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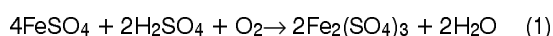
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A KINETIC MODEL OF FERROUS IRON OXIDATION BY ACIDITHIOBACILLUS FERROOXIDANS IN A BATCH CULTURE

The batch oxidation kinetics of ferrous iron by *Acidithiobacillus ferrooxidans* were examined at different oxygen transfer rates and pH in an aerated stirred tank and a bubble column. The microbial growth, oxygen consumption rate and ferrous and ferric iron were monitored during the biooxidation. A kinetic model was established on the basis of the Michaelis–Menten kinetic equation for bacterial growth and the constants estimated from experimental data (maximum specific growth rate 0.069 h^{-1} , saturation constant 2.9 g/dm^3 , and biomass yield coefficient based on ferrous iron 0.003 g.d.w./gFe). Values calculated from the model agreed well with the experimental ones regardless of the bioreactor type and pH conditions.

Key words: *Acidithiobacillus ferrooxidans*, biooxidation, mathematical model.

The biological oxidation of ferrous sulphate by *Acidithiobacillus ferrooxidans* (previously called *Thiobacillus ferrooxidans*) has been shown to be a significant step in the bioleaching of sulphide minerals and the treatment of acid mine drainage. This bioreaction also has beneficial application in the desulphurization of coal and hydrogen sulphide from gases. *A. ferrooxidans* is a chemolithotrophic bacterium that is able to oxidize the ferrous iron in sulphuric acid solution [1]:



Since the acid is removed from the reaction medium, the pH increases as the biooxidation proceeds. In general, the ferric iron formed has an extremely low solubility at $\text{pH} > 2.5$, causing the precipitation of ferric iron compounds. The formation of precipitates depends highly on the pH, and a pH lower than 1.8 seems to limit the ferric iron precipitation [2].

Several models describing the kinetics of ferrous iron oxidation by *A. ferrooxidans* have been published. In general, most of the models were derived from experimental conditions and include a number of parameters that affect the bacterial growth [3–7]. One of the simplest mathematical models of batch ferrous iron biooxidation combines the Monod equation and the substrate (ferrous iron) consumption:

$$\frac{dX}{dt} = \mu X = \mu_{\max} \frac{S}{K_S + S} X \quad (2)$$

$$\frac{dS}{dt} = -\frac{1}{Y_{X/S}} \frac{dX}{dt} \quad (3)$$

with product (ferric iron) formation

$$\frac{dP}{dt} = -\frac{dS}{dt} - k_P P \quad (4)$$

where X, S and P are the biomass, ferrous iron and ferric iron concentrations, respectively, t is time, μ_{\max} the maximum specific growth rate, K_S the saturation (Monod) constant, k_P the ferric iron precipitation rate constant, and $Y_{X/S}$ the biomass yield coefficient based on ferrous iron. Thus, the ferric iron formation should be growth associated. Equation (4) also includes the precipitation of ferric iron, which occurs at $\text{pH} > 2$ [2].

The maximum specific growth rate and the saturation (Monod) constant can be calculated from the Lineweaver–Burk equation, derived from equation (2). The biomass yield coefficient and the ferric iron precipitation rate constant can be calculated from equation (3) and the linearized form of equation (4), respectively.

The oxygen consumption rate is directly related to the biomass growth during ferrous iron biooxidation by the well-known equation:

$$\text{OUR} = q_{\text{O}_2} X \quad (5)$$

where OUR is the oxygen uptake rate, and q_{O_2} is the specific oxygen uptake rate.

A mathematical model of ferrous iron oxidation by *A. ferrooxidans*, based on equations (2) to (5), was used in this paper to describe variations of the biomass, ferrous iron, ferric iron and oxygen concentrations during a batch bioprocess. The main goal was to estimate the applicability of the proposed model for ferrous iron biooxidation under different pH conditions in different types of bioreactors.

MATERIAL AND METHODS

Microorganism and cultivation conditions

The strain of *A. ferrooxidans* B5, isolated from the copper mine Bor (Serbia), was grown on the mineral

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salts medium 9K with an initial ferrous ion concentration of 9 g/L [8]. The inoculum (10% v/v) was taken from the late exponential phase of biooxidation. The pH was adjusted to 1.8 and 2.0 using 5 M H₂SO₄. The temperature in all experiments was maintained at 28°C. The biooxidation was run in a bubble column (made of plexyglass; operating volume: 9 L; internal diameter: 9.2 cm; air sparger: polyacetate canvas; and air flow rate: 60 L/h) and an aerated, stirred tank (made of glass; the operating volume: 8 L; the internal tank diameter: 22 cm; the liquid height: 22 cm; the air flow rate of 250 L/h; and the agitation speed: 300 min⁻¹) equipped with two 8-cm-diameter turbine impellers (the distance of the lower impeller from the vessel bottom: 8 cm; the spacing between impellers: 8 cm; and the gas sparger: a nozzle bellow the impeller) with four 2 x 2 flate blades and fitted with four 3-cm-wide baffles.

