Chemical Engineering Journal 99 (2004) 77–87

Chemical Engineering Journal

www.elsevier.com/locate/cej

A semiempirical model for bacterial growth and bioleaching of *Acidithiobacillus* spp.☆

Hsuan-Liang Liu*, Chun-Hsiung Teng, Yang-Chu Cheng

Department of Chemical Engineering, National Taipei University of Technology, No. 1 Sec. 3 Chung-Hsiao E. Rd., Taipei 10643, Taiwan, ROC

Accepted 31 October 2003

Abstract

A semiempirical model for bacterial growth and bioleaching of *Acidithiobacillus* spp. was developed based on the concept of transport phenomena. Only one mathematical equation and three parameters evaluated from the experimental data are required to describe the complicated bioleaching process, regardless of the source and concentration of the microorganism, the concentration, composition, and physical characteristics (particle size, shape, distribution, and porosity, etc.) of the solid substrate, the concentrations of the leaching products and byproducts, and the leaching conditions (pH, temperature, agitation, etc.). In order to evaluate the semiempirical model, five kinetic models proposed previously were compared. The results of mean deviations show that our model fits their experimental data explicitly well, even better than the corresponding models. Furthermore, the semiempirical model can be successfully used to predict the bacterial growth and bioleaching behaviors in different leaching systems, i.e. direct, indirect, direct and indirect, and simultaneous leaching.

Keywords: Semiempirical model; Bioleaching; *Acidithiobacillus* spp.; Transport phenomena; Kinetic model; Mean deviations

1. Introduction

Microorganisms play an important role in the sulfur cycle in the biosphere. *Acidithiobacillus* spp. (e.g., *A. thiooxidans* and *A. ferrooxidans*), which are capable of chemoautotrophic growth using energy obtained from the oxidation of inorganic sulfur compounds, has been the most widely considered group of microorganism in terms of bioleaching applications due to their acidophilic characteristics [1,2]. In extremely acidic conditions, metals in contaminated soils are solubilized due to the destruction of metal–soil complexes. The bioleaching mechanism of *A. ferrooxidans* follows the direct and indirect processes, whereas that of *A. thiooxidans* only adopts the indirect approach [3–7]. The direct process is a result of direct bacterial attack on sulfide minerals, whereas the indirect one involves ferric iron which acts as a chemical leaching agent and is supplied by regeneration from the reaction product (ferrous iron) through biological oxidation by *A. ferrooxidans*. The contributions of the two leaching mechanisms depend strongly on the types of sulfide mineral and on the operating conditions. Although the bacterial leaching techniques have been intensively applied to the recovery of copper and uranium from low-grade ores and the removal of pyrite sulfur from coal, the kinetics of bioleaching due to the direct and indirect mechanisms are poorly understood.

Although useful information is available on the mechanism of the sulfur oxidation by the acidithiobacilli [8–10], little work has been done to investigate the kinetics of the bacterial sulfur oxidation as well as the kinetics of the direct and indirect bioleaching. The microbial oxidation of elemental sulfur is considered to take place with the adsorption of bacteria onto the solid substrate and their subsequent growth on the solid surface. Furthermore, some researchers have centered on the adsorption of acidithiobacilli onto elemental sulfur [11–13]. However, the amounts of cells attached to the surface of solid substrate are considered to be relatively insignificant compared to those suspended in the liquid medium.

Many kinetic models have been proposed to investigate the bioleaching of metal sulfide concentrates by *A. ferrooxidans* [14,15], the growth and elemental sulfur oxidation in batch culture of *A. ferrooxidans* [16], the batch bacterial dissolution of pyrite particles by *A. ferrooxidans* [17], the elemental sulfur oxidation by *A. thiooxidans* in batch slurry reactors [18], and the simultaneous leaching of zinc sulfide

☆ The C/C++ programs of the semiempirical model proposed in this study and of the kinetic models proposed previously are available on request.

* Corresponding author. Tel.: +886-2-2771-2171x2542; fax: +886-2-2731-7117.
E-mail address: f10894@ntut.edu.tw (H.-L. Liu).

1385-8947/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.cej.2003.10.010

Nomenclature

a_B	surface area covered by unit cell (m^2/cell)
A	total surface area of solid particles (m^2)
A_L	constant for X_L estimation (dimensionless)
A_M	constant for M estimation (dimensionless)
A_T	constant for X_T estimation (dimensionless)
B_L	constant for X_L estimation (per day)
B_M	constant for M estimation (per day)
B_T	constant for X_T estimation (per day)
C_L	constant for X_L estimation (dimensionless)
C_M	constant for M estimation (dimensionless)
C_T	constant for X_T estimation (dimensionless)
d_0	initial diameter of solid particle (m)
D_{EST}	the estimated values according to the kinetic models
D_{EXP}	experimental data
f	weight fraction of zinc in mineral (dimensionless)
$[Fe^{2+}]$	ferrous iron concentration (kg/m^3)
$[Fe^{3+}]$	ferric iron concentration (kg/m^3)
$[Fe^{3+}]_0$	initial concentration of ferric iron in liquid phase (kg/m^3)
h_L	transfer coefficient constant for X_L estimation (m^3/cells per day)
h_T	transfer coefficient constant for X_T estimation (m^3/cells per day)
J	ratio of atomic weight of Fe to Zn (dimensionless)
k_c	reaction rate constant of ferric iron leaching (m/day)
K_A	equilibrium constant for cell adsorption (m^3/cell)
K_L	constant, maximum free bacteria concentration (cells/m^3)
K_M	constant, for M estimation (kg/m^3)
K_T	constant, maximum total bacteria concentration (cells/m^3)
K_{ZF}	rate constant of dissolution of ZnS by Fe^{3+} (m^3/h kg)
M	concentration of substrate leached ($=W_0 - W$) (kg/m^3)
MD	mean deviation
N	number of experimental runs (dimensionless)
t	time (day)
V	total volume of solid–liquid mixture (m^3)
W	concentration of substrate in solid–liquid mixture (kg/m^3)
W_0	initial concentration of substrate in solid–liquid mixture (kg/m^3)
X_a	bacteria adsorbed per unit surface of substrate (cells/m^2)
X_{am}	maximum adsorption capacity per unit surface area of particles (cell/m^2)
X_L	concentration of free bacteria in liquid phase (cells/m^3)
X_{L0}	initial concentration of free bacteria in liquid phase (cells/m^3)
X_T	concentration of total bacteria in solid–liquid mixture (cells/m^3)
X_{T0}	initial concentration of total bacteria in solid–liquid mixture (cells/m^3)
Y	growth yield on solid substrate (cells/kg)
Y_A	growth yield of bacteria on solid mineral (cells/kg mineral)
Y_L	growth yield of bacteria on ferrous iron (cells/kg Fe)
[Zn]	concentration of zinc in liquid phase (kg/m^3)

