Growth of *Thiobacillus ferrooxidans*: a Novel Experimental Design for Batch Growth and Bacterial Leaching Studies

P. I. HARVEY AND F. K. CRUNDWELL*

School of Process and Materials Engineering, University of the Witwatersrand, Johannesburg, South Africa
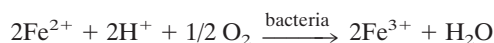
The concentrations of ferrous and ferric ions change dramatically during the course of the batch experiments usually performed to study the kinetics of the bacterial oxidation of ferrous ions and sulfide minerals. This change in concentration of the iron species during the course of the experiment often makes it difficult to interpret the results of these experiments, as is evidenced by the lack of consensus concerning the mechanism of bacterial leaching. If the concentrations of ferrous and ferric ions were constant throughout the course of the batch experiment, then the role of the bacteria could be easily established, because the rate of the chemical leaching should be the same at a given redox potential in the presence and in the absence of bacteria. In this paper we report an experiment designed to obtain kinetic data under these conditions. The redox potential is used as a measure of the concentrations of ferrous and ferric ions, and the redox potential of the leaching solution is controlled throughout the experiment by electrolysis. The effects of ferrous, ferric, and arsenite ions on the rate of growth of *Thiobacillus ferrooxidans* on ferrous ions in this redox-controlled reactor are presented. In addition, the growth of this bacterium on ferrous ions in batch culture was also determined, and it is shown that the parameters obtained from the batch culture and the redox-controlled batch culture are the same. An analysis of the results from the batch culture indicates that the initial number of bacteria that are adapted to the solution depends on the concentrations of ferrous and arsenite ions.

Bacterial oxidation of minerals is important in the formation of acid mine drainage and in the extraction of gold, copper, and uranium from ores. *Thiobacillus ferrooxidans*, which is the bacterium generally associated with these processes, is able to oxidize ferrous ions in solution and reduced sulfur compounds. It is a prime cause of acid mine drainage, a result of the enhanced rates of oxidation that are achieved in the presence of these bacteria. *T. ferrooxidans* was first isolated from acid mine drainage (10). These enhanced rates of oxidation of sulfide minerals are commercially exploited in the bacterial leaching process for the pretreatment of refractory gold ores (21). Full-scale biooxidation plants for the pretreatment of refractory gold ores have been commissioned in South Africa, Australia, Brazil, and Ghana (14).

An understanding of the mechanisms of the oxidation of iron and reduced sulfur compounds by *T. ferrooxidans* could be profitably used in the prevention of acid mine drainage and in the optimization of leaching operations. Iron is commonly present in mineral ores, and one of the key questions that requires further clarification concerns the role that iron plays in bacterial leaching. One of the two dominant points of view is that iron plays no part in the leaching reaction (4, 15). In this case, the bacterial leaching of a mineral, e.g., ZnS, may be represented as follows (16, 29):

$$ZnS + 1/2\ O_2 + 2H^+ \xrightarrow{\text{bacteria}} Zn^{2+} + S + H_2O$$

On the other hand, iron may play a central role in the leaching reaction. This may be represented as the chemical leaching of the mineral by ferric ions and the bacterial oxidation of ferrous ions (16, 29):

$$ZnS + 2Fe^{3+} \rightarrow Zn^{2+} + S + 2Fe^{2+}$$

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

During the course of a batch leaching experiment, the concentrations of ferrous and ferric ions change, and hence the rates of both chemical leaching and bacterial oxidation of ferrous ions change. Thus, it is difficult to determine the contribution of chemical leaching by ferric ions to the overall leaching reaction. Evidence for these difficulties is the lack of consensus in the literature concerning which of the two mechanisms described above dominates the bacterial leaching of minerals (4, 7, 15, 16, 29).

The key to obtaining good kinetic data is to allow the concentrations of as few of the reacting species as possible to change during the course of the reaction. In practice, this is usually achieved by ensuring that those reactants whose concentrations should not vary are present in stoichiometric excess. This is often not possible in studies of bacterial growth in batch culture; hence the importance of the development of other reactor techniques for the study of bacterial growth, such as continuous culture (chemostat) and fed-batch culture.

Unfortunately, fed-batch and chemostat reactor techniques are not easily adapted to studies of bacterial leaching. The concentrations of the ferrous and ferric species in leaching experiments could be controlled in a fed-batch reactor by the addition of an oxidant or a reductant, as required. In our studies of the chemical leaching of zinc sulfide, we maintained the concentration of the ferric ions in solution by oxidizing the ferrous ions produced in the leaching reaction with hydrogen peroxide (11–13). However, the addition of an oxidant or a reductant to the bacterial leaching vessel may interfere with the rate of bacterial growth. An alternative to the addition of chemical agents is to oxidize or reduce the ferrous and ferric ions directly by electrolysis during the course of bacterial leaching experiments. Thus, the concentrations of ferric and

