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The effect of mass transfer on bio-oxidation of ferrous iron using *Thiobacillus ferrooxidans*

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ABSTRACT

Bio-oxidation studies were carried out using *Thiobacillus ferrooxidans* in a bioreactror. The effect of gas-liquid mass transfer of oxygen and liquid-bacterial surface transfer of ferrous were established. The maximum oxygen and ferrous transfer rates were evaluated for each set of experiment and the same were compared with the maximum oxygen and ferrous consumption rate by the microorganisms. It was observed that neither oxygen nor ferrous transfer was not rate controlling step. Ferrous iron oxidation by *Thiobacillus ferrooxidans* in the present study was reaction controlled.

Keywords: *Thiobacillus ferrooxidans*; Mass transfer; Bioreactor; Bio-oxidation

1. INTRODUCTION

Thiobacillus ferrooxidans is one of the most important biomining bacteria from the industrial application aspect (Leduc and Ferroni, 1994). Although kinetic aspects and the molecular biology of the microorganism have been studied widely, knowledge on the various important parameters such as concentrations of Fe(II), Fe(III), oxygen, and carbon dioxide, that affect the growth conditions are not extensively studied (Nemati et al., 1998). For successful industrial application of bioleaching, a steady bacterial metabolism as well as the smooth operation of the equipments need to be ensured (Mazudos et al., 1999).

In several metallurgical processes, mass transfer of certain components from one phase to the other is a very important aspect. *T. ferrooxidans* being an aerobic microorganism, utilizes oxygen as a substrate for the biooxidation of iron. Thus it is essential to predict the gas-liquid mass transfer of oxygen in order to derive the bio-oxidation kinetics and to design an optimal bioreactor. The transfer of oxygen from the gas phase to the liquid phase has to be adequate.

Similarly the presence of Fe(II) in the medium is another important substrate that governs the growth, metabolism of the bacteria and the reaction kinetics. Hence it is necessary to determine the mass transfer rate of Fe(II) also from the liquid phase to the bacterial surface.

There are several factors such as the stirring speed, the airflow rate, volume of the reactor and base area that affect the mass transfer rate. Taking the above aspects into account, the oxygen consumption rate and ferrous consumption rate as well as their transfer rates have been estimated (Boon, 1996).

In the present study the mass transfer rates of oxygen and Fe(II) have been studied in a 2L bioreactor varying different parameters that affect the growth and reaction kinetics.

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2. EXPERIMENTAL

The bacterium *T. ferrooxidans* was used in all the experiments. Pure strain of *T. ferrooxidans* was received from Prof. K.A. Natarajan, Indian Institute of Sciences, Bangalore, India. *T. ferrooxidans* strain was revitalized by inoculating it in fresh 9K medium (Silvermann and Lundgren, 1959). Adaptation studies were carried out by sub culturing the bacterium in a particular culture medium for several times till the growth rate of the bacteria attained a stationary phase. Fe(II) and Fe(III) in solution were analysed volumetrically (Vogel, 1959).

Iron oxidation studies were carried out in a 2L bioreactor at constant temperature. The pH of the solution in the bioreactor was maintained by addition of sulfuric acid. Mixing and gas dispersion was achieved by a six-blade turbine impeller rotating at a specific speed and located at 2cm above the base of the bioreactor. The air flow rate to the bioreactor was controlled by a mass flow controller. An oxygen probe measured the dissolved oxygen of the solution.
Unless otherwise specified all the experiments were carried out in following conditions: 2L of 9K media including 200ml of inoculum, pH 2.0, 120rpm and temperature 35°C.

3. RESULTS AND DISCUSSION

3.1. Gas to liquid mass transfer

3.1.1. Determination of oxygen consumption rate

Oxidation of ferrous iron can be described by the following overall reaction:

$$2FeSO_4 + 1/2\ O_2 + H_2SO_4 \rightarrow Fe_2(SO_4)_3 + H_2O \quad (1)$$

The reaction is first order with respect to the concentration of Fe(II) in the medium.The maximum oxygen consumption ($O_2^{CR\ max}$) rate can be derived from eq. 2:

$$O_2^{CR\ max} = 1/4\ k'\ [Fe(II)] \quad (2)$$

Where k' is the first order reaction rate constant.