Greek letters

α	fraction of zinc sulfide dissolved (dimensionless)
μ, μ_A	specific growth rate of bacteria on solid surface (per day)
μ_m	maximum specific growth rate of bacteria on liquid phase (h^{-1})

and manganese dioxide in the presence of iron-oxidizing bacteria [19]. Li [20] studied the effect of temperature on the mechanism of simultaneous leaching of zinc sulfide concentrate and manganese and obtained the activation energy for this process. In addition, Yukawa et al. [21] presented a kinetic model for the batch leaching of $CuFeS_2$, in which the rate of direct bioleaching was assumed to be proportional to the concentration of free cells in the solution. However, their model predicted much higher leaching rates than the experimentally observed results. In the previous models proposed by Gormely et al. [22] and Chang and Myerson [23], it was assumed that the growth rate of bacteria adsorbed on the mineral is proportional only to the concentration of adsorbed cells. In contrast, Konishi et al. [24] claimed that in a batch reactor, the growth rate of bacteria adsorbed on a FeS_2 surface is proportional to the product of the concentration of adsorbed cells and the fraction of adsorption sites unoccupied by cells. They also emphasized that the observed sigmoidal shape of leaching curves can be predicted only by this rate equation. However, the effect of particle size on the growth kinetics has not yet been addressed experimentally and theoretically in their model.

Many factors such as the source and concentration of the microorganism, the concentration, composition, and physical characteristics (particle size, shape, distribution, and porosity, etc.) of the solid substrate, the concentrations of the leaching products and byproducts, and the leaching conditions (pH, temperature, agitation, etc.) have been shown to affect the bioleaching process significantly, thus restricting the universal applications of the above-mentioned kinetic

models. In addition, too many assumptions were made to simplify the mathematical models, resulting in poor fitting of the experimental data. The purposes of this work are: (1) to obtain a semiempirical model for bacterial growth and bioleaching of *Acidithiobacillus* spp. based on the concept of transport phenomena and (2) to compare our model with the previous kinetic models proposed by Asai et al. [17], Konishi et al. [15,16], Gourdon and Funtowicz [18], and Kai et al. [19]. The results show that our model can fit data well from different experimental sets, even better than the original models. In addition, the number of parameters required to fit the mathematical equation is significantly reduced in our model. Both bacterial growth and bioleaching of *Acidithiobacillus* spp. can be predicted using the same form of mathematical equation, with the only difference being the parameters calculated to fit the equation. In conclusion, the semiempirical model proposed in this study dramatically reduces the complexity of predicting the bioleaching process.

2. Mathematical model

2.1. The concentration of cells suspended in the solution

Based on the concept of transport phenomena, the growth rate of bacteria can be considered as the product of driving force and transfer coefficient as follows:

$$\frac{dX_T}{dt} = (\text{transfer coefficient})(\text{driving force}) \tag{2.1}$$

where X_T is the total cell concentration at time t. If the maximum amount of cell obtained is K_T, then the driving force in Eq. (2.1) becomes $(K_T - X_T)$. We further assumed that the transfer coefficient is proportional to the cell concentration at time t, that is, $h_T X_T$, where h_T is a constant, therefore Eq. (2.1) becomes

$$\frac{dX_T}{dt} = h_T X_T (K_T - X_T) \tag{2.2}$$

Eq. (2.2) describes the growth kinetics of the bacterial well, such that:

(1) When $X_T = 0$, then $dX_T/dt = 0$. It indicates that the growth rate is equal to zero when no cells exist.

(2) When K_T is extremely higher than X_T, the growth of the microorganism enters the exponential growth phase, such as

$$\frac{dX_T}{dt} = h_T X_T (K_T - X_T) \cong h_T K_T X_T \tag{2.3}$$

(3) When $X_T > 0.5K_T$, the growth of the microorganism reaches the decelerated growth phase, such as

$$\frac{d^2X_T}{dt^2} = h_T(K_T - X_T) - h_T X_T = h_T(K_T - 2X_T),$$

$$\frac{d^2X_T}{dt^2} < 0 \quad \text{for } X_T > 0.5K_T \tag{2.4}$$

(4) When $X_T = K_T$, the growth rate becomes zero and the cell concentration reaches its maximum (stationary growth phase), such as

$$\frac{dX_T}{dt} = h_T X_T (K_T - X_T) = 0 \quad \text{for } X_T = K_T \tag{2.5}$$

By integrating Eq. (2.2), an equation of total cell concentration versus time is obtained

$$\ln\left(\frac{X_T}{K_T - X_T}\right) = K_T h_T t + C_T \tag{2.6}$$

$$\frac{X_T}{K_T - X_T} = \exp(K_T h_T t)\exp(C_T) = A_T \exp(B_T t) \tag{2.7}$$

$$X_T = \frac{K_T A_T \exp(B_T t)}{1 + A_T \exp(B_T t)} \tag{2.8}$$

where $A_T = \exp(C_T)$ and $B_T = K_T h_T$. A simple three-parameter equation is thus obtained to describe the total concentration of bacteria versus time. The total amount of cells (X_T) is the sum of the amount of cells attached to the solid substrates (X_A) and the amount of cells suspended in the liquid medium (X_L). We assumed that X_A is negligible compared to X_L, thus X_T in Eq. (2.8) can be replaced by X_L as follows:

$$\frac{X_L}{K_L - X_L} = \exp(K_L h_L t)\exp(C_L) = A_L \exp(B_L t) \tag{2.9}$$

$$X_L = \frac{K_L A_L \exp(B_L t)}{1 + A_L \exp(B_L t)} \tag{2.10}$$

where $A_L = \exp(C_L)$ and $B_L = K_L h_L$. This is again a simple three-parameter equation to describe the concentration of bacteria suspended in the liquid medium.

2.2. The amount of solid substrate leached by the microorganism

If the initial concentration of solid substrate and total amount of cells are W_0 and X_{T0}, respectively, and the growth yield of bacterial on solid substrate is assumed to be a constant, Y, the following equations can be obtained based on mass balance:

$$\frac{dX_T}{dt} = -Y\frac{dW}{dt} \tag{2.11}$$

$$M = W_0 - W = \frac{1}{Y}(X_T - X_{T0}) \cong \frac{1}{Y}(X_L - X_{L0}) \tag{2.12}$$

where M is the amount of solid substrate leached. According to Eq. (2.10) and setting $t = 0$, one gets

$$X_{L0} = \frac{K_L A_L}{1 + A_L} \tag{2.13}$$

By substituting Eqs. (2.10) and (2.13) into Eq. (2.12), one gets

$$M = \frac{1}{Y}\left(\frac{K_L A_L \exp(B_L t)}{1 + A_L \exp(B_L t)} - \frac{K_L A_L}{1 + A_L}\right) \tag{2.14}$$

