Technical Progress Report for the Project

ELECTROCHEMISTRY OF Thiobacillus ferrooxidans
REACTIONS WITH PYRITE

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1. STATEMENT AND OBJECTIVES

The objective of this project is to provide the fundamental information on the mechanisms of bacterial leaching of pyrite. The knowledge of how bacterial leaching of pyrite functions is essential for design and development of a technology for coal cleaning with bacteria. The features of major electrochemical techniques will be examined to find out if any of them can provide a diagnostic information on the mechanisms of related reactions.

2. SCIENTIFIC AND TECHNICAL PROGRESS

2.1 GENERAL

This report discusses the research results obtain during the study of effect of environmental conditions on the chemical activity of T. ferrooxidans. More specifically, the environmental conditions mean the effect of heavy metals, particularly silver, on the activity of bacteria. The chemical activity of bacteria is expressed in terms of its oxidizing ability of ferrous ion. The method of measuring the activity of bacteria is based on the variation of solution redox potentials with time. Our technique is unique compared to the traditional chemostat techniques because it allows the measurement of the bacteria's activity due to its oxidizing property only, excluding the bacteria growth.

The redox potential measurement methods do follow already developed, traditional, kinetic models, and this kind of analysis will be reported in the next trimester. This is an encouraging news because the redox potential technique can be utilized to extract standard kinetic information plus it provides additional information which are not available in standard techniques.

This report represents a paper in preparation which will be submitted for review for publication after its completion. Compared to the previous report (September, 91-January, 92), this report adds some more experimental information with respect to the

effect of substrate on the bacterial activity. Also, this report introduces mercury as another metal whose effect on the activity of bacteria will be studied.

EFFECT OF SILVER AND SOME OTHER HEAVY METALS ON THE IRON OXIDIZING ABILITY OF THIOBACILLUS FERROOXIDANS.

Introduction

Because of its great technological potential, bio-leaching involving Thiobacillus ferrooxidans and other autotrophic bacteria has received much attention (1, 2) over the past few decades. Consequently, the scientific literature is observed contain an increasing number of reports which bear the testimony to the importance of this developing technology. For example, microbial oxidation has already been observed to be useful for liberating precious metals, especially gold, locked in the refractory sulfide matrix, and thereby rendering the cyanidation in heap leaching practice possible (3). Reportedly (3), bio-oxidation is also applicable for dump leaching of low grade refractory copper ores such as CuFeS_2 . Lately, active consideration is given to use bio-leaching for the removal of pyritic sulfur from coal and help control environmental pollution (i.e. acid rain caused by SO_2 contained in the exhaust gases from coal power plants).

A critical review of the literature would reveal that the role of T. ferrooxidans, in bio-leaching of mixed metal sulfides is to catalyze the oxidation of iron sulfide(s) to Fe(III) sulfate. However, previous work do not specify which component of the microbial cell plays key role in bio-leaching. Earlier reports (4, 5) have mentioned that a c type cytochrome may be responsible for the transport of electrons from Fe(II) to oxygen, whereas a more recent communication (6) has cited that a 92K glycoprotein may be the most important component of the ferrous iron oxidizing system of the T. ferrooxidans. While there is a lack of agreement regarding the identity of the cell active sites responsible for Fe(II) (or sulfur) oxidation, two alternative mechanisms are generally suggested for the microbial leaching of metal sulfides. According

to the direct mechanism, a physical contact between the bacteria and the sulfide mineral surface is required (7). In the indirect mechanism, the bacteria generated Fe(III) acts as a lixiviant for the solubilization of metals from the mineral (3). In actual fact, however, both of the mechanisms may operate simultaneously in a complementary manner.

An important side effect of bio-leaching may be co-dissolution of other heavy metal ions, which, if accumulated to a certain level, may influence the activity of the T. ferrooxidans cells. In a more drastic case, the heavy metals may kill the bacteria and thus stop bio-leaching. On the other hand, some heavy metal may exert an inhibitory effect on bio-leaching by deactivating bacteria the living catalysts. The deactivation process may involve blocking or damaging of a significant fraction of the cell active sites by heavy metal ions. In another possible deactivation process, the presence of a particular heavy metal may enhance the formation of toxic species from dissolved oxygen e.g. $O_2^{\cdot -}$. Oxygen toxicity through the superoxide (i.e. $O_2^{\cdot -}$) formed in the biological systems is already known in the literature (8).