Since *T. ferrooxidans* is aerobic in nature the oxygen consumption rate (O_2^{CR}) is affected by the bacterial activity,

$$O_2^{CR} = \frac{O_2^{CR\ max}.[O_2]_L}{[O_2]_L + kO_2} \quad (3)$$

In the above equation kO_2 stands for the affinity co-efficient of bacteria for oxygen and $[O_2]_L$ is dissolved oxygen concentration in the media. For *T. ferrooxidans* kO_2 is about 10% of the oxygen concentration in air-saturated water. The oxygen consumption rate had been derived for each set of experiment.

3.1.2. Determination of the mass transfer co-efficient of oxygen

The volumetric mass transfer co-efficient of oxygen (k_La) in the bioreactor was determined from the following correlation (Reit, 1979).

$$k_La = 2.0.\ 10^{-3}.\ (P/V)^{0.7}.Vs^{\ 0.2} \quad (4)$$

where $500<P/V<10^4 W/m^3$ and $2<V<4400L$.

In the above equation P/V is the power input per unit of medium volume (W/m^3) and Vs is the superficial gas velocity in the bioreactor. In the bioreactor k_La value was found to be $0.1301 s^{-1}$.

3.1.3. Gas-liquid mass transfer rate of oxygen

Mass transfer rate of oxygen from the gas phase to the liquid phase can be expressed as follows:

$$O_2{}^{TR}_{gas \rightarrow liquid} = k_L a\,([O_2]_s - [O_2]_L) \quad (5)$$

$[O_2]_s$ is the dissolved oxygen concentration of the liquid phase in equilibrium with the gas phase. Due to oxidation reaction the actual oxygen concentration in the outlet gas phase will be different from that in the inlet air and can be derived from the gas phase balance for oxygen as:

$$Q_{out}\,[O_2]_G = Q_{in}[O_2]_{air} - V.O_2{}^{CR} \quad (6)$$

Where, $[O_2]_G$ and $[O_2]_{air}$ are oxygen concentration in the gas phase and air respectively, Q is air flow rate and V is the reactor volume.

From the above equation (by assuming $Q_{in} = Q_{out}$),

$$[O_2]_G = [O_2]_{air} - V.O_2{}^{CR}/\,Q_{in} \quad (7)$$

Henry's law constant (K_H) for gas solubility is often used in the form of dimensionless air/water partition co-efficient $K_{A/W}$ (i.e. K_H/RT):

$$K_{A/W} = [O_2]_{air} / [O_2]_S{}^{air} = [O_2]_G / [O_2]_S \quad (8)$$

where $[O_2]_S{}^{air}$ is the equilibrium concentration of oxygen in liquid saturated with air. From the eqs (7) and (8),

$$[O_2]_s = [O_2]_S{}^{air} . \{1 - V.O_2{}^{CR} / Q_{in}[O_2]_{air}\} \quad (9)$$

Equilibrium solubility of oxygen in pure water is different from actual solution containing different ions. In such cases solubility can be estimated by the method of Krevelen and Hoffijzer (1948). This relates the Henry's law constant in the solution (K_H) to that in pure water ($K_{H/W}$).

$$Log_{10}(K_H/K_{H,W}) = hI \quad (10)$$

$$I = \text{ionic strength} = 1/2\Sigma C_i Z_i{}^2 \quad (11)$$

where C_i is the concentration of ions of valency Z_i. The quantity h is the sum of contributions due to the species of positive and negative ions and to the species of gas i.e. $h = h_+ + h_- + h_g$. Calculated K_H values from eq. 10 was used to estimate $[O_2]_S{}^{air}$ from Henry's law.

