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THE EFFECT OF AS(III) ON THE GROWTH OF *THIOBACILLUS FERROOXIDANS* IN AN ELECTROLYTIC CELL UNDER CONTROLLED REDOX POTENTIALS

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ABSTRACT

Thiobacillus ferrooxidans oxidises ferrous ions in solution during the bacterial leaching process. In batch experiments that are usually performed to determine the kinetics of the bacterial oxidation of ferrous ions, the concentrations of both the ferrous and ferric ions change as a result of the bacterial activity. This change in concentration makes it difficult to interpret the data from these experiments, particularly if the concentrations vary through reaction regimes where different mechanisms govern the bacterial activity. In this study the concentrations of ferrous and ferric ions are maintained at their initial values by controlling the redox potential of the solution in which the reaction occurs. The redox potential is controlled by reducing the ferric ions produced by the bacteria. In order to achieve this, the bacteria are grown in the cathode compartment of an electrolysis cell. The current to the cell is varied by the controller, depending on whether more or less reduction of ferric ions is required. The current required to reduce the ferric ions in order to maintain the redox potential at a constant value is a measure of the bacterial activity and the rate of bacterial growth. The effect of ferrous, ferric and arsenite concentrations on the rate of bacterial growth are presented. It is shown that growth rate is strongly dependent on the concentration of both the ferric and the arsenite ions in solution. Copyright © 1996 Elsevier Science Ltd

Keywords
Leaching; bioleaching; bacteria; redox reactions; biotechnology

INTRODUCTION

In the last ten years a number of full-scale plants for the bacterial leaching of refractory gold ores have been commissioned [1]. Refractory gold ores frequently contain arsenic, usually in the form of arsenopyrite (FeAsS), and bacterial leaching of these ores results in the formation of arsenite (As^{3+}) and arsenate (As^{5+}) species in solution [2]. Arsenite is oxidised to arsenate by ferric ions only in the presence of bacteria and pyrite [3,4] and during bacterial leaching a substantial amount of the arsenic in solution is precipitated as ferric arsenate ($FeAsO_4$) [5].

Arsenic in solution inhibits the growth of the bacteria. Arsenate, the least poisonous of the arsenic compounds, substitutes for phosphate, and results in the uncoupling of oxidative phosphorylation by the formation of an unstable ADP–arsenate complex [6]. Energy, which would normally result in the formation of ATP, is expended, and the rate of growth is inhibited. Arsenite derives its toxicity from

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deactivating enzymes that have thiol groups as their active centres by binding to these thiol groups [6]. As a result, arsenite inhibits the oxidative decarboxylation of pyruvate and alpha-ketogluterate, which upsets the functioning of the citric acid cycle [6].

Only a few studies on the effect of arsenic on the rate of growth of *Thiobacillus ferrooxidans* have been performed, and most of these studies are limited to determining the toxicity level of arsenic. Tuovinen *et al.* [7] found arsenic to be toxic between 50 and 100 mg/L. Braddock *et al.* [8] determined that that arsenite is not oxidised by the bacteria to arsenate, and in continuous culture they observed no effect on the rate of bacteria growth at either 1.33 or 2.67 mM arsenite. Collinet and Morin [9] determined that the growth of both *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* is inhibited with 5 g/L arsenite and 40 g/L arsenate. Barrett *et al.* [2] noted that arsenic at concentrations of 30 mM As(III) was a major toxin to a moderately thermophilic mixed culture, and that of concentrations of 90 mM As (III) were found in dead cultures.

In this paper, we report an investigation of the effect of arsenite toxicity on the growth of *Thiobacillus ferrooxidans*. We have developed a new experimental design in order to obtain accurate data on the rate of bacterial growth. Previous work on the growth of *Thiobacillus ferrooxidans* has generally been conducted in batch experiments in which the concentrations of both ferric and ferrous ions change substantially throughout the experiment [10]. This makes it difficult to interpret the experimental data, particularly when the rate of growth is dependent on the concentrations of both ferrous and ferric ions in solutions, and when there are other ions present in the solution that affect the rate of growth. As a result, we have developed a batch experimental apparatus in which the redox potential is maintained at a constant level throughout the experiment, thus keeping the concentrations of both ferrous and ferric ions at a constant level. The principle of the operation of this apparatus is also described in this work.

