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Use of *Thiobacillus ferrooxidans* for iron oxidation and precipitation

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Fe(II) oxidation reaction was carried out using an acidophilic microorganism, *Thiobacillus ferrooxidans.* Four different parameters such as pH, Fe(II), Fe(III) and biomass concentration were studied. The oxidation reaction follows a pseudo first order rate equation. Apparent reaction rate constants were calculated. Unified rate equation was developed using the four parameters. Along with oxidation, a part of the iron also was precipitated. The extent of Fe(III) precipitation in each case was calculated.

Keywords: biooxidation, iron precipitation, rate equation, *Thiobacillus ferrooxidans*

Introduction

In hydrometallurgical processing of ores, the leach liquor usually contains Fe, as the same is associated with most of the sulfidic as well as oxidic ores (Chen & Cabari 1986). The coextracted iron has to be removed before further processing of leach liquor to recover the metal values. For removal of iron it is initially oxidized to Fe(III) which is separated from the leach liquor either by precipitation or by using ion exchange technique (Ritcey 1986). Instead of using the above chemical technique, the problem of iron control in leach liquor can be dealt with by using acidophilic microorganisms like *Thiobacillus ferrooxidans* (Toro *et al.* 1988). The said acidophilic bacteria have been widely used to recover metal values from low grade ores (Baily & Hansford 1988). Though most of the time the bacteria were used to leach out metal values from ores, their role in oxidation and precipitation of iron is difficult to ascertain, especially in absence of ores. The Fe oxidation reaction by *Thiobacillus ferrooxidans* has been studied in the pure system but the objectives are different such as evaluation of media composition on the Fe oxidation rate (Alexander *et al.* 1987, Kurosawa *et al.* 1994), evaluation of μ_m and specific growth rate using the Monod equation without bacterial count (Lacey & Lawson 1970). The kinetic parameters of ferrous oxidation by *Thiobacillus ferrooxidans*, such as evaluation of rate constants, dependence factor, rate equation, etc. are limited (Pesic & Oliver 1989).

Bearing in mind the importance of this former work, the present study deals with the kinetic parameters of Fe(II) oxidation and evaluation of Fe precipitation rate by the bacterium, *Thiobacillus ferrooxidans.*

Materials and methods

Microorganism

Pure strain of *Thiobacillus ferrooxidans* was received from Professor K.A. Natarajan, Department of Metallurgical Engineering, Indian Institute of Science, Bangalore. The bacteria were cultured in 9K medium (Silverman & Lundgren 1959). The strain was subcultured repeatedly for adaptation purposes.

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Experimental conditions

The experiments were carried out in 250 ml Erlenmeyer flasks containing 90 ml medium and 30 ml inoculum. The flasks were shaken constantly in a Julabo SW 20-C rotary shaker. Samples were collected at regular intervals to analyze the ferrous and ferric concentrations and to check the pH of the culture media. The basic experimental conditions were pH 2.0, temperature 37°C, initial Fe(II) of 0.9 g and Fe(III) of 0.3 g.

Analysis

Ferrous iron analysis was carried out by using decinormal solution of potassium dichromate and barium diphenyl sulphonate as indicator. Orthophosphoric acid was added to reduce the ferrous-ferric oxidation potential. Total iron analysis was done by reducing ferrous to ferric form, by adding stannous chloride. Mercuric chloride was added to stabilize the excess of stannous chloride and was titrated as above. The ferric concentration was obtained by deducting levels of ferrous concentration from total iron values.

Results and discussion

Effect of ferrous iron concentration

Ferrous iron concentration was varied from 1 to 7.5 g l^{-1} to find out the effect of Fe(II) on the activity of the microorganism. The iron oxidation rate increased with the increase Fe(II) initial concentration up to 5 g l^{-1} (0.62 g total Fe(II) in solution) and beyond that it decreased. The iron oxidation rates for different initial Fe(II) concentrations are shown in Table 1.