The maximum oxygen transfer rate was also calculated for each experimental conditions (considering $([O_2]_L) = 0$). With the increase in Fe(II) in the medium, the dissolved oxygen concentration of liquid phase in equilibrium with gas phase ($[O_2]_s$) was observed to decrease with simultaneous decrease of $O_2{}^{TRmax}$. In Figure 1, the maximum oxygen transfer rate ($O_2{}^{TRmax}$), maximum oxygen consumption rate ($O_2{}^{CRmax}$) and oxygen consumption rate ($O_2{}^{CR}$) are plotted against the Fe(II) concentration. It is evident that the ratio of $O_2{}^{TR}/\,O_2{}^{CR}$ is much higher than 1 and the ratio increases with the advance of biooxidation reaction. In each case a very little deviation is observed between $O_2{}^{CRmax}$ and $O_2{}^{CR}$. These facts suggest that the oxygen mass transfer was high in the bioreactor and the biooxidation of Fe(II) using *T. ferrooxidans* was a reaction controlled process in this investigation.

In Figure 2, $[O_2]_s$ and $[O_2]_L$ are plotted against ferrous concentration in the medium. There was very little decrease in the equilibrium concentration and the actual concentration of oxygen in the liquid phase with increase of ferrous iron concentration in the medium. It can be implied that sufficient oxygen was always available in the medium.

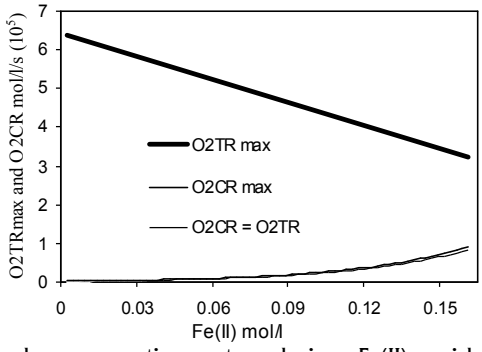

Figure 1. Oxygen transfer and consumption rates during Fe(II) oxidation [Conditions: Air flow rate 200ml/min, 120rpm, Temperature 35°C]

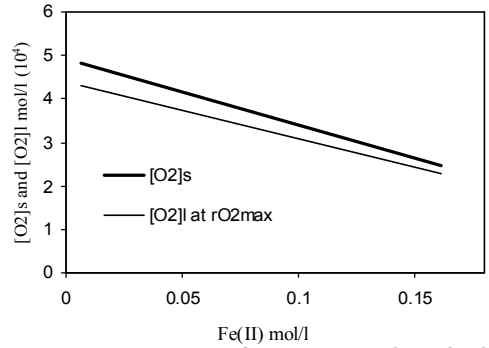

Figure 2. Actual and equilibrium concentration of oxygen in liquid phase [Conditions: Air flow rate 200ml/min, 120rpm, Temperature 35°C]

3.2. Liquid to solid mass transfer

Mass transfer from the bulk phase to the reaction surface is also of considerable importance in heterogeneous reactions for example, transfer of oxygen, carbon dioxide and dissolved substrate like Fe^{2+} or reduced sulfur compounds from the bulk phase to the bacterial cell wall. Transport of species 'i' from bulk phase to the solid surface can be described as:

$$\phi_i = K_{ci} (C^b_i - C^s_i) \quad (12)$$

where K_{ci} is the mass transfer co-efficient and ϕ_i is the mass transfer rate. C^b_i and C^s_i are concentrations of species 'i' in bulk and on the solid surface respectively.

For liquid to solid mass transfer, K_c can be calculated on semi theoretical basis from the physical properties of aqueous and solid phase and diffusion coefficient of the reactant. Basically the semitheoretical correlation (Harriot 1962) takes the form,

$$Sh = 2 + 0.6\ Re_p^{1/2}\ Sc^{1/3} \quad (13)$$

where Sh = Sherwood number($K_c d/D_i$), Re_p = Reynolds number($\rho d v_s/\mu$) and Sc = Schmidt number($\mu/\rho D_i$), where, d = diameter of particle(m), D = diffusivity (m^2/s), v_s = slip velocity (m/s), μ = absolute viscosity(kg/m/s) and ρ = density (kg/m^3).