EXPERIMENTAL

The apparatus used was a electrolysis cell, made of PVC, and divided into two compartments, each of 2 litre capacity, by a semipermeable cationic membrane. The cathode was made of platinum, and the anode of lead. The cathode compartment was stirred by means of an impeller attached to an overhead stirrer. Air was bubbled through the reactor from an oil-free compressor line. The cathode compartment was covered by a PVC lid. The electrolysis cell was placed in a constant-temperature bath.

Experiments were performed at a pH of 1.8, and the oxygen concentration was maintained at 5.8 ppm. The pH was measured and controlled using a Gallenkamp pH controller and the concentration of oxygen was measured with a Degussa oxygen meter. Very little pH adjustment was required from the controller. The temperature was maintained at 35°C. The redox potential was measured using a platinum electrode and a silver/silver chloride reference electrode. The redox potential was measured using a PC30 analog/digital control card in an IBM compatible personal computer. The current to the electrolysis cell was supplied by a 30 Amp power supply, and the current was controlled by a signal from the personal computer implementing a proportional, integral and derivative (PID) control law.

The bacterial culture used was a pure strain of *Thiobacillus ferrooxidans* (strain FC1) that was adapted for growth on arsenic-containing ores and was obtained from Dr Rawlings (Microbiology, University of Cape Town) via Dr E. Lawson (Microbiology, University of the Witwatersrand, Johannesburg). The bacteria were grown in a 9K medium [11] prior to the growth experiment. The bacterial culture was replaced at regular intervals by culture from the original sample in order to minimise the possibility of contamination. Prior to initiating a redox-controlled batch experiment, the cells were extensively adapted to the exponential growth phase. This was achieved by the batch sub-culturing of the inoculum in the 9K medium by repeatedly removing 75% of the culture solution once the concentration of ferrous ion reached less than 0.5 g/L and replacing this solution with fresh 9K medium. Once the bacteria had begun to rapidly oxidised the ferrous ions in solution, the proportion of solution replaced with fresh 9K medium was reduced to 25%, and since the rate of growth was rapid at this stage, the sub-culturing procedure was required on a daily basis.

Analytical grade reagents were used. The concentration of ferrous ion was determined by titration with potassium dichromate with diphenylamine sulphonate used as the indicator. The concentration of ferric ion was determined by the reduction of the ferric ions to ferrous ions by stannous chloride, and determining the concentration of ferrous ions. The concentration of arsenite was determined by titration with cerrium sulphate with ferroin used as the indicator.

RESULTS AND DISCUSSION

Description of operation of the experimental apparatus

The concept of the experimental design that we have developed is to perform the bacterial growth experiments in the cathode compartment of an electrolysis cell, and to control the concentrations of ferrous and ferric ions by adjusting the current to the electrolysis cell. A schematic diagram of the apparatus is shown in Figure 1. Bacteria oxidise the ferrous ions to ferric ions, and the ferric ions are reduced at the platinum cathode. The concentration of ferrous and ferric ions is maintained at the initial value of the experiment by maintaining a constant redox potential in the solution by manipulating the current to the cathode.