The chemical equation for Fe oxidation can be written as

$$\text{Fe}^{2+} + \tfrac{1}{2}\text{O}_2 + 2\text{H}^+ \xrightarrow{\text{Bacteria}} \text{Fe}^{3+} + \text{H}_2\text{O} \qquad (1)$$

Assuming the oxidation reaction to be of first order, the iron oxidation rate can be written as:

$$\text{Rate} = -\text{d}(\text{Fe}^{2+}) / \text{d}t = k(\text{Fe}^{2+}) \qquad (2)$$

where k is the specific reaction rate constant. After integrating, Equation 2 can be written as:

$$-\ln\ (\text{Fe})_t / (\text{Fe})_0 = kt \qquad (3)$$

where $(\text{Fe})_t$ is concentration of Fe(II) at time t, $(\text{Fe})_0$ is initial Fe(II) concentration and t is time in h.

If the reaction is first order, then a plot of ln $(\text{Fe})_t / (\text{Fe})_0$ versus time would give a straight line and from the slope, k can be calculated. Figure 1 shows the plot of ln $(\text{Fe})_t / (\text{Fe})_0$ versus time for different initial Fe(II) concentrations and the correlation coefficient for each line was observed to be more than 0.9. Therefore iron oxidation rate by the microorganism can be concluded to be of first order.

Table 1. Iron oxidation and other kinetic parameters for different oxidation conditions

	Rate of Fe(II) oxidation (mg^{-1} h)	k, reaction rate constant (h^{-1})	Iron precipitated after 100 h (mg)	Dependence factor
Initial Fe(II) (g)				
0.16	9.16	–	80	–1.50
0.27	18.05	–	143	
0.61	29.83	0.06304	350	
0.82	22.12	0.04906	400	
0.96	14.76	0.03146	473	
Initial Fe(III) (g)				
0.342	13.4	0.02299	400	–0.81
0.39	10.8	0.02189	350	
0.414	8.7	0.01934	300	
Initial pH				
1.25	7.5	0.013	575	1.14
1.5	17.9	0.02095	540	
1.75	18.1	0.02428	500	
2.0	27.0	0.03224	473	
2.5	13.2		425	
Biomass				
4.7×10^4	11.85	0.02252	–	0.60
9.4×10^4	15.9	0.0322	–	
1.4×10^5	37.5	0.04424	–	

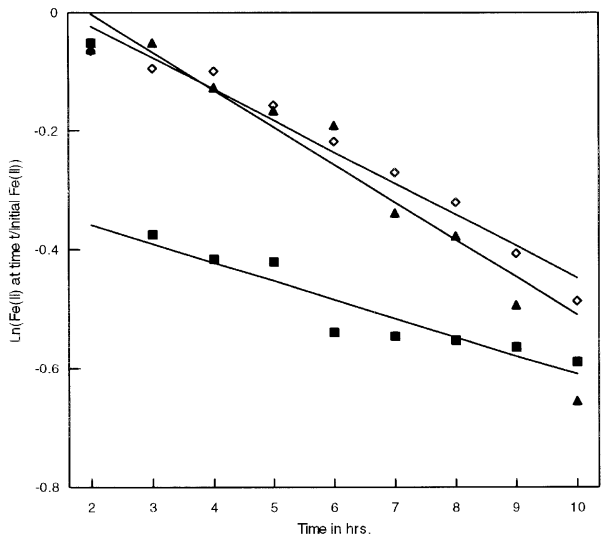

Figure 1. First order reaction plot as a function of initial Fe(II) concentration (g): (■), 0.96; (▲), 0.61; (◇), 0.82.

Values of k calculated for different initial Fe(II) concentrations are shown in Table 1. The dependence factor for Fe oxidation by the bacteria for Fe(II) concentration can be calculated from the slope of a plot between ln$k$ versus ln(Fe(II)$_0$). Figure 2 shows a plot of ln$k$ versus ln(Fe(II)$_0$). The slope of the curve was found to be 1.50. Therefore the oxidation rate depends on the first power of initial Fe(II) concentration.