A major difficulty in the calculation of particle (i.e bacteria) Reynolds number lies in the choice of correct slip velocity. If laminar flow conditions are assumed then slip velocity will be for free settling particle and the terminal velocity can be calculated from Stoke's law as

$$v_s = (\rho_s - \rho_l)\ g d^2 / 18\mu \quad (14)$$

In Table 1, the values of different dimensionless numbers and mass transfer coefficient are listed. Following standard values have been used for the calculation : D_{O2} = 2.5 x $10^{-9}m^2/s$ (in water at 25ºC), μ_{water} = 8.9 x 10^{-4}kg/m/s (at 25ºC), size of bacteria = $1*10^{-6}$ x $0.5*10^{-6}$ m^2/bacteria, ρ (bacteria) = 1013kg/m^3,ρ (water) = 1000kg/m^3

Table1
Calculated values of mass transfer coefficients

Species	v_s (m/s)	Re	Sc	Sh	Kc (m/s)
O_2	7.96 x 10^{-9}	8.94 x 10^{-9}	356	2	5 x 10^{-3}
Fe^{2+}	7.96 x 10^{-9}	8.94 x 10^{-9}	1237.8	2	1.44 x 10^{-3}

Under equilibrium condition the flux of the species 'i' from the bulk phase to the reaction surface, ϕ_i (mol/m^2/s) is equal to the consumption rate of the species 'i', iCR (mol/m^2/s).

$$\phi_i = iCR \tag{15}$$

The bacterial ferrous iron consumption rate per bacterial surface area, FCR (mol/m^2/s) can be estimated from the simplified kinetic equation given below (Boon, 1996):

$$FCR = \{q_{Fe2+,max} / (1+ [K_s/K_i])*([Fe^{3+} / Fe^{2+}]_{bulk})\}/A_{bact}. \tag{16}$$

where $q_{Fe2+,max}$ is the maximum bacterial specific ferrous iron consumption rate which is 9.6molFe^{2+}/C-mol/h for *T. ferrooxidans,* 'K_s/K_i' is 0.05 for *T. ferrooxidans* and A_{bact} is the specific surface area of *T. ferrooxidans* per mole of carbon, which is estimated to be 960m^2/C-mol .

The effect of mass transfer rate of Fe(II) (from the bulk to the bacterial surface) on the Fe(II) oxidation rate has been studied in detail varying different parameters as mentioned below.

3.2.1. Initial ferrous iron concentration

The initial Fe(II) concentration was varied from 54 to 161mol/m^3 at pH 2.0. The maximum ferrous transfer rate (FTR) was calculated from eq.12 (by putting the surface concentration of Fe(II) i.e. $C^s_{Fe(II)}$ = 0) and the consumption rate was calculated from eq. 16. The ferrous transfer rate always showed a higher trend compared to the consumption rate (FCR). With the initiation of the Fe(II) oxidation reaction, Fe(II) concentration in the bulk medium decreased along with decrease in the FCR and the FTR. From Figure 3, it is observed that during the oxidation reaction there is no mass transfer limitation at the bacterial surface.

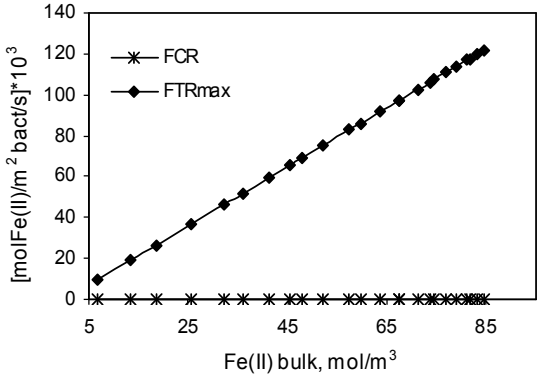

Figure 3. Fe(II) consumption rate and maximum transfer rate at 84.5mol/m^3 initial Fe(II) [Conditions: Initial pH 2, Temperature 35ºC]

3.2.2. Initial pH

The initial pH of the medium was varied from pH 1.25 to 2.5 while keeping initial Fe(II) concentration at 28mol/m^3. It was observed that in all pH conditions there was a decrease in ferrous consumption rate as well as transfer rate with the decrease in bulk Fe(II) concentration. Also there is a wide difference in ferrous transfer rate and consumption rate till the completion of reaction as seen in Figure 4. No iron precipitation was observed during the oxidation process.