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* Corresponding author. Mailing address: School of Process and Materials Engineering, University of the Witwatersrand, Private Bag 3, WITS, 2050, South Africa. Phone: 27-11-716-2413. Fax: 27-11-339-7213. E-mail: FKC@chemeng.chmt.wits.ac.za.
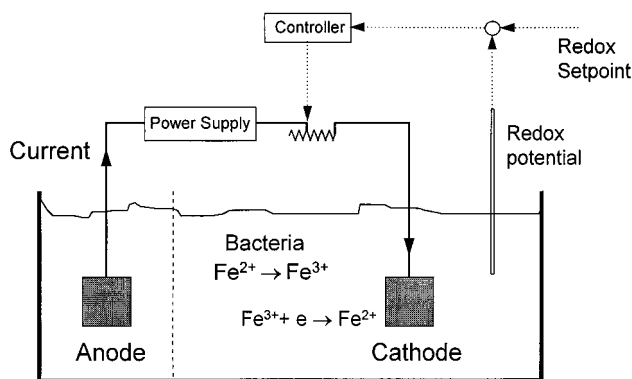
FIG. 1. Schematic diagram of the experimental apparatus. The bacteria are grown in the cathode compartment of a divided electrolytic cell. The redox potential in this compartment is controlled at the setpoint level by changing the current to the cathode.

ferrous ions may be controlled by adjusting the electrolysis current.

The aim of this paper is to report the development of a new experimental technique for kinetic studies of bacterial leaching in which the concentrations of the iron species are controlled. The redox potential of the solution, which is dependent on the ratio of the concentrations of ferrous and ferric ions, is controlled by electrolysis throughout the course of the batch experiment. For this reason we refer to the apparatus as a redox-controlled reactor.

In this work, we evaluated the redox-controlled reactor by studying the growth of *T. ferrooxidans* on ferrous ions. We show that the same values of the growth parameters for the bacterial oxidation of ferrous ions are obtained from data from the redox-controlled reactor and from the batch reactor. The kinetics of bacterial leaching of sulfide minerals with the redox-controlled reactor will be described in subsequent work.

MATERIALS AND METHODS

In batch experiments that are usually performed to determine the kinetics of bacterial oxidation of ferrous ions, the concentrations of the ferrous and ferric ions change over a wide range. This change in concentration may be countered by the electrolytic reduction of the ferric ions that are produced by the bacteria. A schematic diagram of the apparatus is shown in Fig. 1. The apparatus is an electrolytic cell, and the reactions of interest occur in one compartment of the cell. A feedback loop, which consists of the measurement of the iron species in solution, the controller, and a variable resistor, is used to control the ferrous and ferric ion concentrations at the setpoint value. In this experiment, the redox potential of the solution, given by the Nernst equation $E = E^0 + (RT/F)$ ln ($[Fe^{3+}]/[Fe^{2+}]$), is used as a measure of the concentrations of ferrous and ferric ions. The current to the electrode is adjusted automatically by the controller in order to maintain the redox potential at the setpoint value. Since the only reaction in the present study is the bacterial oxidation of ferrous ions to ferric ions, the reaction at the electrode in the bacterial growth compartment is the reduction of ferric ions to ferrous ions. (Note that the redox potential is used to determine the concentrations of ferrous and ferric ions only for automatic control purposes.)

Conceptually, the redox-controlled reactor is a fed-batch reactor. Instead of using the controlled addition of a nutrient as it is depleted in the fed-batch reactor, we control the addition of the electrons which regenerate the substrate (ferrous ions) that is consumed by the bacteria.

The rate of ferrous ion oxidation, $r_{Fe^{2+}}$, measured in units of grams/liter · second, is given by $r_{Fe^{2+}} = (I/F)(M_{Fe}/V)$, where I is the current (in amperes), F is Faraday's constant (96,500 C/mol), M_{Fe} is the molecular mass of iron, and V is the volume (in liters) of the cathode compartment.

The specific growth rate of the bacterial culture, μ, can be calculated from the rate of ferrous ion oxidation by $\mu = r_{Fe^{2+}}Y/N = (I/F)(YM_{Fe}/NV)$ if it is assumed that the rate of oxidation of the substrate utilized for maintenance processes is small (1, 6). Y is the bacterial yield coefficient, and N is the bacterial concentration. Therefore, the growth rate of the bacterial culture can be easily calculated from the measurement of the current.

Thus, the two advantages of this apparatus that arise in the study of the oxidation of ferrous ions are that (i) there is no change in the concentrations of the ferrous and ferric ions in solution during the experiment and (ii) the current that is supplied to the electrolysis cell is a direct measure of the rate of bacterial oxidation of ferrous ions.

Apparatus. The electrolysis cell was made of polyvinyl chloride (PVC) and was divided into two compartments by a semipermeable cationic membrane. Each compartment of the divided cell had a capacity of 2 liters. The bacterial oxidation of ferrous ions occurs in the cathode compartment. This compartment was stirred by an impeller driven by an overhead stirrer. Air from an oil-free line was sparged into the cathode compartment. Electrodes for the measurement of pH and redox potential and an oxygen probe were suspended in the solution of the cathode compartment. The cathode compartment was covered by a PVC lid.

