentration, and cell number of inoculum size. However, the lag time, which corresponds to the time required to obtain the maximum conversion rate, is affected by initial ferrous iron concentration and initial cell number, but not by initial pH.

Measuring ferrous iron concentration during its oxidation in 9K medium is a quick and simple method for predicting the relative potential of various strains of *T. ferrooxidans* in microbial leaching operations. However, according to Norris (*16*), developing better strains of *T. ferrooxidans* may not improve leaching rates if mineral dissolution is slower than iron oxidation in solution. Therefore, as better iron-oxidizing strains of the bacterium are identified, other improvements in microbial leaching operations need to be developed.

Based on the data presented in this paper, a low initial ferrous iron concentration and a large inoculum volume (initial cell number) are recommended for comparative studies of various strains of *T. ferrooxidans*. A low initial pH (1.8–2.0) is also recommended to prevent ferrous iron precipitation. In addition, before determining its maximum rate of oxidation of ferrous iron, each strain of *T. ferrooxidans* grown for a comparative study should be washed thoroughly to obtain cell suspensions containing a minimum amount of ferric iron.

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