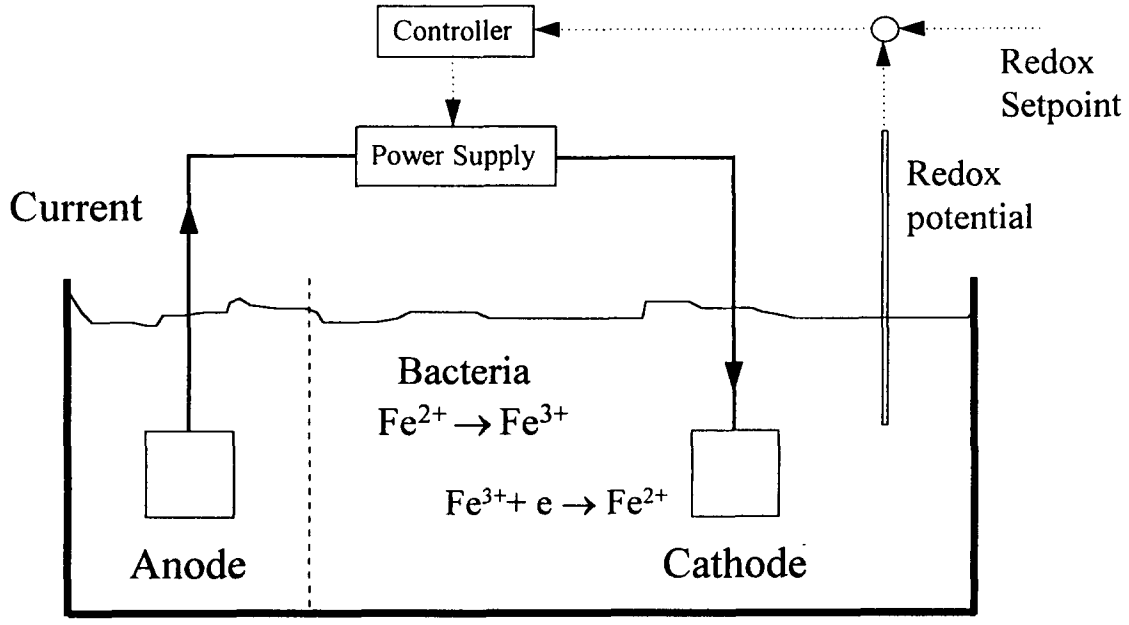

Fig.1 Schematic diagram of the experimental apparatus. The redox potential, hence the concentrations of ferrous and ferric ions, is controlled at a constant value, given by the setpoint value, by manipulating the current to the cell

Thus the redox potential of the solution is measured as an indication of the concentrations of the ferrous and ferric ions and this measurement is used by the controller to adjust the current to the electrolysis cell.

It is essential to realise that this experimental design is not the same as that described by Hubner [12], Taya *et al.*[13], Natarajan [14,15], nor Blake *et al.* [16], who all performed experiments in an electrolysis cell. In their experiments, none of these workers attempted to control the concentration of iron species in solution by varying the current to the cell. Rather, they used one of the two usual strategies for the control of electrolysis cells, that is, either the current to the electrolysis cell was constant, called galvanostatic operation, or the potential of the cathodic electrode or across the cell was constant, called potentiostatic operation. For example, Hubner [12] and Blake *et al.* [16] used a constant current to the cell (galvanostatic operation) while Natarajan [14,15] controlled the potential of the cathode at a predetermined level

(potentiostatic control). In this work, we measured and controlled the redox potential of the solution, experiments that are more similar in concept to the chemical leaching experiments at constant redox potential used by ourselves [17–19].

The advantage of this experiment is that the current is a direct measure of the rate of the oxidation of ferrous ions, given by Faraday's law:

$$r'_{Fe^{2+}} = \frac{I}{F} \tag{1}$$

where $r'_{Fe^{2+}}$ is the rate of oxidation of ferrous ions (mol/s), I is the current (Amps), and F is Faraday's constant (96500 C/mol). From the mass balance over the cathode compartment, the specific rate of ferrous ion oxidation, $r_{Fe^{2+}}$ (g/L.hr), is given by:

$$r_{Fe^{2+}} = \frac{I}{F}\frac{M_{Fe^{2+}}}{V} \tag{2}$$

where $M_{Fe^{2+}}$ is the molecular mass of iron, and V is the volume of the cathode compartment. The specific growth rate of the bacterial culture, μ, can be calculated from the rate of ferrous ion oxidation by:

$$\mu = \frac{r_{Fe^{2+}}Y}{N} \tag{3}$$

where Y is the bacterial yield coefficient and N is the bacterial number. It has been assumed that the amount of substrate utilised for maintenance processes is small. Thus the growth rate of the bacterial culture can be easily calculated from the measurement of the current using Eqs. (2) and (3).

The current obtained from an experimental run is therefore an instantaneous and continuous measurement of the rate of bacterial growth during the batch growth in which the concentrations of the substrate and the product in solution are constant. Measurements of the current during the batch experiment are shown in Figure 2. The current rises during the initial stages, indicating that the rate of bacterial growth is increasing during this period, then during the later stages, the current reaches a plateau, indicating that the bacterial growth rate has reached its maximum value and has entered the exponential growth phase. The growth rate was determined using Eqs. (2) and (3) from the current plateau representing the exponential growth phase.