It is obvious that the growth yield of bacteria on solid substrate, Y, is not constant. Besides, there are two Y values to be considered in bioleaching system: one is from direct leaching, Y_1, and the other from indirect leaching, Y_2. Thus, Eq. (2.14) is not considered a good model to predict the amount of solid substrate leached precisely. Instead of regarding Y as an adjustable parameter in the curve-fitting exercise based on the above theoretical calculations, we audaciously assume that the behavior of bioleaching is similar to that of bacterial growth. Thus, the amount of solid substrates leached by the microorganism can be expressed as the similar form of Eq. (2.10), that is

$$M = \frac{K_M A_M \exp(B_M t)}{1 + A_M \exp(B_M t)} \tag{2.15}$$

where $A_M = \exp(C_M)$. Considering when $t = 0$, the amount of solid substrates leached should be zero, thus Eq. (2.15) is modified as follows:

$$M = \frac{K_M A_M \exp(B_M t)}{1 + A_M \exp(B_M t)} - \frac{K_M A_M}{1 + A_M} \tag{2.16}$$

Again, Eq. (2.16) is a simple three-parameter equation to describe the amount of solid substrate leached by the microorganism. Interestingly, Eq. (2.16) has been shown to fit the five sets of experimental data better than Eq. (2.14), particularly in the beginning of the leaching experiments (data not shown).

2.3. Evaluation of the mathematical models

The mean deviation (MD) is the first measure of dispersion that we will use that actually uses each data value in its computation. It is the mean of the distances between each value and the mean. It gives us an idea of how spread out from the center the set of values is. Thus, it was used to evaluate the proposed semiempirical model and the previous kinetic models. MD is defined as follows:

$$\mathrm{MD} = \frac{1}{N}\sum_{1}^{N} |D_{\mathrm{EXP}} - D_{\mathrm{EST}}| \tag{2.17}$$

where N is the number of experimental runs, and D_{EXP} and D_{EST} are the experimental data and the estimated values according to these models, respectively.

2.4. Experimental data

The experimental data used in the present study were extracted from previous work by Asai et al. [17], Konishi et al. [15,16], Gourdon and Funtowicz [18], and Kai et al. [19]. All these work studies the kinetic models using *A. ferrooxidans*, except for the work by Gourdon and Funtowicz [18], in which *A. thiooxidans* was used.

3. Results and discussion

The proposed semiempirical model in this study and the previous kinetic models are summarized in Table 1. It is obvious that our model is the simplest one to describe the bacterial growth and bioleaching among these models, with the number of parameters required to fit the mathematical

Table 1
The proposed semiempirical model in this study and the previous kinetic models

Model	Mathematical equations	Remarks
Semiempirical model	$X_L = \frac{K_L A_L \exp(B_L t)}{1 + A_L \exp(B_L t)}$, $M = \frac{K_M A_M \exp(B_M t)}{1 + A_M \exp(B_M t)} - \frac{K_M A_M}{1 + A_M}$	Model proposed in this study
Konishi et al. [16]	$\frac{dX_T}{dt} = \mu\left(\frac{W_0}{V}\right)\left(\frac{W}{W_0}\right)^{2/3}\frac{K_A X_{m0} X_L}{(1 + K_A X_L)^2}$, $-\frac{dW}{dt} = \mu\left(\frac{W_0}{Y}\right)\left(\frac{W}{W_0}\right)^{2/3}\frac{K_A X_{m0} X_L}{(1 + K_A X_L)^2}$	Direct leaching by *A. ferrooxidans*
Gourdon and Funtowicz [18]	$\frac{dX_T}{dt} = \mu\frac{6K_A X_{am} W_0}{d_0 \rho}\left(\frac{W}{W_0}\right)^{2/3}\frac{X_L}{1 + K_A X_L}$, $W = W_0 - \frac{V}{3}(C - C_0)$	Direct leaching by *A. thiooxidans*
Konishi et al. [15]	$\frac{dX_T}{dt} = \mu_A\frac{K_A X_{am} X_L}{(1 + K_A X_L)^2}\frac{A}{V} + 2Y_L k_c[Fe^{3+}]_0\frac{A}{V}(1 - a_B X_a)$, $\frac{d[Zn]}{dt} = \frac{f}{Y_A}\mu_A\frac{K_A X_{am} X_L}{(1 + K_A X_L)^2}\frac{A}{V} + \frac{k_c[Fe^{3+}]_0 A(1 - a_B X_a)}{JV}$	Direct and indirect leaching by *A. ferrooxidans*
Kai et al. [19]	$\frac{dX_T}{dt} = \mu_A\frac{K_A X_{am} X_L}{(1 + K_A X_L)^2}\frac{A}{V} + \mu m\frac{X_L[Fe^{2+}]}{K_s + [Fe^{2+}]}$	Direct and indirect leaching by *A. ferrooxidans*
Asai et al. [17]	$\frac{dX_T}{dt} = \mu\left(\frac{W_0}{V}\right)\left(\frac{W}{W_0}\right)^{2/3}\frac{K_A X_{m0} X_L}{(1 + K_A X_L)^2}\left(1 + \frac{fY_L}{Y_A}\right)$, $\frac{M}{V} = \frac{f(X_T - X_{T0})}{Y_A + fY_L}$	Simultaneous leaching by *A. ferrooxidans*

equation being reduced to 3 (i.e., K_L, B_L, and C_L for predicting the cell concentration in the liquid medium and K_M, B_M, and C_M for predicting the amount of solid substrate leached). The comparisons of the proposed semiempirical model with the previous kinetic models are described in the following sections.

3.1. The comparison of the semiempirical model with the kinetic model proposed by Konishi et al. [16]

Several experiments were conducted to examine the adsorption behavior of *A. ferrooxidans* onto elemental sulfur by Konishi et al. [16]. They found that the free cells in the liquid phase were extensively adsorbed on the surface of sulfur and the extent of adsorption depended strongly on the sulfur–liquid loading ratio. The concentration of adsorbed cells, X_A, approached a limiting value, whereas the concentration of free cells in the liquid, X_L, continued to increase with time. They also demonstrated that elemental sulfur was completely oxidized to sulfuric acid by mass balance with respect to sulfur. To describe cell growth and related substrate consumption, Konishi et al. [16] established a metabolic stoichiometry based on the similar approaches, in which the kinetics of batch bioleaching of mineral pyrite (FeS_2) [17,24], coal pyrite [25], and sphalerite (ZnS) [15] were described. According to these models, the growth rate of adsorbed cells on a solid substrate is assumed to be directly proportional to the product of the concentration of adsorbed cells and the fraction of adsorption sites unoccupied by cells, which is in contrast to the assumption made by Yukawa et al. [21], in which the rate of direct bioleaching was assumed to be proportional to the concentration of free cells in the solution.