The redox potential was measured by using a platinum electrode and a silver–silver chloride reference electrode. The redox potential, which is the voltage between the platinum and the reference electrodes, was measured by using a high-impedance source and a PC 30 analog/digital control card (Eagle Electronics) installed in an IBM-compatible personal computer. The current to the electrolysis cell was supplied by a 30-A, 20-V power supply. The current through the electrolysis cell was manipulated by an output signal from the PC 30 card that changed the value of a variable resistor placed in series with the power supply and the electrodes. A computer program was written that compared the measured redox potential with the setpoint value and, on the basis of this measurement, determined the value of the output signal to the variable resistor by using the proportional, integral, and derivative control law. The redox potential was maintained to within 1% of the setpoint.

The pH of the solution in the cathode compartment was measured and controlled by a Gallenkamp pH controller at a value of 1.8. However, very little pH adjustment was required from the pH controller, because the electrolysis cell is pH balanced. Protons are consumed in the cathode compartment; however, an equal amount are produced in the anode compartment, where the electrode reaction is the evolution of oxygen. Since the semipermeable-permeable membrane between the anode and the cathode compartments is a cationic membrane, the current through the cell is carried principally by the transfer of protons from the anode compartment to the cathode compartment, thus neutralizing any changes in the pH that occur as a result of bacterial activity.

The concentration of oxygen was measured with a Degussa oxygen meter. The concentration of oxygen was controlled manually at a value of 5.8 ppm. The electrolysis cell was placed in a constant-temperature bath, which maintained the temperature at 35°C.

The results obtained with the electrolysis cell were compared with those obtained from batch experiments. The vessel for batch experiments was essentially the same as the cathode compartment of the electrolysis cell. The apparatus used was a 2-liter PVC vessel that was covered by a PVC lid. The vessel was stirred by an impeller driven by an overhead stirrer, and air was sparged into the solution. pH, oxygen, and redox electrodes were suspended in the solution. The concentration of oxygen was controlled manually at a value of 5.8 ppm. The pH was controlled by the addition of acid at a value of 1.8, which was regulated by a Gallenkamp pH controller. The vessel was placed in a constant-temperature bath, which maintained the temperature at 35°C.

Bacterial culture. The bacterial culture used was a pure strain of *T. ferrooxidans* (strain FC1) that was obtained from D. Rawlings at the University of Cape Town, Cape Town, South Africa. The bacteria were cultured in a batch reactor in 9K medium (30) under conditions similar to the conditions used for the experiments. The 9K medium contains, per liter, 3 g of $(NH_4)_2SO_4$, 0.1 g of KCl, 0.5 g of K_2HPO_4, 0.5 g of $MgSO_4 \cdot 7H_2O$, 0.01 g of $Ca(NO_3)_2$, and 44.8 g of $FeSO_4 \cdot 7H_2O$. The pH of the medium was adjusted to 1.8 by the addition of H_2SO_4. The bacteria were maintained in the exponential growth phase by subculturing on a daily basis. The inoculum was replaced at regular intervals by culture from the original sample to reduce the possibility of contamination.

The redox-controlled experiments were conducted in the same culture medium but with the iron present at various concentrations. The experiment was begun by adding inoculum from the batch reactor to the growth compartment of the redox-controlled reactor. The inoculum was 10% (by volume) of the reactor size. The initial cell numbers were typically on the order of 10^7 cells/ml.

The total concentration of bacteria was determined by counting the number of bacteria in a four-by-four grid of a hemacytometer. The standard deviation for the cell number was 1.15% of the mean (10 replicates).

The yield coefficient, Y, was determined from ratio of the change in the total concentration of bacteria (cells per milliliter) to the change in the concentration of ferrous ions (grams per liter) in the exponential growth phase for eight independent batch experiments. An average value for Y of 8.4×10^{10} cells/g of Fe was obtained (standard deviation, 0.5×10^{10} cells/g of Fe [eight replicates]).

Reagents and analysis. Analytical-grade reagents were used for all experiments.

The concentration of ferrous ion was determined by titration with potassium dichromate. Diphenylamine sulfonate was used as the indicator (33). The standard deviation for the determination of the concentration of ferrous ions in solution was 1.8% of the mean (10 replicates). The concentration of ferric ions was determined by reducing the ferric ions to ferrous ions with stannous chloride and determining the amount of ferrous ions that were reduced.