Effect of ferrous and ferric ions on the rate of growth

The growth rate of the bacterial culture at different concentrations of ferrous and ferric ions is represented in Figure 3. Each data point in this Figure represents one batch experimental run, as shown in Figure 2. The rate of the bacterial growth is analysed in terms of the Monod equation, given by:

$$\mu = \mu_{max}\left(\frac{[Fe^{2+}]}{[Fe^{2+}] + K_s(1 + K_i[Fe^{3+}])}\right) \tag{4}$$

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

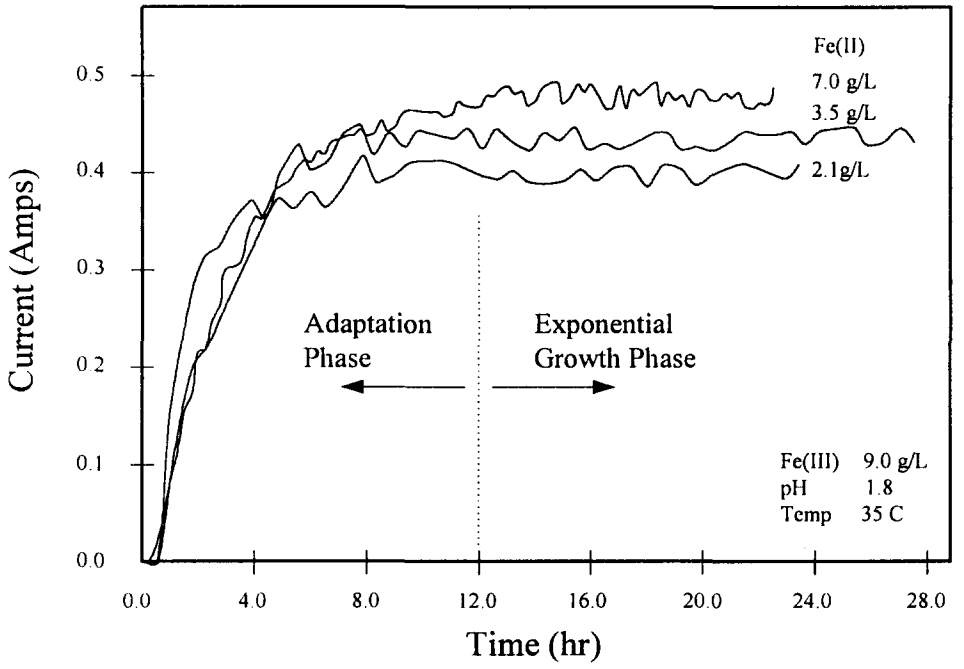

Fig.2 Typical results from the apparatus. The current rises as the bacteria move through the adaptation phase or lag phase and the rise time of the controller, then it remains constant once the bacteria are in the exponential growth phase. The current obtained in the exponential growth phase is used to calculate the growth rate, given by Eqs. (2) and (3)