In order to evaluate the semiempirical model proposed in this study and the kinetic model proposed by Konishi et al. [16], the data of the concentration of free cells and the amount of sulfur leached were extracted from their experiments. Fig. 1 shows the results of our model fitting to their experimental data emphasizing the effects of medium pH, initial sulfur–liquid loading ratio, and initial total cell concentration on bacterial growth and sulfur oxidation. The parameters used to fit their experimental data are given in Table 2. It is obvious that our model fits their experimental data explicitly well. The values of MD after fitting their experimental data by our model range from 0.0171×10^{15} to 0.1320×10^{15} cells/m^3 for the free cell concentration and from 0.0798 to 0.2161 kg S/m^3 for the sulfur oxidized as listed in Table 2. These values are all smaller than the corresponding values after fitting with the kinetic model proposed by Konishi et al. [16], indicating that our model fits the experimental data even better than their original kinetic model.

3.2. The comparison of the proposed model with the kinetic model proposed by Gourdon and Funtowicz [18]

Gourdon and Funtowicz [18] proposed a kinetic model of elemental sulfur oxidation by *A. thiooxidans* in batch

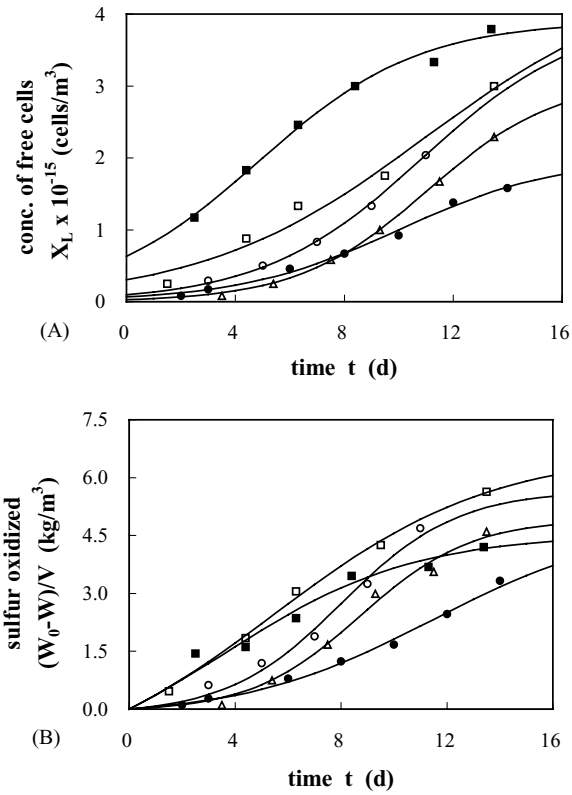

Fig. 1. The experimental data from Konishi et al. [16] after fitting with the semiempirical model proposed in this study (solid curves): (A) the concentration of free bacteria in the liquid medium; (B) the sulfur oxidized. Symbols: the initial sulfur–liquid loading ratio of 10 kg/m^3, pH 2.0–0.9 at an initial total cell concentration of 1.01×10^{13} cells/m^3 (●); the initial total cell concentration of 1.26×10^{12} cells/m^3 (△); 1.01×10^{13} cells/m^3 (○); 1.26×10^{14} cells/m^3 (□); 1.35×10^{15} cells/m^3 (■) and medium pH 2.0–0.7 at an initial sulfur–liquid loading ratio of 20 kg/m^3.

slurry reactors by integrating some of the commonly accepted assumptions made for the modeling of metal sulfide bacterial oxidation in aqueous suspension. They assumed that the growth is proportional only to the concentration of attached bacteria, which is in contradiction with the assumption of Konishi et al. [16] who considered that the growth rate of bacteria attached to solid surfaces of metal sulfides or elemental sulfur is proportional to the product of the concentration of attached bacteria and the fraction of adsorption sites unoccupied by bacteria. The attachment of the cells onto the surface of the sulfur particles was considered following Freundlich isotherm. The model considers that sulfur particles have a uniform mass distribution in the range of particle sized used and that the particle size may only decrease during incubation due to sulfur oxidation and sulfate solubilization. However, their experimental results have shown that this assumption is not valid during incubation when sulfur concentration is relatively high.

In order to evaluate the semiempirical model proposed in this study and the kinetic model proposed by Gourdon and Funtowicz [18], the data of the concentrations of free cells and sulfate were extracted from their experiments. The

Table 2
The parameters used to fit the experimental data by Konishi et al. [16] and the mean deviations after fitting by our model and the kinetic model proposed by Konishi et al. [16]

Experimental data sets	Parameters			MD ($\times 10^{15}$ cells/m^3)	
	K_L ($\times 10^{15}$ cells/m^3)	B_L (per day)	C_L (dimensionless)	Our model	Konishi et al. [16]
Set 1 (●)	2.00	0.34	−3.4	0.0346	0.1094
Set 2 (○)	4.00	0.34	−3.7	0.0242	0.1365
Set 3 (△)	3.10	0.42	−4.65	0.0171	0.1091
Set 4 (□)	4.60	0.24	−2.25	0.1320	0.1331
Set 5 (■)	3.90	0.34	−1.65	0.0634	0.2915
	Parameters			MD (kg S/m^3)	
	K_M (kg S/m^3)	B_M (per day)	C_M (dimensionless)	Our model	Konishi et al. [16]
Set 1 (●)	5.00	0.28	−3.2	0.0798	0.1767
Set 2 (○)	5.80	0.46	−3.7	0.1926	0.3226
Set 3 (△)	5.00	0.48	−4.2	0.1521	0.1685
Set 4 (□)	8.50	0.24	−1.3	0.0717	0.1368
Set 5 (■)	6.20	0.28	−1.0	0.2161	0.2983

results of our model fitting to their experimental data are shown in Fig. 2 and the parameters used to fit their experimental data are given in Table 3. Our model did not fit their experimental data of bacterial growth well compared to the results of data fitting by their original model, with the MD value (0.5402×10^{15} cells/m^3) slightly higher than that after curve fitting by their model (0.4338×10^{15} cells/m^3). In contrast, our model fit their experimental data of sulfate production better than their model, with the MD value (0.1868 kg Zn/m^3) slightly lower than that after fitting by their model (0.2200 kg Zn/m^3). As shown in Fig. 2, the rate of sulfate production by sulfur oxidation increases in the first few days of incubation. Attached cells grow on the sulfur particles and the total number of attached cells in the reaction volume increases. The rate of sulfur conversion therefore increases proportionally and the free cell concentration in the liquid medium also increases due to the detachment of part of the cells. In our model, the attachment of the bacteria onto the surface of sulfur particles was assumed to be negligible comparing to the amount of free cells in the liquid medium. Furthermore, the last data point measured by Gourdon and Funtowicz [18] was taken at the 80th day of incubation; it could be the experimental error to reduce the accuracy of our curve fitting to their data.