The concentration of the arsenite ions in solution was determined by titration with cerium sulfate (33). The end point was detected with ferroin. The calcula-
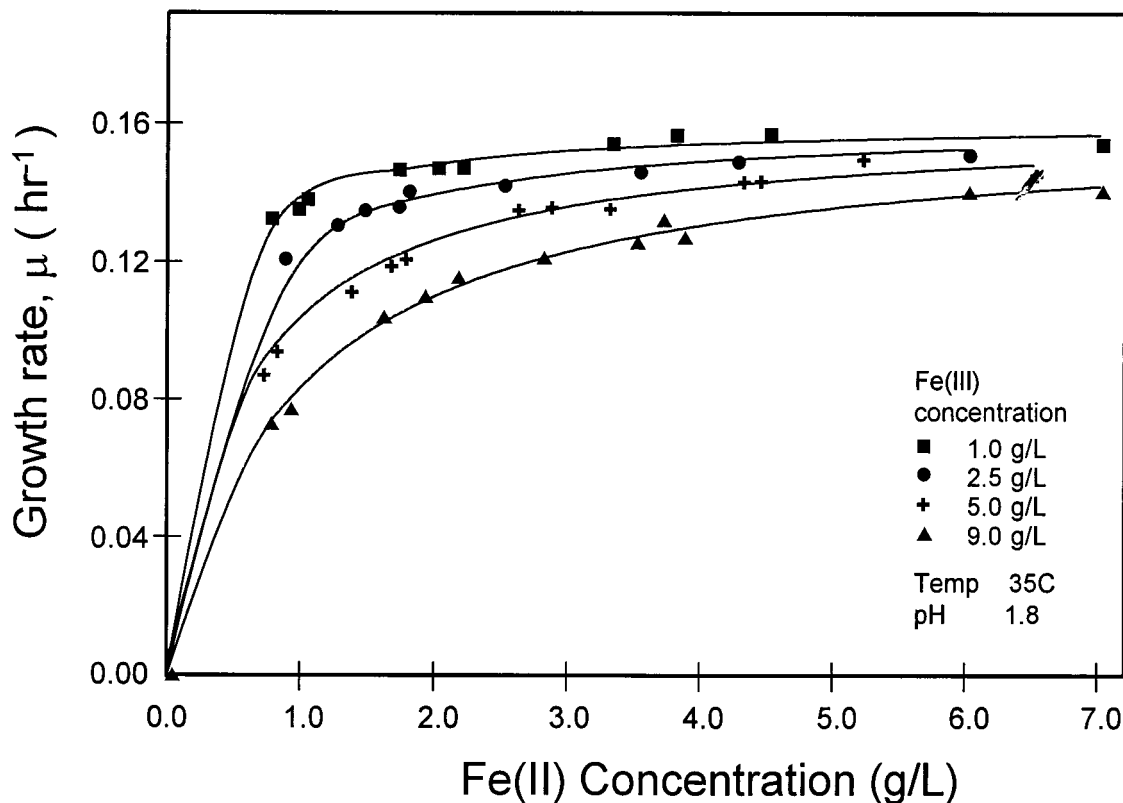
FIG. 2. Effect of the concentrations of ferrous and ferric ions in solution on the rate of bacterial growth of *T. ferrooxidans* in the redox-controlled reactor (17). The points represent the experimental data, and the lines represent the Monod equation with the parameters given in Table 1. Each point represents a separate experiment in the redox-controlled reactor.

tion of the concentration of arsenite ions required adjustment for the concentration of ferrous ions in solution.

RESULTS

Bacterial growth on ferrous ions in the redox-controlled electrolysis cell. The current that is required to reduce ferric ions in order to maintain the redox potential at a given value is directly proportional to the rate of bacterial oxidation of ferrous ions. Thus, the current is an instantaneous and continuous measurement of the rate of bacterial activity during the experiment. The rate of oxidation of ferrous ions is calculated from the value of the current at a particular value. From the determination of the bacterial concentration, N, determined at the same time as the rate of oxidation of ferrous ions, the rate of bacterial growth was calculated (four replicates per experiment). Cell counts in the exponential phase of growth in the redox-controlled reactor were typically on the order of 10^8 cells/ml. By performing experiments with different concentrations of ferrous, ferric, and arsenite ions in solution, we can determine the effect that these ions have on the rate of bacterial growth. Altogether, 44 redox-controlled experiments were performed, and the results are presented below.

(i) Effect of ferrous and ferric ions on the rate of bacterial growth on ferrous ions. The effect of ferrous and ferric ions on the rate of bacterial growth on ferrous ions in the redox-controlled reactor is shown in Fig. 2. These results indicate that the rate of growth reaches a maximum with increased concentrations of the substrate (ferrous ions) and that the rate is inhibited by the product (ferric ions). Similar results have been reported by other researchers (see reference 19 for a review).

The experimental results shown in Fig. 2 may be described by the Monod growth equation. The form of the Monod equation for product inhibition by ferric ions is given as follows (1, 6, 29):

$$\mu = \mu_{max} \left\{ \frac{[Fe^{2+}]}{[Fe^{2+}] + K_s(1 + K_i[Fe^{3+}])} \right\}$$

where μ_{max} is the maximum growth rate of the bacteria, and K_s and K_i are constants.