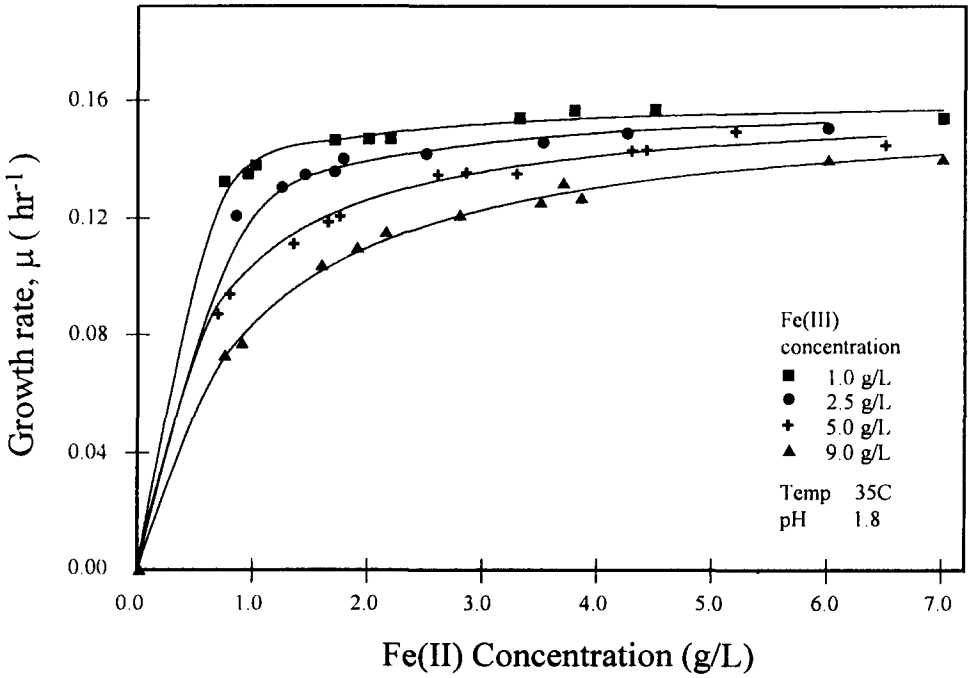

Fig.3 The rate of bacterial growth of *Thiobacillus ferrooxidans* at various concentrations of ferrous and ferric ions in solution. The points represent the data and the lines represent Eq. (4) with the parameters given in Table 1. Each point represents a batch experiment

The results presented in Figure 3 were analysed by Lineweaver–Burk plots and by non-linear minimization of the parameters. The inverse of Eq. (4) can be written as:

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{1}{[Fe^{2+}]} \frac{K_s(1+K_i[Fe^{3+}])}{\mu_{max}} \tag{5}$$

Thus a double-reciprocal plot (Lineweaver–Burk plot) of $1/\mu$ against $1/[Fe^{2+}]$ should be a straight line with slope $\{K_s(1+K_i[Fe^{3+}])\}/\mu_{max}$ and y-intercept $1/\mu_{max}$. The constants K_s and K_i can be easily evaluated from the variation of the slope with $[Fe^{3+}]$.

A double-reciprocal plot of the data represented in Figure 3 is shown in Figure 4, indicating that Eq. (5) is a good description of the data. The values of the parameters obtained from these results are given in Table 1.

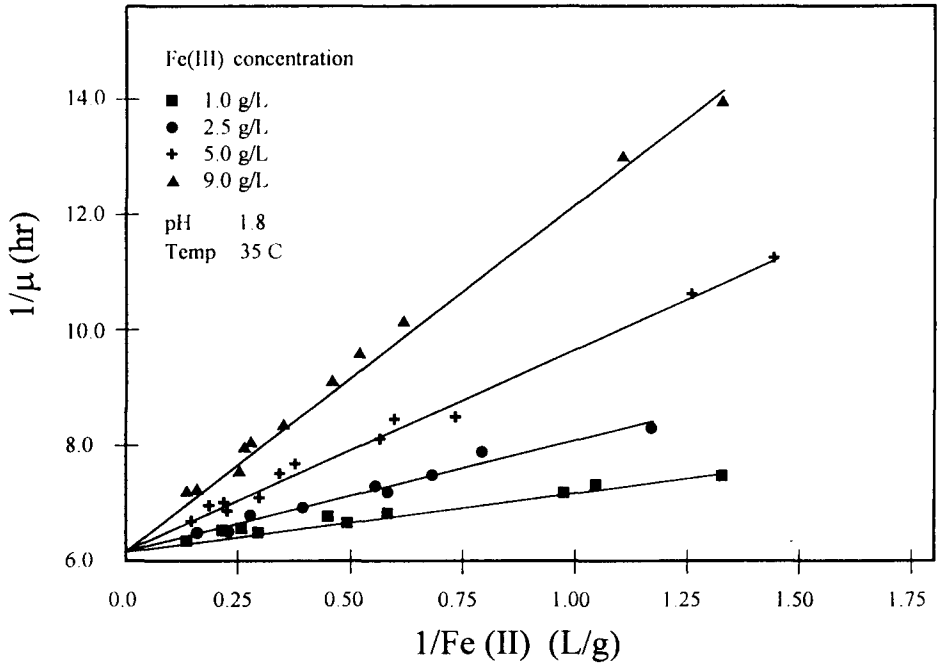

Fig.4 The Lineweaver–Burk plot of the data plotted in Figure 3

TABLE 1 Kinetic parameters for the growth of *Thiobacillus ferrooxidans* in a constant redox-potential reactor

	μ_{max} (hr^{-1})	K_s (g/L)	K_i (L/g)
L–Burk plot	0.162	0.062	1.643
Non-linear	0.161	0.073	1.287

In addition, Eq. (4) was fitted to the data represented in Figure 3 by the minimization of the least squares errors, given by:

$$S = \sum_{i,j} (\mu_{i,j} - \mu'_{i,j})^2 \tag{6}$$

where $\mu'_{i,j}$ is the data at the i^{th} ferrous ion concentration and at the j^{th} ferric ion concentration, and $\mu_{i,j}$ is

the calculated growth rate from Eq. (4) at the corresponding ferrous ion and ferric ion concentrations. This function was minimized by the downhill simplex method of Nelder and Mead [20] using the routine 'amoeba' written in C [21].