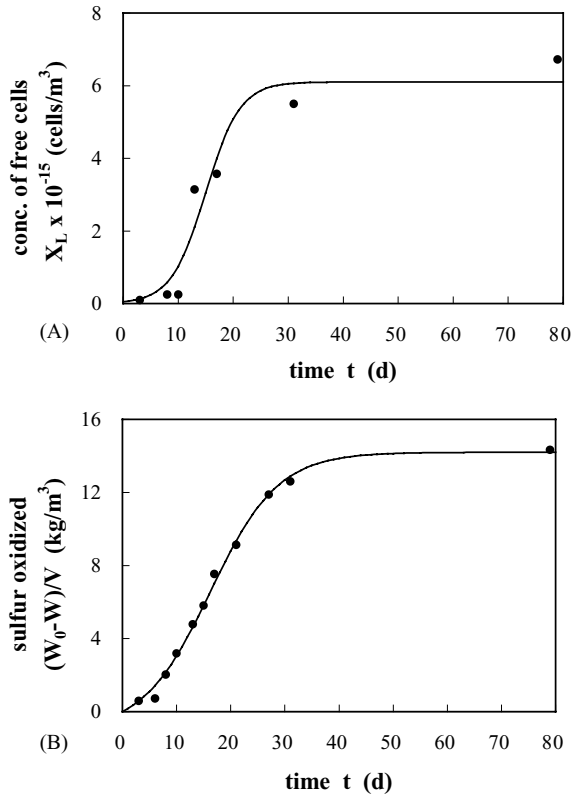

Fig. 2. The experimental data from Gourdon and Funtowicz [18] after fitting with the semiempirical model proposed in this study (solid curves): (A) the concentration of free bacteria in the liquid medium; (B) the sulfate production. The experiments were carried out at 30 °C in the aerated slurry reactor with the initial concentrations of 20 kg S/m^3 and $X_{L0} = 1.25 \times 10^{13}$ cells/m^3. Closed circles (●) indicate the experimental data.

3.3. The comparison of the proposed model with the kinetic model proposed by Konishi et al. [15]

The theoretical approach reported in [15] was to generate a bioleaching model allowing for two mechanisms: the direct and indirect bacterial actions. In the direct action, bacteria multiply on the sulfide mineral which acts as a solid substrate; part of the cells are released from the solid surface into the liquid medium. When an iron-containing medium is used in the bioleaching, ferric iron leaching occurs with the formation of ferrous iron, which is readily oxidized by *A. ferrooxidans*. According to this leaching scheme, bacterial growth occurs not only on the sulfide mineral but also in the liquid medium containing soluble iron. Thus, the total growth rate is expressed as the sum of the growth rate of adsorbed bacteria and the rate of free

Table 3
The parameters used to fit the experimental data by Gourdon and Funtowicz [18] and the mean deviations after fitting by our model and the kinetic model proposed by Gourdon and Funtowicz [18]

Experimental data sets	Parameters			MD ($\times 10^{15}$ cells/m^3)	
	K_L ($\times 10^{15}$ cells/m^3)	B_L (per day)	C_L (dimensionless)	Our model	Gourdon and Funtowicz [18]
Set 1 (●)	6.10	0.32	−4.80	0.5402	0.4338
	Parameters			MD (kg S/m^3)	
	K_M (kg S/m^3)	B_M (per day)	C_M (dimensionless)	Our model	Gourdon and Funtowicz [18]
Set 1 (●)	15.25	0.16	−2.60	0.2055	0.3025

bacterial in the liquid medium. After all, they developed a very complicated mathematical model, which includes several key parameters such as the adsorption equilibrium constant, the maximum adsorption capacity, the reaction rate constant, the specific growth rate, and the growth yield of adsorbed bacteria. Many of these parameters are difficult to be measured, making this model somewhat user-unfriendly.

In order to evaluate the semiempirical model proposed in this study and the kinetic model proposed by Konishi et al. [15], the data of the concentrations of free cells and zinc leached in solution were extracted from their experiments. Fig. 3 shows the results of our model fitting to their experimental data. These experiments were conducted to investigate the effects of initial ferric iron concentration and initial solid–liquid loading ratio on bacterial growth and on bioleaching rate. The parameters used to fit their experimental data are given in Table 4. It is obvious that our model fits their experimental data explicitly well with the MD values ranging from 0.0132×10^{14} to 0.3568×10^{14} cells/m^3 and from 0.0141 to 0.0518 kg Zn/m^3 for the concentrations of free cells and zinc leached in solution, respectively. All these MD values are much smaller than the corresponding MD values after fitting the experimental data by the original model proposed by Konishi et al. [15]. Although the kinetic model proposed by Konishi et al. [15] has taken into account the effects of the direct bacterial action and the ferric iron leaching on the leaching rate, several key parameters required to fit the rate expressions for the bacterial growth and leaching were not experimentally determined in their model, including the specific growth rate and the growth yield. Although the theoretical approach by Konishi et al. [15] has made them examine quantitatively the effects of various operating variables in the same time, the goodness of their model depends strongly on the accurate estimation of these parameters by the complicated mathematical equations proposed, leading to large MD values after fitting the experimental data by their kinetic model to evaluate the concentrations of free bacteria and zinc leached in solution. In contrast, only three parameters are required in our semiempirical model and they are directly determined from the experimental data sets, making our model more realistic on predicting the experimental results.

3.4. The comparison of the proposed model with the kinetic model proposed by Kai et al. [19]

Kai et al. [19] obtained a simplified kinetic equation for the oxidative dissolution of zinc sulfide based on the models proposed by Verbaan and Huberts [26] and Konishi et al. [15]. Their equation considered particle shrinkage by