The parameters of the Monod growth equation can be obtained either from a double-reciprocal plot of the data or by fitting the parameters to the data by nonlinear minimization of the sum of squared errors (27, 28). The lines in Fig. 2 represent the Monod growth equation with the parameters given in Table 1. An examination of Fig. 2 indicates that the correspondence between the numerical fit and the data is excellent.

The values of μ_{max} that are reported in the literature (19) range between 0.05 and 1.3 h^{-1}, while those for K_s range between 0.0 and 1.0 g/liter and those for K_i range between 0.5 and 2.3 liters/g. Therefore, the values of the parameters ob-

TABLE 1. Kinetic parameters for the growth of *T. ferrooxidans* in the redox-controlled reactor

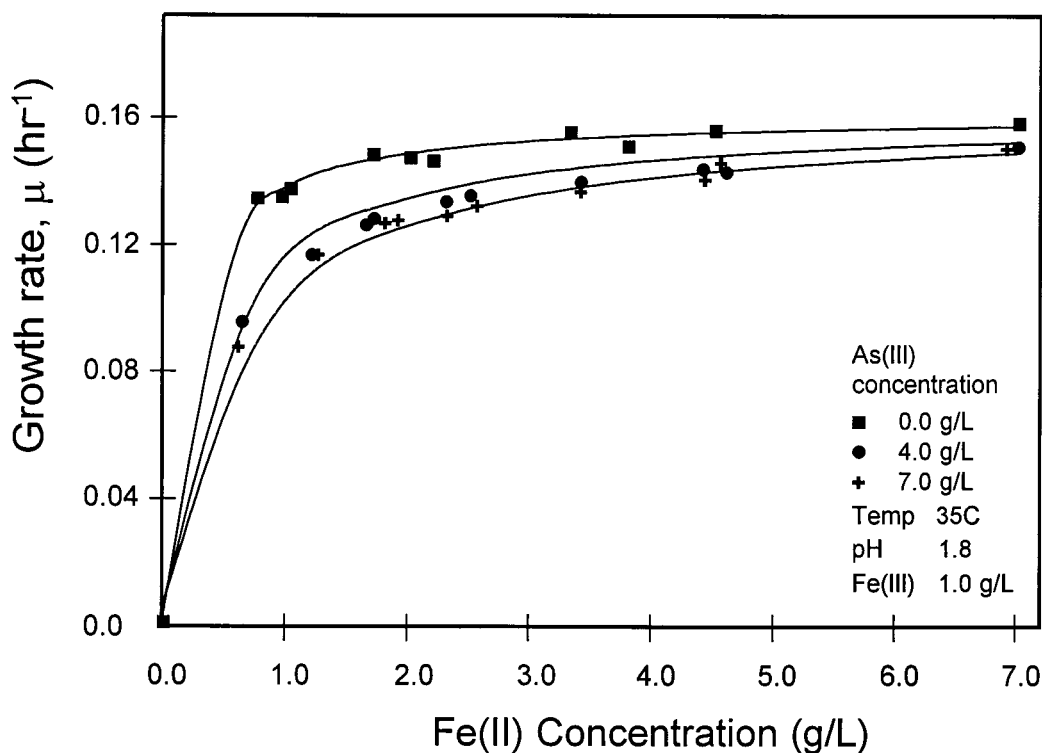| Arsenite	μ_{max} (h^{-1})	K_s (g/liter)	K_i (liters/g)	K_a (liters/g)
Absent	0.16	0.073	1.29	
Present	0.16	0.073	1.29	0.046

FIG. 3. Effect of arsenite ions on the rate of bacterial growth of *T. ferrooxidans* at various concentrations of ferrous ions in solution in the redox-controlled reactor (17). The points represent the data, and the lines represent the modified Monod equation with the parameters given in Table 1.

tained from the data from the redox-controlled reactor are consistent with those reported in the literature.

(ii) Effect of arsenite ions on the rate of growth in the redox-controlled reactor. A successful application of bacterial leaching technology has been the pretreatment of refractory gold ores (14). These ores often contain arsenopyrite (FeAsS), and the bacterial leaching of arsenopyritic ores results in the formation of arsenite [As(III)] and arsenate [As(V)] species in solution (3, 21). The oxidation of arsenite to arsenate by ferric ions occurs only in the presence of bacteria and pyrite (23, 24), and a substantial proportion of the arsenic in the ore is precipitated as ferric arsenate ($FeAsO_4$) (21).

Previous studies of the effect of arsenic compounds on the rate of growth of *T. ferrooxidans* are limited (8, 9, 17, 32). Collinet and Morin (9) reported that the growth of both *T. ferrooxidans* and *Thiobacillus thiooxidans* is inhibited with 5 g of arsenite per liter and 40 g of arsenate per liter.