The lines on Figure 3 represent Eq. (4) with the fitted parameters. The parameters are given in Table 1, and an inspection of Figure 3 indicates that there is good correspondence between the numerical fit and the data.

Values of μ_{max} reported in the literature [10] range between 1.3 and 0.05 hr^{-1}, while those for K_s range between 1.0 and 0.0 g/L, those for K_i range between 2.3 and 0.5 L/g. It is clear from these results that parameters obtained from the data from the redox-controlled apparatus are consistent with those reported in the literature.

The values of the parameters obtained by the two methods of parameter estimation are essentially the same, except for the values of the parameter K_i. The double-reciprocal plot gives undue emphasis to points at lower concentrations of ferrous ions, and the use of linear-regression analysis of these Lineweaver–Burk plots violates the assumption of normal distribution of errors made in the derivation of the linear regression analysis. For these reasons, the values of the parameters from the non-linear minimization technique are more acceptable.

Effect of arsenite concentrations

The inhibition of the rate of bacterial growth by arsenite is significant, as shown in Figure 5. During these experiments the concentration of arsenite remained constant and was not oxidised to arsenate. The effect of arsenite on the rate of bacterial growth can be represented by a modified form of the Monod equation:

$$\mu = \mu_{max}\left(\frac{[Fe^{2+}]}{[Fe^{2+}]+K_s(1+K_i[Fe^{3+}])+K_a[As^{3+}]}\right) \tag{7}$$

where K_a is a constant. The data represented in this Figure may be analysed in a manner similar to the data in the absence of arsenite, that is, by analysis of the double-reciprocal plot, and by non-linear regression.

The double-reciprocal plot for the data represented in Figure 6, and the values of the parameters obtained from the Lineweaver–Burk analysis of this plot are given in Table 2.

The lines in Figure 5 represent Eq. (7) fitted to the data by non-linear regression. The parameters μ_{max}, K_s and K_i are the same as those obtained from the data represented in Figure 3. An inspection of Figure 5 indicates that there is good correspondence between the fit of the equation and the experimental data.

TABLE 2 Kinetic parameters for the growth of *Thiobacillus ferrooxidans* in the presence of arsenite in a constant redox-potential reactor

	μ_{max} (hr^{-1})	K_s (g/L)	K_i (L/g)	K_a (L/g)
L–Burk plot	0.160	0.062	1.922	0.046
Non-linear	0.161	0.073	1.287	0.057

The values of the parameters obtained from the Lineweaver–Burk analysis and the non-linear minimization differ with respect to the values for K_i and K_a, emphasising the difficulty that this method has when evaluating more complex growth functions than the Monod equation for growth on a single substrate. The non-linear minimization technique has the advantage that the same values of the parameters obtained in the absence of arsenite can be used to obtain the value of K_i when arsenite is present, ensuring that the overall

set of parameters is consistent. Thus the lines represented in Figures 3 and 5 are all described by Eq. (7) with the non-linear parameters given in Table 2.