A reverse case may also be possible in which the bacteria's metal leaching activity may be enhanced in presence of a heavy metal. This is likely to happen if a conducive metallic species is initially added into the leach system or released in situ from the mineral by the indirect leaching mechanism.

Tuovinen and Kelly (9), have studied the effect of UO_2^{2+} on the iron oxidation by Thiobacillus ferrooxidans. It was found that the presence of UO_2^{2+} species partially inhibited the oxidizing activity of the bacteria by blocking the cell active sites. Addition of a complexing agent like EDTA, or a non-toxic cation (e.g. Mg^{2+}), reduces the inhibitory action due to uranyl cation. Imai et al (4) found that mercuric and mercurous ions are even more toxic than uranyl or any other heavy metal ions studied, e.g. lead, arsenic,

zinc, molybdenum, copper, cadmium etc. The observed variation in toxicity of heavy metals might have resulted from differences in the modes of interactions between the cell active sites and various heavy metals ions. On the basis of the above information it is likely that UO_2^{2+} may be physically sorbed onto the cell so that it can be exchanged with a divalent cation such as Mg^{2+} . On the other hand, Hg(II) may directly interact with the T. ferrooxidans cells (4) forming a chemical bond with the iron oxidase.

Silver, another heavy metal, is often cited as toxic to the T. ferrooxidans (4, 10-13). For example, previous workers (11, 12) have reported that in an aqueous FeSO_4 solution of low pH, growth of the T. ferrooxidans is decreased and their ferrous iron oxidizing ability lost to a significant extent by the presence of approximately 0.10 ppm Ag^+ . However, if a metal sulfide such as e.g. CuFeS_2 , is used in the solid form as the bacteria's sole nutrient, then the presence of a significant quantity of silver may not cause any harm to the bacteria's metal sulfide bio-leaching activity (14-16). On the contrary, the bio-leaching of CuFeS_2 is observed to be enhanced at pH 1.25 in the presence of 30 mg/L Ag^+ (16). The positive effect of silver has been explained (14, 16) in terms of the fact that Ag^+ forms Ag_2S to generate the galvanic couple, $\text{Ag}_2\text{S}/\text{CuFeS}_2$, in which Ag_2S acts as a cathode facilitating anodic dissolution of CuFeS_2 .

In the light of the literature reports mentioned above, it is conceivable that the apparent dual role of silver in the ferrous iron oxidizing ability of T. ferrooxidans is due to differing experimental conditions. Consequently, it was decided to study the effect of silver and a number of other heavy metals on the iron oxidizing activity of the T. ferrooxidans by extending the experimental parameters beyond those considered in the earlier investigations.

In our earlier report, we have described the effect of a number of experimental parameters on the Fe(II) oxidizing ability of the bacteria. Since then we have upgraded our results by doing additional experiments on the effects of concentrations of microbial cell and Fe(II) on the bacterial oxidation of Fe(II). Moreover, the work has been extended to study the effect of mercury, another heavy metal, on the Fe(II) oxidizing activity of T. ferrooxidans. The results obtained will be described herein.

Experimental

Materials and equipment

The equipment and the method of growing T. ferrooxidans (strain ATCC 13598) are reported in reference 17. Only reagent grade chemicals were used.

Procedure

(a) Experiments without heavy metals

Deionized water, 250mL, of a desired pH was transferred into the bio-reactor to which 25 μ L of 1M Fe₂(SO₄)₃ and 250 μ L of 1M FeSO₄ were added. The biooxidation of Fe(II) was initiated by the addition of 2mL of concentrated T. ferrooxidans cells. The chemical activity of the bacteria was monitored by measuring the redox potential (vs SCE) of the solution. A specially built pyrite electrode served as the indicator electrode.

(b) Experiments with heavy metals

The experimental procedure was similar to the preceding section except that a desired concentration of a heavy metal (e.g. silver and mercury) was added before the addition of bacteria to the system.

To study the effect of conditioning the bacteria with mercury, 2mL of concentrated bacteria was added to a solution (250mL) mercury (0.025-10 mg/L). When the conditioning period, 5-120 minutes, was completed, requisite amounts of ferrous and ferric iron (to make 0.001M and 0.0001M respectively in a 250mL reactor) were added and redox potential monitoring started.