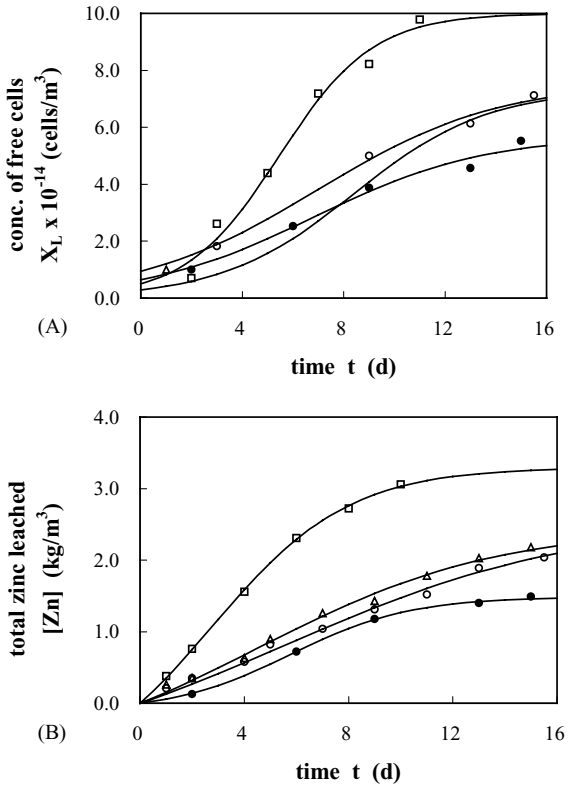

Fig. 3. The experimental data from Konishi et al. [15] after fitting with the semiempirical model proposed in this study (solid curves): (A) the concentration of free bacteria in the liquid medium; (B) the concentration of zinc leached in solution. Symbols: the initial sulfur–liquid loading ratio of 10 kg/m^3 at an initial total cell concentration of 1.5×10^{13} cells/m^3 and $[Fe^{3+}]_0 = 0$ kg/m^3 (●); the initial total cell concentration of 1.1×10^{13} cells/m^3 and $[Fe^{3+}]_0 = 0.3$ kg/m^3 (△); the initial total cell concentration of 0.95×10^{13} cells/m^3 and $[Fe^{3+}]_0 = 0.5$ kg/m^3 (○); and the initial sulfur–liquid loading ratio of 20 kg/m^3 at an initial total cell concentration of 2.0×10^{13} cells/m^3 and $[Fe^{3+}]_0 = 0.5$ kg/m^3 (□).

Table 4
The parameters used to fit the experimental data by Konishi et al. [15] and the mean deviations after fitting by our model and the kinetic model proposed by Konishi et al. [15]

Experimental data sets	Parameters			MD ($\times 10^{14}$ cells/m^3)	
	K_L ($\times 10^{14}$ cells/m^3)	B_L (per day)	C_L (dimensionless)	Our model	Konishi et al. [15]
Set 1 (●)	5.70	0.30	−2.05	0.1782	0.6975
Set 2 (○)	7.60	0.28	−1.95	0.1658	0.5888
Set 3 (△)	7.35	0.38	−3.20	0.0132	0.2042
Set 4 (□)	10.0	0.54	−2.95	0.3568	0.6256
	Parameters			MD (kg Zn/m^3)	
	K_M (kg Zn/m^3)	B_M (per day)	C_M (dimensionless)	Our model	Konishi et al. [15]
Set 1 (●)	1.60	0.44	−2.55	0.0141	0.0808
Set 2 (○)	3.50	0.18	−1.00	0.0518	0.0814
Set 3 (△)	3.35	0.22	−1.00	0.0513	0.1215
Set 4 (□)	4.45	0.38	−1.05	0.0256	0.1342

dissolution, which is different from the previous assumption that the particle size is constant with time [16]. By adopting the model proposed by Konishi et al. [15] to fit the experimental results, the model proposed by Kai et al. [19] is similar to the Michaelis–Menten mechanism of enzyme catalysis. Their kinetic model shows that the dissolution rates of zinc sulfide and manganese dioxide are enhanced by the presence of the iron-oxidizing bacterium *A. ferrooxidans* in the simultaneous leaching system. It is considered that the bacterial oxidation of zinc sulfide and elemental sulfur yielded on the surface of it might be effective for the enhancement. The removal of elemental sulfur might also improve the galvanic reaction rate. The leaching of zinc sulfide by *A. ferrooxidans* has been intensively studied [27]. Boon et al. [28] have pointed out that the direct mechanism is negligible based on their redox-stat experiments. In contrast, Konishi et al. [15] have shown that the direct leaching could not be ignored when the concentration of irons was low. In the study of Kai et al. [19], the extent of leaching during the simultaneous dissolution with *A. ferrooxidans* was determined.

In order to evaluate the semiempirical model proposed in this study and the kinetic model proposed by Kai et al. [19], the data of the concentrations of free cells and zinc leached in solution were extracted from their experiments. Fig. 4 shows the results of our model fitting to their experimental data. The parameters used to fit their experimental data are given in Table 5. It is obvious that our model fit their experimental data well, with the MD values for the concentrations of free cells and zinc leached being 0.0341×10^{14} cells/m^3 and 0.0230 kg Zn/m^3, respectively. These values are smaller than those after fitting by the original model proposed by Kai et al. [19], indicating that our model is superior to the original one. Consequently, the semiempirical model proposed in this study could predict the dissolution of zinc sulfide during the simultaneous leaching. This model could predict the dissolution even in the presence of *A. ferrooxidans*. Since our model is relatively simple comparing to the theoretical calculations by Kai et al. [19], it would be useful for the design of a leaching process and for the optimization of operating conditions of simultaneous leaching systems.

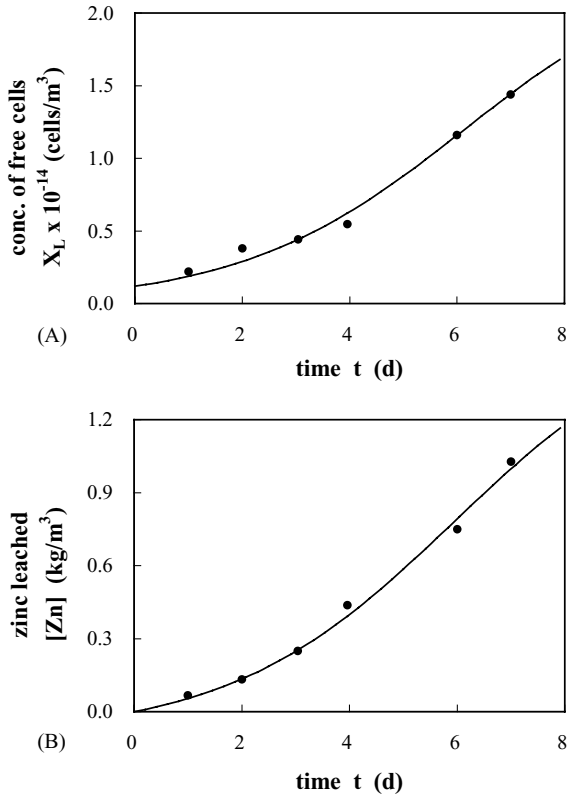

Fig. 4. The experimental data from Kai et al. [19] after fitting with the semiempirical model proposed in this study (solid curves): (A) the concentration of free bacteria in the liquid medium; (B) the concentration of zinc leached in solution. The experiments were carried out with the initial concentrations of 10 kg ZnS/m^3, $X_{T0} = 1.0 \times 10^{13}$ cells/m^3, and $[Fe^{3+}]_0 = 0.1$ kg/m^3 at 30 °C. Closed circles (●) indicate the experimental data.