Ferrous, ferric and total iron

The total iron concentration in the culture medium was measured by atomic absorption spectrophotometry (AAS PYE UNICAM, Phillips). The ferrous and ferric iron concentrations were calculated from the total iron concentration and the redox potential. A combined redox electrode (HANNA HI 3932) was used for the redox measurements. The pH was monitored by a pH meter (HANNA HI 9025).

Biomass

The biomass was determined by measuring the weight of the dried (80°C, 24 h) membrane filter (Millipore, NVC 45218, pore size 0.45 mm) after vacuum filtration of the sample.

Oxygen uptake rate

The oxygen uptake rate was measured by the dynamic method [9]. For this measurement, samples of the fermentation broth were taken periodically from the fermenter and transferred into a measuring cell equipped with a magnetic stirrer. The sample was sparged with air using a syringe, the cell was closed by an oxygen probe (HANNA HI8043), and then the dissolved oxygen level in the fermentation broth was monitored. The time constant of the oxygen probe was 9 s, and the measurement lasted 5 to 10 minutes. Thus, since the condition of $\Delta t/\tau_E > 20$ was fulfilled, the oxygen probe dynamics could be for sure ignored without affecting estimation of the oxygen uptake rate [9]. The oxygen uptake rate was calculated from the slope of the linear part of the relationship between the dissolved oxygen level and time.

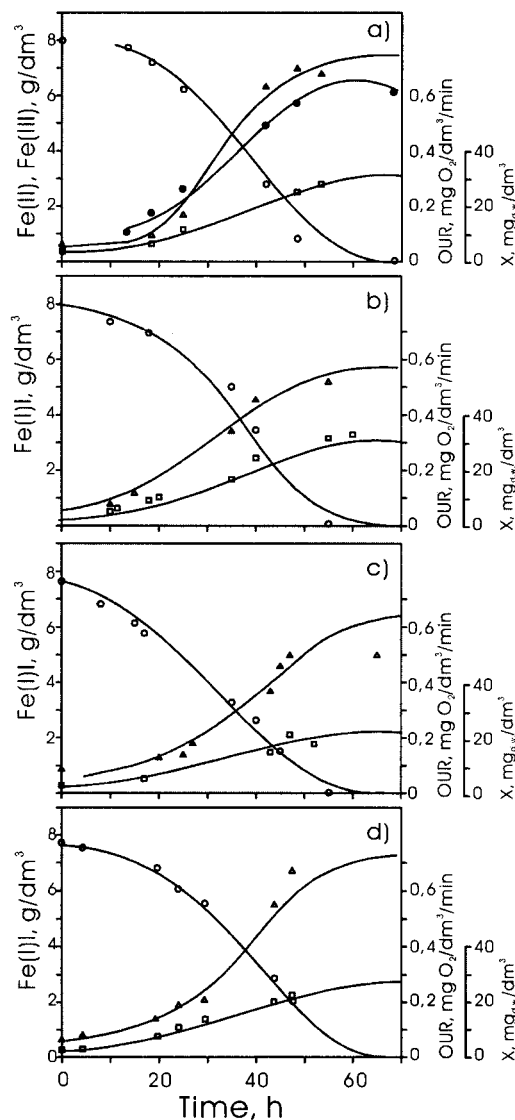


Figure 1. The concentration of ferrous iron (○), ferric iron (●), biomass (□) and OUR (Δ) during the biooxidation of ferrous sulphate (lines: prediction by the model) bubble column: a) without pH control (approximately pH 2.5), b) pH 1.8, and c) pH 2.0; aerated, stirred tank: d) pH 2.0.

RESULTS AND DISCUSSION

Variations of the biomass, ferrous iron and ferric iron concentrations and oxygen uptake rate during biooxidation in both types of bioreactors, without and with controlled pH conditions are shown in Figure 1. The experimental data and calculated values agree quite well, confirming the proposed model. The values of the biomass, ferrous iron and ferric iron (only for the experiment carried out at pH 2.5) concentrations predicted by the model, as well as the oxygen uptake rate (initial concentrations $X_0 = 2.5\text{--}3\text{ g/dm}^3$, $S_0 = 8\text{--}9\text{ g/dm}^3$ and $P_0 = 0.9\text{ g/dm}^3$) were in accordance with the experimental data (correlation coefficient $R = 0.990$ to

Table 1. The values of μ_{\max} and $Y_{X/S}$ during ferrous iron oxidation by *A. ferrooxidans* in a bubble column and stirred aerated tank at different pH

Bioreactor	pH	μ_{\max} , h ⁻¹	$Y_{X/S}$, g _{d.w.} /g _{Fe}
Bubble column	2.5	0.069	0.0027
	2.0	0.067	0.0035
	1.8	0.065	0.0028
Stirred aerated tank	2.0	0.068	0.0030

0.999) regardless of the bioreactor type and pH conditions. Precipitation of the ferric iron was observed only in the experiment at pH 2.5 (Figure 1a). In experiments at constant pH (1.8 and 2.0; Figure 1b, c and d) precipitation was not observed. Because cells of *A. ferrooxidans* did not assimilate ferrous iron [10], the ferric iron concentration in the solution corresponded to the oxidized ferrous iron. The ferric iron concentration in these experiments was not calculated and not incorporated in the proposed model.