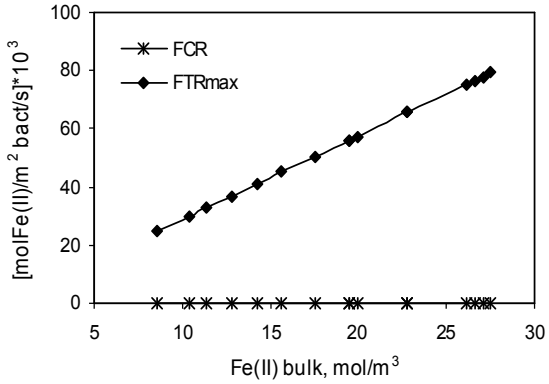

Figure 4. Fe(II) consumption rate and maximum transfer rate at initial pH 1.5 [Conditions: Initial Fe(II) 27.5mol/m^3, Temp. 35°C]

3.2.3. Initial ferric iron concentration

The initial Fe(III) concentration was varied from 10mol/m^3 to 27mol/m^3 keeping initial Fe(II) at 33mol/m^3 and pH at 2. In all the cases a marked difference was observed between the ferrous consumption rate and transfer rate. The transfer rate showed a higher trend and both the rates were observed to decrease with the decrease in bulk Fe(II) concentration (Figure 5).

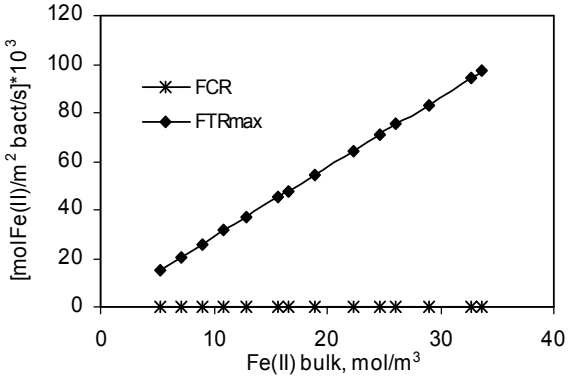

Figure 5. Fe(II) consumption rate and maximum transfer rate at initial Fe(III) 10.9mol/m^3 [Conditions: Initial Fe(II) 33mol/m^3, pH 2]

3.2.4. Initial biomass concentration

The initial bacterial concentration was varied from $2.3x10^4$ to $3.4x10^{11}$cells/ml. The initial Fe(II) concentration was maintained between 30 to 40mol/m^3. The initial pH was maintained at 2.0. It was observed that at higher bacterial concentration when the bacterial lag phase was nearly negligible, the ferrous transfer rate was amazingly higher than the ferrous consumption rate. With the decrease in Fe(II) concentration in the bulk both the rates were observed to decrease. From Figure 6, it can be observed that there was no mass transfer limitation of ferrous ions at the bacterial surface.

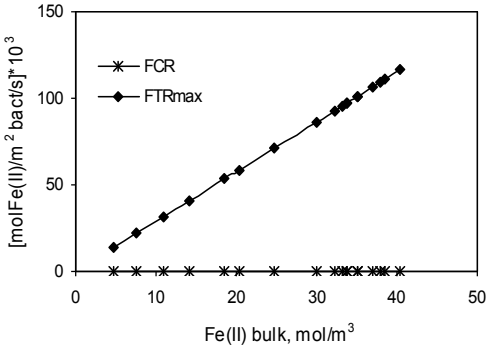

Figure 6. Fe(II) consumption and maximum transfer rate at initial log biomass conc. 4.4 [Conditions: Initial Fe(II) 40mol/m^3, pH 2]

4. CONCLUSIONS

The transfer rate of oxygen from air to the liquid medium was much higher than the oxygen consumption rate by the microorganism. Ferrous ions transfer rate from the bulk to the bacterial surface was also very high in comparison to consumption rate. In all the sets of experiment that were carried out by varying Fe(II), Fe(III) concentration, pH and bacterial concentration a wide difference was noted between the ferrous consumption rate and the ferrous transfer rate. It can be concluded that ferrous iron oxidation by *T. ferrooxidans* is not controlled by gas-liquid mass transfer of oxygen or by liquid to bacterial surface mass transfer by ferrous ions rather it a reaction controlled process.

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