Table 5
The parameters used to fit the experimental data by Kai et al. [19] and the mean deviations after fitting by our model and the kinetic model proposed by Kai et al. [19]

Experimental data sets	Parameters			MD ($\times 10^{14}$ cells/m^3)	
	K_L ($\times 10^{14}$ cells/m^3)	B_L (per day)	C_L (dimensionless)	Our model	Kai et al. [19]
Set 1 (●)	2.40	0.48	−2.95	0.0341	0.0585
	Parameters			MD (kg Zn/m^3)	
	K_M (kg Zn/m^3)	B_M (per day)	C_M (dimensionless)	Our model	Kai et al. [19]
Set 1 (●)	1.75	0.48	−2.85	0.0230	0.0519

3.5. The comparison of the proposed model with the kinetic model proposed by Asai et al. [17]

A kinetic model was proposed to describe the batch growth in the dissolution of pyrite caused by the direct microbial action by Konishi et al. [24], in which the growth rate of adsorbed bacteria was assumed to depend on the fraction of adsorption sites unoccupied by bacteria and to be proportional to the dissolution rate of pyrite as a solid substrate. Unlike the assumptions made by Konishi et al. [24] that the key parameters: specific growth rate of bacteria on solid surface, the growth yield of bacteria on pyrite sulfur, and the equilibrium constant for cell adsorption are independent of particle size, the maximum adsorption capacity per unit weight of solid particle is a function of particle size in the model proposed by Asai et al. [17]. The modified model was then used to simulate the dissolution behavior of pyrite particles by *A. ferrooxidans* in a batch bioreactor for different operating variables such as the initial particle size, the initial cell concentration, and the initial pyrite–liquid loading ratio. Experimental studies were made on the adsorption of bacteria on pyrite particles as well as the bacterial dissolution of pyrite. The Langmuir isotherm was then used to fit the adsorption data. Their kinetic model shows that the equilibrium constant was independent of the particle size, whereas the maximum adsorption capacity per unit weight of pyrite increased with decreasing particle size. The evaluated kinetic parameters were found to be independent of the initial particle size.

In order to evaluate the semiempirical model proposed in this study and the kinetic model proposed by Asai et al. [17], the data of the concentrations of free cells and total iron leached in solution were extracted from their experiments. Fig. 5 shows the results of our model fitting to their experimental data. The parameters used to fit their experimental data are given in Table 6. It is obvious that our model fit their experimental data well with the MD values for the concentrations of free cells and iron leached being ranging from 0.0818×10^{14} to 0.4430×10^{14} cells/m^3 and 0.0167 to 0.0577 kg Fe/m^3, respectively. These values are smaller than those after fitting by the original model proposed by Asai et al. [17], indicating that our model fits the experimental data even better than the original model. According to Asai et al. [17], four complicated mathematical equations were required to establish the respective effects of the initial particle size, the initial cell concentration, and the initial solid–liquid loading ratio on bioleaching. In contrast, only one simple equation with three parameters is enough to evaluate these effects on bioleaching in our semiempirical model, indicating that the proposed model in this study can be successfully used to predict the bacterial dissolution behavior for different operating conditions.

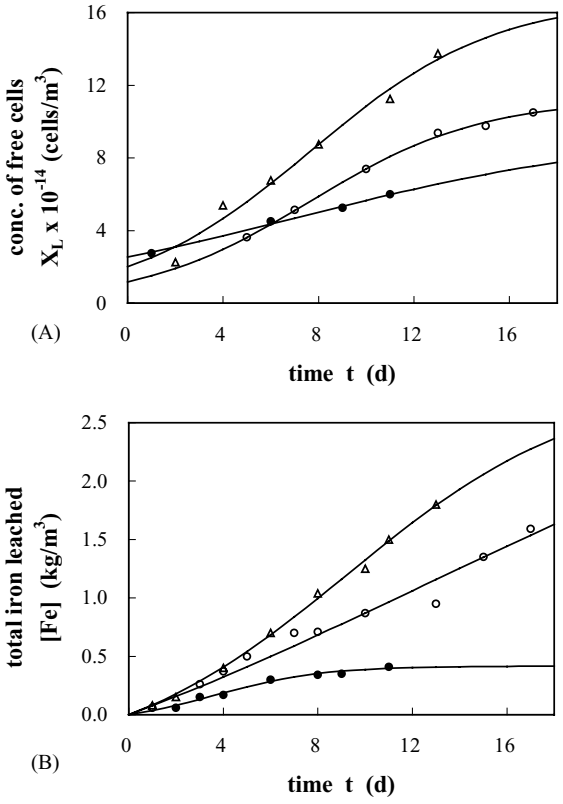

Fig. 5. The experimental data from Asai et al. [17] after fitting with the semiempirical model proposed in this study (solid curves): (A) the concentration of free bacteria in the liquid medium; (B) the concentration of total iron leached in solution. Symbols: 10 kg pyrite/m^3, particle size 149–177 μm, $[Fe]_{T0} = 0.35$ kg/m^3, and $X_{T0} = 2.53 \times 10^{14}$ cells/m^3 (●); 10 kg pyrite/m^3, particle size 63–88 μm, $[Fe]_{T0} = 0.35$ kg/m^3, and $X_{T0} = 1.02 \times 10^{14}$ cells/m^3 (△); 10 kg pyrite/m^3, particle size 53–63 μm, $[Fe]_{T0} = 0.35$ kg/m^3, and $X_{T0} = 1.02 \times 10^{14}$ cells/m^3 (○).

Table 6
The parameters used to fit the experimental data by Asai et al. [17] and the mean deviations after fitting by our model and the kinetic model proposed by Asai et al. [17]

Experimental data sets	Parameters			MD ($\times 10^{14}$ cells/m^3)	
	K_L ($\times 10^{14}$ cells/m^3)	B_L (per day)	C_L (dimensionless)	Our model	Asai et al. [17]
Set 1 (●)	9.45	0.14	−1.00	0.0818	0.5407
Set 2 (○)	11.6	0.28	−2.15	0.0844	0.2171
Set 3 (△)	16.8	0.26	−2.00	0.4430	0.4891
	Parameters			MD (kg Fe/m^3)	
	K_M (kg Fe/m^3)	B_M (per day)	C_M (dimensionless)	Our model	Asai et al. [17]
Set 1 (●)	0.50	0.44	−1.60	0.0167	0.0609
Set 2 (○)	4.80	0.08	−1.00	0.0577	0.0689
Set 3 (△)	3.30	0.20	−1.85	0.0242	0.1031

4. Conclusions

In this study, a semiempirical model for bacterial growth and bioleaching of *Acidithiobacillus* spp. was developed based on the concept of transport phenomena. Only three parameters are required to fit the mathematical equation. This model was used to fit the experimental data from previous studies [15–19] and was compared with the corresponding kinetic models. The results show that our model describes the complicated bioleaching process explicitly well, regardless of the source and concentration of the microorganism, the concentration, composition, and physical characteristics (particle size, shape, distribution, and porosity, etc.) of the solid substrate, the concentrations of the leaching products and byproducts, and the leaching conditions (pH, temperature, agitation, etc.). In addition, our model can be applied to predict the bacterial growth and bioleaching behaviors in different leaching systems, i.e. direct, indirect, direct and indirect, and simultaneous leaching. However, the disadvantage in applying the proposed model is that no obvious physical meanings can be obtained regarding these three parameters. Although the sensitive analysis shows that no direct correlations were observed among these three parameters (data not shown), probably due to the limited experimental data sets, no conclusion can be made whether these three parameters are correlated to one another when more experimental data sets are available.