Standard experimental conditions

The iron oxidizing activity of the Thiobacillus ferrooxidans has been studied under a variety of experimental conditions. The standard conditions are as follows:

pH	1.7
temperature	25°C
concentration of Fe(II)	0.001M
concentration of Fe(III)	0.0001M
no heavy metal ion	
<u>T. ferrooxidans</u> cells	2mL

Two parallel sets of experiments were conducted, one with and the other without a heavy metal, to demonstrate the effect of a heavy metal on the activity of the T. ferrooxidans. Results are discussed below.

RESULTS and DISCUSSION

Results of experiments without heavy metals

Effect of cell concentration

T. ferrooxidans absorb UV-VIS light at 590nm (7). This property was utilized to establish a correlation between the reaction rate and

the cell concentration of T. ferrooxidans. At first, optical densities of the bacterial cells of various concentrations at 590 nm (i.e. OD590s) were measured. Subsequently, the corresponding concentrations of the bacteria were used in Fe(II) oxidation experiments (under standard conditions mentioned above) to determine the rate constants from the measured change of redox potential with time (17). According to the results represented by Fig. 1, the Fe(II) oxidation with T. ferrooxidans is first order with respect to the concentration of the bacteria up to OD590=2.82. However, beyond OD590=2.82 the reaction rate decreases with increasing cell density because of substrate limitation, an effect, explained in the following section.

Effect of Fe(II) concentration

Three series of experiments were performed with T. ferrooxidans cell concentrations given by OD590=1.41, 2.8 and 5.85. In each series of experiments, parameters other than Fe(II) concentration were maintained constant. The results are represented by the curves with closed symbols in Fig. 2. In general, it is observed (Fig. 2) that the rate constant versus concentration of Fe(II) curves shift towards right as the microbial cell density is increased from OD590=1.41 to OD590=5.85. This happens because the reaction under study requires an optimum substrate i.e. Fe(II) to bacterial cell ratio. If the substrate concentration is lower than optimum, then the substrate concentration will be a limiting factor for the reaction rate. As a result, the rate constant will be lower than what it would be if optimum substrate to cell ratio is used. However, if more substrate is present in the system than optimum, then the reaction will be cell limited. Under this condition, more Fe(II) species will be available to compete for the active sites on the microbial cell. Competition among the Fe(II) ions for the active sites on the cell will intensify with an increase in Fe(II) concentration in the system. This, in turn, may lead to a reduction

in the average duration of attachment of a particular Fe(II) ion to the bacterial cell. Consequently, a situation may arise where only a fraction of the Fe(II) species present in the system will be in direct contact with the cells for the whole of time duration required for oxidation to Fe(III). As an immediate effect, the reaction rate will decrease. Reduction of reaction rate in this manner is termed as competitive inhibition by Fe(II) and is well known in the literature (18).

Our results discussed above suggest that for maximum ferrous iron oxidizing activity of the bacteria, the ratio of concentrations of the microbial cell and ferrous ions must be optimized. According to our findings, if 2mL of T. ferrooxidans cells of OD590=1.41 are used in a reactor volume of 250mL, then the optimum concentration of Fe(II) will be 0.00037M approximately. On counting the bacteria by fluorescence microscopy we have found that 2mL bacteria of OD590=1.41 corresponds to approximately 7.72×10^{10} cells of the T. ferrooxidans in our reactor. If the number of cells in the reactor is increased to 15.44×10^{10} and 32.26×10^{10} (corresponding to OD590=2.8 and 5.85), the optimum Fe(II) concentration will be 0.001M and 0.002M respectively. The rate constant under optimum conditions is observed to be 0.06 min^{-1} approximately and represents the maximum point on the curves in Fig. 2 (closed symbols). The portion of each curve right to the maximum point represent inhibitory range and is attributable to competitive inhibition by Fe(II) species (18). In addition, product inhibition due to ferric ions in a competitive as well as noncompetitive manner is possible (18). On the other hand, the left side of the curves corresponds to Fe(II) limitations and is called stimulation region. The stimulation region of the curve represented by the closed squares was used for the Lineweaver-Burk analysis (Fig. 3) and Direct plot analysis (Fig. 4). Both analyses show that the maximum possible value of the 1st order rate constant should be 0.069 min^{-1} under the conditions used in our work. These analyses also suggest that the Fe(II) concentration corresponding to the half-maximal rate constant (i.e. K_m) is 0.000154M

approximately. This results could not be compared with those of the other researchers because no other study was conducted in a similar way.