Along with Fe(II) oxidation, a part of the Fe is precipitated during bacterial oxidation (Toro *et al.* 1988). The solution chemistry of Fe is rather complicated (Bhapu 1986) and iron is usually precipitated as jarosite, goethite, hematite or hydroxide. It was observed that the amount of iron precipitated increased with increase of initial Fe(II) concentration. Table 1 shows the amount of Fe precipitated for different Fe(II) concentration after 96 h.

Effect of ferric concentration

The Fe(III) concentration was varied from 2.85 to 3.65 g to determine the effect of Fe(III) concentration on the bacterial Fe(II) oxidation rate. It was observed that Fe(II) oxidation rate decreased with increase of initial Fe(III) concentration as shown in Table 1. This decrease in oxidation rate may be due to an inhibitory effect of Fe(III) on the growth and activities of the bacterium. Since the oxidation rate depends not only on initial Fe(II) but also on Fe(III) concentration, the oxidation reaction is not exactly first order but follows a pseudo first order rate equation. To find out the reaction rate constant, ln $(\mathrm{Fe})_t / (\mathrm{Fe})_0$ is plotted against time, t, as shown in Figure 3. The reaction rate constant for different initial Fe(III) concentration is shown in Table 1. To determine the dependence factor ln$k$ is plotted against ln [Initial Fe(III) concentration] and the slope was found to be 0.81 from Figure 4. Fe precipitation was observed to decrease with increase of Fe(III) concentration as shown in Table 1.

Effect of pH

The nature of protein synthesis by the bacteria depends on pH (Amaro *et al.* 1991); hence, pH is an important factor in determining the biooxidation

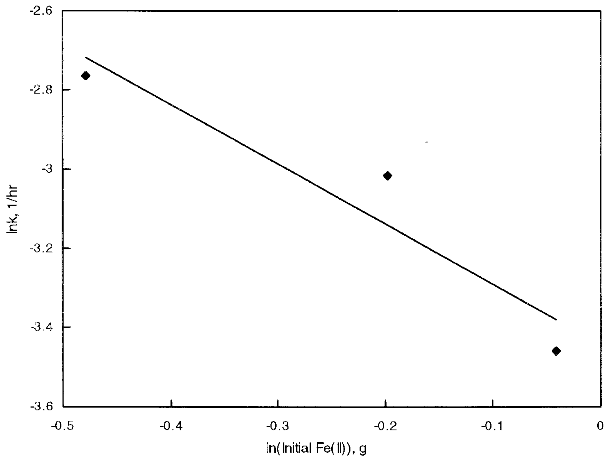

Figure 2. Determination of dependence factor with respect to initial Fe(II).

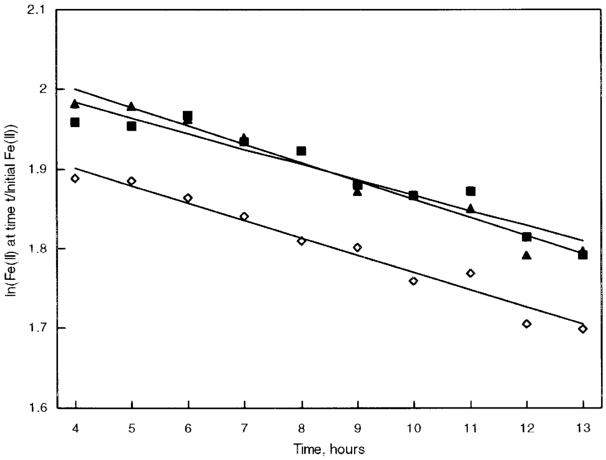

Figure 3. First order reaction rate plot with respect to initial Fe(III) concentration (g): (■), 0.342; (▲), 0.39; (◇), 0.414.