We recently presented results for the effect of arsenite ions on the rate of growth of *T. ferrooxidans* obtained in the redox-controlled reactor (17). The results from reference 17 are summarized in Fig. 3. Titration for arsenite ions in solution indicated that the arsenite ion concentration remained constant throughout the experiment; that is, arsenite was not oxidized to arsenate during the course of the experiment. The results represented in Fig. 3 indicate that the rate of bacterial growth is strongly inhibited by arsenite ions in solution. The effect of arsenite ions on the rate of bacterial growth can be described by a modified form of the Monod equation:

$$\mu = \mu_{max} \left\{ \frac{[Fe^{2+}]}{[Fe^{2+}] + K_s(1 + K_i[Fe^{3+}]) + K_a[As^{3+}]} \right\}$$

where K_a is a constant. The data were analyzed by numerically fitting the parameter K_a to the data by nonlinear minimization

of the sum of squared errors (27, 28). The same values of the parameters μ_{max}, K_s, and K_i that were obtained in the absence of arsenite ions were used in the evaluation of K_a. The lines in Fig. 3 represent the modified Monod equation with the values of the parameters given in Table 1. An examination of Fig. 3 indicates that the correspondence between the equation and the data obtained with the redox-controlled reactor is excellent.

Batch studies of the growth of *T. ferrooxidans.* It is important to establish that the growth of the bacteria in the redox-controlled reactor is not influenced by the technique itself, for example, that the application of a current to the solution does not influence the bacterial growth. In this study we determined this by comparing the growth parameters obtained from the data from the redox-controlled reactor with the parameters obtained from studies of batch growth of *T. ferrooxidans* for the oxidation of ferrous ions.

It is common in studies of the bacterial oxidation of ferrous ions to evaluate the growth parameters from the concentration of the substrate rather than from the biomass (see, for example, reference 22). The results from the studies of the oxidation of ferrous ions by *T. ferrooxidans* in the absence and presence of arsenite ions are shown in Fig. 4 and 5, respectively.

The rate of bacterial oxidation of ferrous ions may be described by Monod-type kinetics and by the kinetics of the exponential growth of bacteria. If it is assumed that the rate of substrate utilization for maintenance activities is small, then the following equation describing the rate of change of substrate may be obtained (1):

$$- \frac{1}{\frac{N_0}{Y} + ([Fe^{2+}]_0 - [Fe^{2+}])} \frac{d[Fe^{2+}]}{dt} = \frac{\mu_{max}}{[Fe^{2+}] + K_s(1 + K_i[Fe^{3+}])}$$
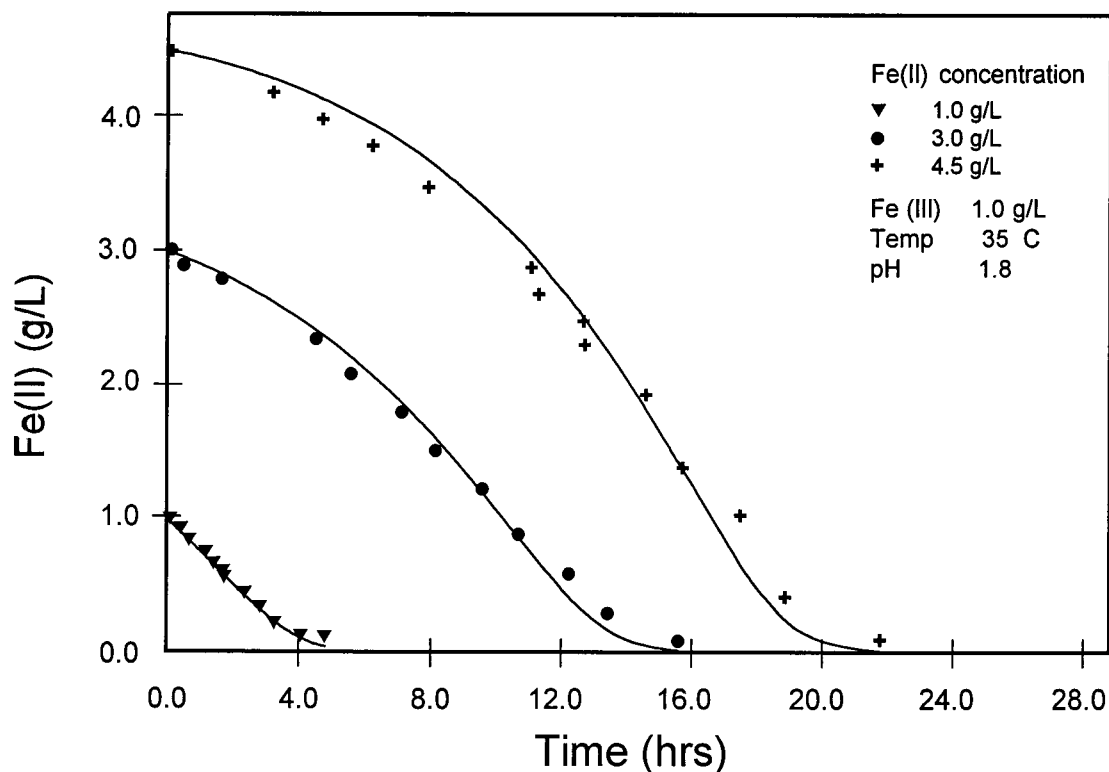
FIG. 4. Effect of the initial concentration of ferrous ions on the oxidation of ferrous ion by *T. ferrooxidans* in a stirred batch reactor. The points represent the experimental data during the batch run, and the lines represent the numerical fit of batch substrate balance to the data.