The parameters of the model, namely $\mu_{\max} = 0.069 \text{ h}^{-1}$, $K_S = 2.93 \text{ g/dm}^3$ and $Y_{X/S} = 0.003 \text{ g}_{d.w.}/\text{g}_{Fe}$, were calculated using equations (2) and (3). The estimated values of μ_{\max} and $Y_{X/S}$ were in a good agreement with the values calculated from the slopes of the linear part of the relationship of the biomass concentration versus time corresponding to the logarithmic growth phase, and that of the total biomass versus the total oxidized ferrous iron, respectively, as can be seen in Table 1. The rate of precipitation, $k_p = 0.1 \text{ h}^{-1}$, was calculated from equation (4). The oxygen uptake rate was calculated using equation (5) by assuming that $q_{O_2} = 0.020 \pm 0.001 \text{ mg O}_2/\text{mg}_{d.w.}/\text{min}$ [11].

According to recently proposed models of ferrous iron oxidation by *A. ferrooxidans*, only the substrate uptake was successfully simulated [2,4,12]. The disagreement between the calculated and experimental values of the ferric iron concentration was explained by insufficient understanding of the process of ferric iron precipitation. The biomass concentration was not included in the models due to the questionable methods of its estimation [4]. Since the experimental values of the oxygen uptake rate were in accordance with the calculated values, this model also confirmed the proposed method for quantifying the *A. ferrooxidans* biomass concentration by measuring the oxygen uptake rate [11].

CONCLUSIONS

The kinetics of ferrous iron oxidation by *A. ferrooxidans* was shown in the present study to be of the

Monod equation type. The model incorporates, for the first time, the rate of ferric iron precipitation. Also, it successfully predicts variations of the biomass, ferrous iron and ferric iron concentrations and the oxygen uptake rate with the progress of ferrous iron oxidation by *A. ferrooxidans* at different pH conditions in the most common bioreactors, namely bubble column and aerated, stirred tank reactors.

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NOTATIONS

- k_p – ferric iron precipitation rate constant (h⁻¹)
- K_S – saturation (Monod) constant (g/dm³)
- P – ferric iron concentration (g/dm³)
- P_0 – initial ferric iron concentration (g/dm³)
- OUR – oxygen uptake rate
- q_{O_2} – specific oxygen uptake rate (mg O₂/mg_{d.w.}/min)
- S – ferrous iron concentration (g/dm³)
- S_0 – initial ferrous iron concentration (g/dm³)
- t – time (h)
- X – biomass concentration (mg_{d.w.}/dm³),
- X_0 – initial biomass concentration (mg_{d.w.}/dm³)
- $Y_{X/S}$ – biomass yield coefficient based on ferrous iron (mg_{d.w.}/g_{Fe})

Greek symbols

- m_{\max} – maximum specific growth rate (h⁻¹)

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IZVOD

KINETIČKI MODEL OKSIDACIJE GVOŽDJE(II)–JONA POMOĆU BAKTERIJE *ACIDITHIOBACILLUS FERROOXIDANS* U ŠARŽNIM USLOVIMA

(rad)

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U radu je proučavana kinetika oksidacije gvožđe(II)–jona pomoću bakterije *Acidithiobacillus ferrooxidans* u bioreaktoru sa mešalicom i barbotажnoj koloni, a pod različitim uslovima aeracije i pH. U toku biooksidacije praćena je promena biomase, potrošnja kiseonika i promena koncentracije gvožđe(II)– i gvožđe(III)–jona. Na osnovu Michaelis–Menten–ove kinetičke jednačine za mikrobni rast i konstanti izračunate na osnovu eksperimentalnih podataka (maksimalna specifična brzina mikrobnog rasta $\mu_{max} = 0,069 \text{ h}^{-1}$, saturaciona konstanta $K_S = 2,9 \text{ g/dm}^3$, koeficijent prinosa biomase u odnosu na supstrat $Y_{X/S} = 0,003 \text{ g}_{d.w.}/\text{g}_{Fe}$) razvijen je matematički model. Vrednosti dobijene numeričkom integracijom jednačina modela pokazuju dobro slaganje sa eksperimentalnim podacima (koeficijenti korelacije $R = 0,990\text{--}0,999$) sa eksperimentalnim podacima nezavisno od vrste korišćenog bioreaktora i pH.

Ključne reči: *Acidithiobacillus ferrooxidans*, biooksidacija, matematički model.