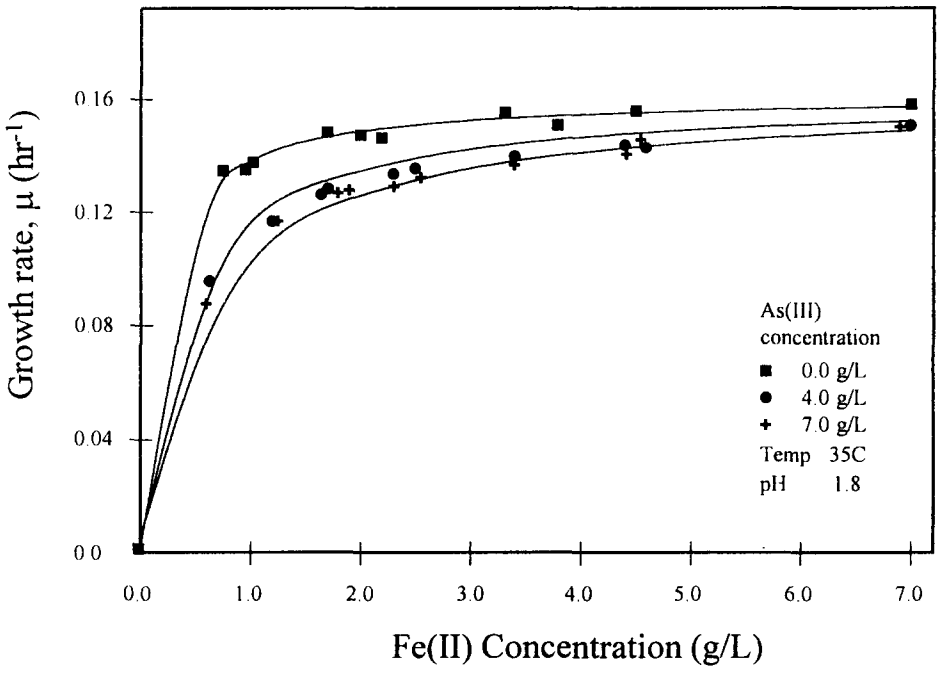

Fig.5 The rate of bacterial growth of *Thiobacillus ferrooxidans* at various concentrations of ferrous and arsenite ions in solution. The points represent the data and the lines represent Eq. (7) with the parameters given in Table 2

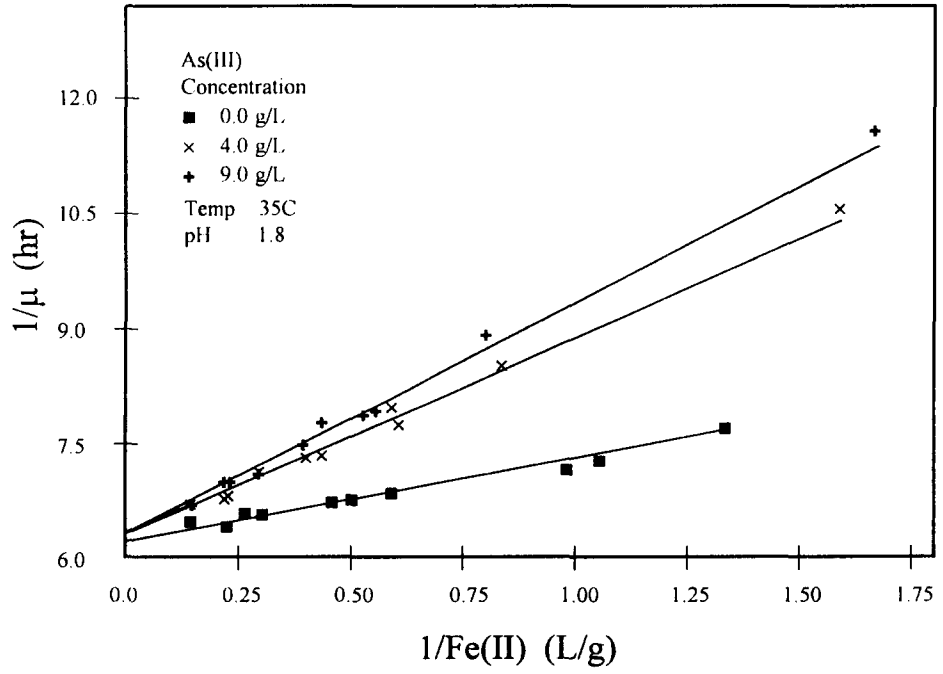

Fig.6 The Lineweaver-Burk plot of the data plotted in Figure 5

CONCLUSIONS

The results presented here indicate that the redox-controlled apparatus is a useful apparatus for the study of bacterial growth. The analysis of the data is clear and the apparatus is easy to operate. The values of the parameters obtained for the growth in a medium without arsenite agree with those reported in the literature, indicating that the presence of the cathode in the growth compartment does not interfere with the nature of bacterial growth.

The effect of the concentration of arsenite on the growth rate of the bacteria has been established using this technique. The growth kinetics were described by the Monod equation with inhibition by ferric and arsenite ions.

A major advantage of the redox-controlled apparatus described in this paper is that controlled experiments in the presence of sulphide minerals can be easily performed, and it should be possible to obtain much information on the mechanism of bacterial leaching from this apparatus.

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