where the subscript 0 indicates the initial value. In addition, the concentration of ferric ions in solution is related to the total concentration of iron by the solution material balance: $[Fe^{3+}] = [Fe^{3+}]_0 + [Fe^{2+}]_0 - [Fe^{2+}]$. Therefore, this differential equation describes the batch oxidation of ferrous ions by *T. ferrooxidans*. The equation was solved numerically by using a Runge-Kutta-Fehlberg routine (2). The parameters were fitted to the data by minimizing the sum of squared errors between the measured data and the model solution (28).

The numerical solution of the equations representing the model and the experimental data are in close agreement, indicating that the model is a good description of the leaching reaction. The parameters for the batch growth of *T. ferrooxidans* are given in Tables 2 and 3. A comparison of the parameters obtained for bacterial growth from the redox-controlled reactor and from the batch vessel shows that they are in close agreement, considering the wide range of these parameters that has been reported in the literature (19).

The mathematical description of the bacterial growth in batch culture has an additional parameter that is not the same as that required in the analysis of the results from the redox-controlled reactor (or continuous culture). This is the parameter N_0/Y. At first sight, it seems that this parameter may be obtained by independent means: the initial concentration of bacteria, N_0, may be determined, and the yield coefficient, Y, may be determined from the rate of increase in the concentration of cells and the corresponding increase in the rate of oxidation of ferrous ion. However, a difficulty arises with the value of the initial concentration of cells, N_0. Counting the initial number of bacteria determines the total number of bacteria, rather than the number of bacteria that are active under the conditions of the experiment. This is also true for other

techniques that determine total biomass. The problem in determining total numbers or total biomass becomes clearer when one considers the effect of arsenite ions on the rate of growth of the bacteria. As the concentration of arsenite increases, the number of bacteria that are adapted to the arsenite decreases. Even though there may be the same total number of bacteria in the inoculum from experiment to experiment, it is expected that as the concentration of arsenite ions increases, the number of adapted bacteria decreases. Thus, the effective value of N_0 should also decrease, reflecting the initial number of bacteria that are active at that concentration of arsenite ions.

Indeed, this is exactly what is obtained from the analysis of the batch data for the growth of *T. ferrooxidans* by the numerical method presented here. The values of the parameter N_0/Y obtained in this study are given in Table 3. The results given in Table 3 indicate that as the concentration of arsenite ions increases, the value of N_0/Y decreases.

An examination of the parameters given in Table 3 indicates that the value of N_0/Y is also dependent on the initial concentration of ferrous ions in solution. This result means that the size of the population that is adapted to higher concentrations of ferrous ions, given by N_0, decreases as the concentration of ferrous ion increases. This effect has not been reported previously and probably would not have been noticed if the batch data were simply analyzed by the conventional method of double-reciprocal plots, that is, by Lineweaver-Burk plots.