Results of Experiments Involving Heavy Metal Ions

Effect of Fe(II) concentration in the presence of silver

It is observable from the rate constant versus Fe(II) concentration plots corresponding to the open symbols in Fig. 2 that the curves representing microbial Fe(II) oxidation in the presence of silver shift towards left as the cell density of the bacteria is decreased from OD590=5.85 to 1.41. It is explainable in terms of the fact that, in general, silver ions deactivate a portion of the microbial cells. As a result, the proportion of the cells effective for Fe(II) oxidation is reduced when 10 mg/L Ag^+ is present in the system. However, since the silver concentration is fixed, it will deactivate a fixed number of cells from each of the three microbial cell concentrations studied. Consequently, the effective number of cells in the concentrations of cells used in our work will follow the sequence

$$\text{OD590}=1.41 < \text{OD590}=2.8 < \text{OD590}=5.85.$$

A reduction in the effective number of cells would mean that a lower concentration of Fe(II) would be necessary to optimize the Fe(II) to cell ratio and hence to maximize the reaction rate. It is for this reason that the rate constant versus Fe(II) concentration curves symbolized by open symbols in Fig. 2 should shift towards left as in cell density of the bacteria is decreased.

Effect of mercuric ion concentration

Computation of the rate constant and subsequent plot against mercury ion concentration (Fig. 5) shows more clearly that the rate of Fe(II) oxidation catalyzed by T. ferrooxidans is maintainable up to a threshold value of mercury concentration, beyond which the rate decays. According to Fig. 5, this threshold value of mercury concentration is dependent on microbial cell density. For instance, for a cell density of OD590=2.699, the maximum concentration of mercury that causes no harm to the microbial Fe(II) oxidation is approximately 0.5 mg/L. On the other hand, a lower OD590 of 2.089, the maximum value of mercury concentration tolerable in the bacterial oxidation of Fe(II) approaches towards 0.25 mg/L.

Oxidation of Fe(II) with bacteria conditioned with mercury

Effect of time

The effect of conditioning period of the bacteria with mercury on the rate constant of Fe(II) oxidation is represented by Fig. 6. In general, Fig. 6 shows that the rate constant of bacterial oxidation of Fe(II) decreases as the duration of conditioning the bacteria with mercury is increased. The adverse effect of conditioning time also increases with increasing concentration of mercury.

CONCLUSIONS

Microbial oxidation of ferrous iron by T. ferrooxidans was studied without addition of a nutrient in the system other than Fe(II). In addition, the duration of experiments was only one hour which was too short a time for the bacteria to grow. Consequently, our results are not growth biased. For this reason, care should be taken while comparing our results with other results, almost all of

which are growth biased. The important conclusions drawn on the basis of our work follow:

(1) Our results suggest that the ratio of concentrations of Fe(II) and the microbial cells in the system is an important parameter for the microbial oxidation of Fe(II). If this ratio is varied from the optimum value, then the rate of reaction will decrease (Fig. 1 and 2).

(2) The presence of silver and mercury has been observed to be detrimental to the Fe(II) oxidation catalyzed by T. ferrooxidans. However, depending on the cell density, the bacteria can maintain their Fe(II) oxidizing activity in the presence of a small amount of mercury (Fig. 5).

(3) Conditioning of the bacteria with a significant concentration (2.5 mg/L or over) of silver is found to be more detrimental to the Fe(II) oxidizing activity of the T. ferrooxidans (Fig. 6).

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Fig. 1 Rate constant of the microbial oxidation of Fe(II) as a function of cell density (in terms of OD₅₉₀) under standard conditions.

Fig. 2 Rate constant of the microbial oxidation of Fe^{2+} ion as a function of ferrous iron concentration (pH=1.7; $T = 25^\circ\text{C}$). Squares: $\text{OD}_{590}=2.8$; triangles: $\text{OD}_{590}=1.41$; circles: $\text{OD}_{590}=5.85$. Closed symbols correspond to experiments without Ag. Open symbols correspond to experiments with 10 mg/L Ag.

Fig. 3 Lineweaver-Burk plot of the rate constant of bacterial oxidation of Fe(II) as a function of Fe(II) concentration.

Fig. 4 Direct linear plot of rate constant of microbial oxidation of Fe(II) as a function of Fe(II) concentration.

Fig. 5 Effect of concentration of mercury on Fe(II) oxidation by *T. ferrooxidans* (pH 1.7 and temperature 25°C).

Fig. 6 Rate constant of microbial oxidation of Fe(II) as a function of pre-conditioning period of the bacteria with mercury at temperature 25C (OD590 2.09 and pH 1.70).

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