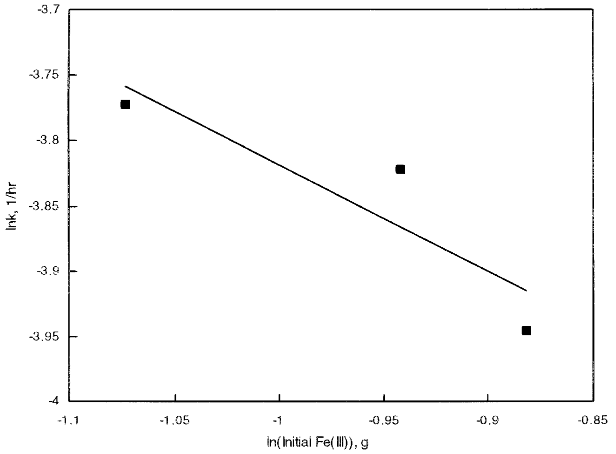

Figure 4. Determination of dependence factor with respect to initial Fe(III) concentration.

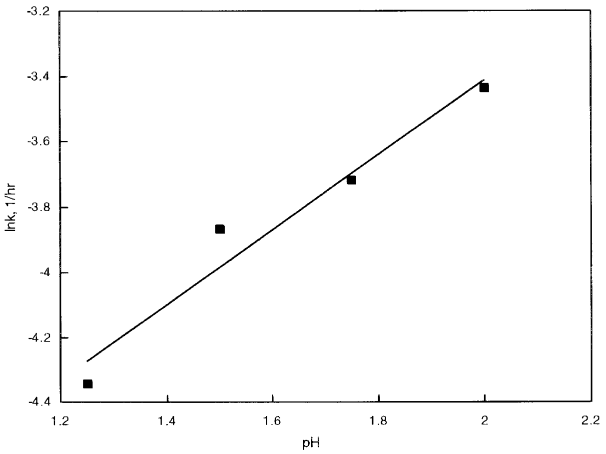

Figure 5. Determination of dependence factor with respect to initial pH.

rate. pH of the initial solution was varied from 1.25 to 2.5. The iron oxidation rate increased up to pH 2.0 and thereafter decreased. The iron oxidation rates for different initial pH levels are shown in Table 1. k values are calculated by plotting $\ln(\mathrm{Fe})_t / (\mathrm{Fe})_0$ versus pH. The dependence factor was calculated from the slope of a curve between ln$k$ versus pH and was found to be 1.14 as shown in Figure 5. The iron precipitation increased with decrease of pH as shown in Table 1.

Effect of initial biomass concentration

The iron oxidation rate also depends on the activity as well as concentration of microorganisms. Biomass concentration was varied from 4.7×10^4 to 2.8×10^5 cells ml^{-1}. It was observed that the lag phase decreased with increase of bacterial concentration. At highest and lowest bacterial concentration, the log phase initiated at 2 and 10 h respectively. The oxidation rate also increased with initial biomass concentration as shown in Table 1. The k value and dependence factor were calculated graphically as done previously. The values are shown in Table 1.

Conclusions

The Fe oxidation rate by the bacterium depends on various factors such as pH, Fe(II), Fe(III) and biomass concentration. The oxidation rate increased with increase of biomass concentration but decreased with increase of Fe(III) concentration. The biooxidation rates increased with increase of Fe(II) level up to 0.6 g after which the rates decreased. As regards pH, the biooxidation rate increased up to 2.0 beyond which it showed a downward trend. Iron precipitation increased with increase of Fe(II), pH and bacterial concentration and reduced with increase of Fe(III) concentration.

The apparent reaction rate constants in each of the cases were calculated by using first order rate equation. The dependence factors of iron oxidation by the bacteria for each of the parameters studied were calculated. Using the dependence factor, the rate constant can be written as:

Rate of iron oxidation =

$$\frac{k'(\mathrm{pH})\ (\text{Biomass concentration})}{(\mathrm{Fe(II)})\ (\mathrm{Fe(III)})} \tag{4}$$

(k' = pseudo 1st order reaction rate constant)

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