It is common in studies of the oxidation of ferrous ions by *T. ferrooxidans* to evaluate the growth parameters from the data for the concentration of ferrous ions in solution by the method of double-reciprocal plots (see, e.g., references 19 and 22) of the batch substrate equation. (The right-hand side of the batch
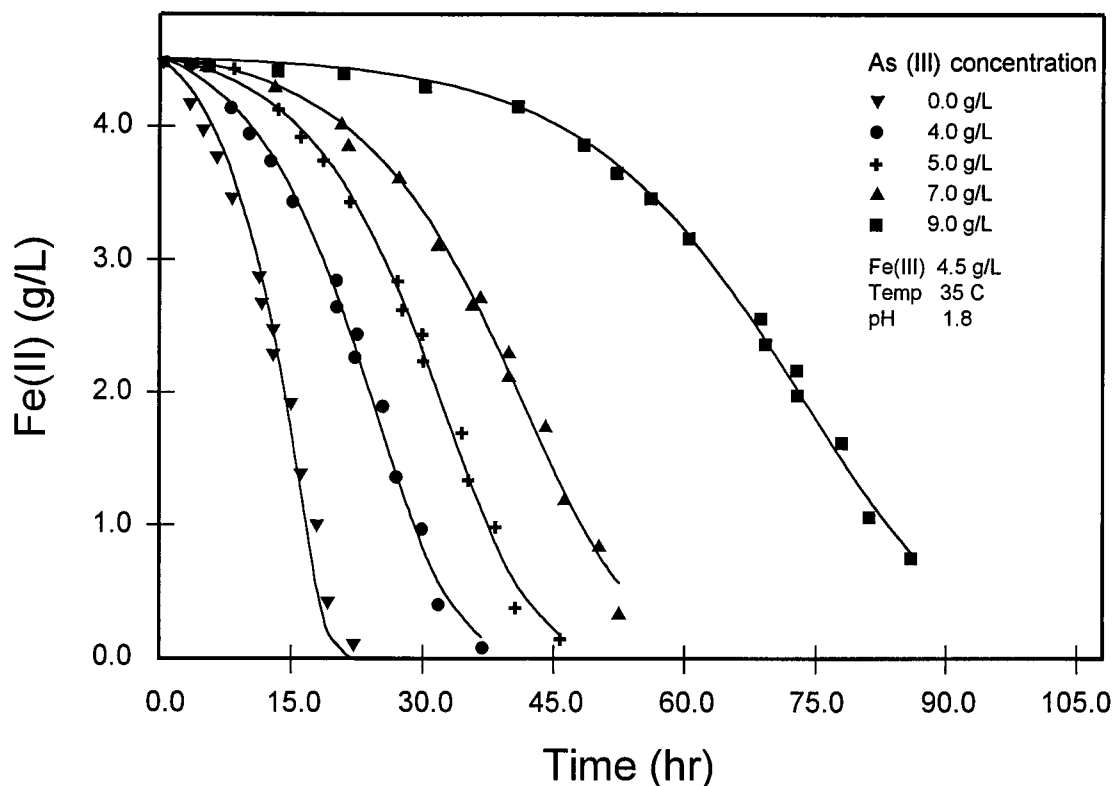
FIG. 5. Effect of the concentration of arsenite ions on the bacterial oxidation of ferrous ions by *T. ferrooxidans* in a stirred batch reactor. The points represent the experimental data during the batch run, and the lines represent the fit of batch substrate balance to the data.

substrate equation represents the specific growth rate, μ, and it is evaluated from the data at each time.) However, if N_0 is dependent on the initial concentration of ferrous ions in solution, it is difficult to evaluate the right-hand side of the batch substrate equation from the data, because the value of the parameter N_0/Y is unknown. Therefore, it is difficult to construct double-reciprocal plots that are reliable, and the parameters obtained from this method of analysis must be treated with caution.

A comparison of Tables 1 and 2 indicates that the values of the parameters obtained from the batch culture and the redox-controlled reactor are in excellent agreement. Therefore, the environment of the redox-controlled reactor does not influence the growth of *T. ferrooxidans* on ferrous ions in solution.

DISCUSSION

Other researchers have performed bacterial growth experiments with *T. ferrooxidans* in an electrolysis cell (5, 18, 20, 25, 26, 31). However, it is important to emphasize that the experiment described here is different in concept, design, and operation from all of those previous experiments. The previous researchers operated their electrolysis cell in one of the two standard modes: either in the potentiostatic mode, in which the

potential of the cell or the potential of one of the electrodes is constant, or in the galvanostatic mode, in which the current through the cell is constant. Hubner (18) and Blake et al. (5) operated the cell in the galvanostatic mode, while Natarajan (25, 26) operated the cell in the potentiostatic mode. None of the previous researchers controlled the concentrations of ferrous and ferric ions in solution by manipulating the current to the electrolysis cell. The redox-controlled reactor is not a standard method for the operation of an electrolysis cell.

The principal feature of this apparatus is that the concentrations of ferrous and ferric ions do not change during the reaction. The growth of *T. ferrooxidans* on ferrous ions in solution in the redox-controlled reactor is consistent with that of batch growth on ferrous ions. Therefore, the electrolysis technique does not affect the growth of the bacteria, and as a result, this apparatus can be used to identify the role of the bacteria and the role of iron in the bacterial leaching reaction. For example, if ferric ions are an important intermediate in the bacterial leaching of ores, the rates of chemical and bacterial

TABLE 2. Kinetic parameters for the growth of *T. ferrooxidans* in batch culture

Arsenite	μ_{max} (h^{-1})	K_s (g/liter)	K_i (liters/g)
Absent	0.16	0.075	1.27
Present	0.16	0.075	1.27

TABLE 3. Additional kinetic parameters for the growth of *T. ferrooxidans* in batch culture

[Fe^{2+}]$_0$ (g/liter)	[As(III)]$_0$ (g/liter)	N_0/Y (g/liter)	K_a (liters/g)
1.0	0.0	1.68	
3.0	0.0	0.60	
4.5	0.0	0.34	
4.5	4.0	0.25	0.48
4.5	5.0	0.13	0.45
4.5	7.0	0.094	0.48
4.5	9.0	0.021	0.60

leaching will be the same at the same redox potential. However, if iron plays a minor or insubstantial role in bacterial leaching, then the rates of chemical leaching and bacterial leaching at the same redox potential should not necessarily be the same. Since the redox potential can be accurately controlled in this novel apparatus, it should be possible to determine the role of iron in the bacterial leaching of ores.

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