Acknowledgements

Financial support (project numbers NSC-90-2214-E-027-006 and NSC-92-2622-E-027-003-CC3) from the National Science Council, Taiwan, ROC, is very much appreciated.

References

[1] W. Krebs, C. Brombacher, P.P. Bosshard, R. Bachhofen, H. Brandl, Microbial recovery of metals from solids, FEMS Microbiol. Rev. 20 (1997) 605–617.

[2] T. Gehrke, J. Telegdi, D. Thierry, W. Sand, Importance of extracellular polymeric substances from *Acidithiobacillus thiooxidans* for bioleaching, Appl. Environ. Microbiol. 64 (1998) 2743–2747.

[3] R.D. Tyagi, D. Couillard, Bacterial leaching of metals from digested sewage sludge, Process Biochem. 22 (1987) 114–117.

[4] R.D. Tyagi, D. Couillard, Bacterial leaching of metals from sludges, in: P.E. Cheremisinoff (Ed.), Encyclopedia of Environmental Control Technology, vol. 3, Gulf Publishing Company, Texas, 1989, pp. 557–591.

[5] D. Couillard, G. Mercier, Bacterial leaching of heavy metals from sewage sludge-bioreactors comparison, Environ. Pollut. 66 (1990) 237–253.

[6] D. Couillard, G. Mercier, Optimum residence time (in CSTR and airlift reactor) for bacterial leaching of metals from sewage sludge, Water Res. 25 (1991) 211–218.

[7] W.H. Rulkens, J.T.C. Grotenhuis, R. Tichy, Methods of cleaning contaminated soils and sediments, in: W. Salomons, U. Förstner, P. Mader (Eds.), Heavy Metals, Springer, Berlin, 1995, pp. 151–191.

[8] S.R. Hutchins, M.S. Davidson, J.A. Brierley, C.L. Brierley, Microorganisms in reclamation of metals, Ann. Rev. Microbiol. 40 (1986) 311–336.

[9] T. Sugio, C. Domatsu, O. Munakata, T. Tano, K. Imai, Role of ferric iron-reducing system in sulfur oxidation by *Thiobacillus ferrooxidans*, Appl. Environ. Microbiol. 49 (1985) 1401–1406.

[10] T. Sugio, W. Mizunashi, K. Inagaki, T. Tano, Purification and some properties of sulfur: ferric-iron oxidoreductase from *Thiobacillus ferrooxidans*, J. Bacteriol. 169 (1987) 4916–4922.

[11] W.I. Schaeffer, P.E. Holbert, W.W. Umbreit, Attachment of *Thiobacillus ferrooxidans* to sulfur crystals, J. Bacteriol. 85 (1963) 137–140.

[12] S. Takakuwa, T. Fujimori, H. Iwasaki, Some properties of cell–sulfur adhesion in *Thiobacillus thiooxidans*, J. Gen. Appl. Microbiol. 25 (1979) 21–29.

[13] R.D. Bryant, J.W. Costerton, E.J. Laishley, The role of *Thiobacillus albertis* glycocalyx in the adhesion of cells to elemental sulfur, Can. J. Microbiol. 30 (1984) 81–90.

[14] V. Sanmugasunderam, R.M.R. Branion, D.W. Duncan, A growth model for the continuous microbiological leaching of zinc sulfide concentrate by *Thiobacillus ferrooxidans*, Biotechnol. Bioeng. 27 (1985) 1173–1179.

[15] Y. Konishi, H. Kubo, S. Asai, Bioleaching of zinc sulfide concentrate by *Thiobacillus ferrooxidans*, Biotechnol. Bioeng. 39 (1992) 66–74.

[16] Y. Konishi, Y. Takasaka, S. Asai, Kinetics of growth and elemental sulfur oxidation in batch culture of *Thiobacillus ferrooxidans*, Biotechnol. Bioeng. 44 (1994) 667–673.

[17] S. Asai, Y. Konishi, K. Yoshida, Kinetic model for batch bacterial dissolution of pyrite particles by *Thiobacillus ferrooxidans*, Chem. Eng. Sci. 47 (1992) 133–139.
[18] N. Gourdon, N. Funtowicz, Kinetic model of elemental sulfur oxidation by *Thiobacillus thiooxidans* in batch slurry reactors, Bioprocess Eng. 18 (1998) 241–249.
[19] T. Kai, Y. Suenaga, A. Migita, T. Takahashi, Kinetic model for simultaneous leaching of zinc sulfide and manganese dioxide in the presence of iron-oxidizing bacteria, Chem. Eng. Sci. 55 (2000) 3429–3436.
[20] J. Li, Mechanism in simultaneous leaching processes of zinc sulphide concentrate and manganese ores, Nonferrous Met. 48 (1996) 90–93 (in Chinese).
[21] T. Yukawa, D. Vanselow, B.J. Ralph, P.L. Rogers, A kinetic model for the bacterial leaching of chalcopyrite, J. Ferment. Technol. 56 (1978) 45–54.
[22] L.S. Gormely, D.W. Duncan, R.M.R. Branion, K.L. Rinder, Continuous culture of *Thiobacillus ferrooxidans* on a zinc sulfide concentrate, Biotechnol. Bioeng. 17 (1975) 31–40.
[23] Y.C. Chang, A.S. Myerson, Growth models of the continuous bacterial leaching of iron pyrite by *Thiobacillus ferrooxidans*, Biotechnol. Bioeng. 24 (1982) 889–896.
[24] Y. Konishi, S. Asai, H. Katoh, Bacterial dissolution of pyrite by *Thiobacillus ferrooxidans*, Bioprocess Eng. 5 (1990) 231–238.
[25] Y. Konishi, T. Kawamura, S. Asai, Removal of inorganic sulfur from coal by *Thiobacillus ferrooxidans*, Biotechnol. Bioeng. 29 (1993) 66–74.
[26] B. Verbaan, R. Huberts, Dissolution of sphalerite (ZnS) in acidic ferric sulfate solution in the presence of manganese dioxide, Miner. Metall. Process. 15 (1988) 29–34.
[27] W.K. Choi, A.E. Torma, R.W. Ohline, E. Ghali, Electrochemical aspects of zinc sulphide leaching by *Thiobacillus ferrooxidans*, Hydrometallurgy 33 (1993) 137–152.
[28] M. Boon, M. Snijder, G.S. Hansford, J.J. Heijnen, The oxidation kinetics of zinc sulphide with *Thiobacillus ferrooxidans*, Hydrometallurgy 48 (1998) 171–186.