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Sensitivity analysis of the semiempirical model for the growth of the indigenous *Acidithiobacillus thiooxidans*

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Abstract

Based on the concept of transport phenomena and driving force, we had previously developed a semiempirical model, in which only one mathematical equation and three parameters (K_L, B_L, and C_L) are required to describe the complicated bacterial growth of the indigenous *Acidithiobacillus thiooxidans*. In order to determine the effects of these parameters, sensitivity analysis based on Morris method was further conducted in this study. The results show that K_L, B_L, and C_L are closely related to the maximal cell concentration, the growth rate in the exponential phase, and the residual time in the lag phase, respectively. The effects of four important cultivation factors: the concentrations of elemental sulfur (S^0), $CaCl_2$, $MnSO_4$, and the initial pH, on these three parameters were also analyzed. The results show that the concentration of elemental sulfur exhibits positive correlations with K_L and C_L; whereas it exhibits a negative correlation with B_L. We have subsequently developed three regression equations to predict the values of these three parameters with the concentration of elemental sulfur as the sole information available. These parameters can be further fed into the semiempirical model for predicting the bacterial growth of the indigenous *A. thiooxidans* with high accuracy.

Keywords: Bioleaching; *Acidithiobacillus thiooxidans*; Semiempirical model; Morris method; Sensitivity analysis

1. Introduction

It is well known that certain microorganisms play an important role in the sulfur cycle in the biosphere. These bacteria can enhance markedly the metal leaching rate from sulfides. Among them, *Acidithiobacillus* spp. (e.g., *Acidithiobacillus thiooxidans* and *A. ferrooxidans*), capable of chemoautotrophic growth using energy obtained from the oxidation of inorganic sulfur compounds, has been the most widely considered group in terms of bioleaching applications due to their acidophilic characteristics [1]. Although the bioleaching techniques have been intensively applied to the recovery of cadmium, cobalt, copper, gold, manganese, nickel, plutonium, silver, uranium, and zinc from ores and concentrates as well as to the removal of pyrite sulfur from coal [2–5], the kinetics of bioleaching regarding the direct and indirect mechanisms [6–9] are poorly understood. Therefore, the development of mathematical models to describe the continuous microbial growth and reactions is still difficult [10].

Previously, several kinetic models have been proposed to predict the bacterial growth and bioleaching behaviors of *Acidithiobacillus* spp. [10–16]. However, most of these models require too many parameters, such as the source and concentration of the microorganism, the concentration, composition, and physical characteristics (i.e., particle size, shape, distribution, and porosity, etc.) of the solid substrate, the concentrations of the leaching products and byproducts, and the leaching conditions (i.e., pH, temperature, agitation, etc.), thus restrict the universal applications of these models. In addition, too many assumptions are made to simplify the mathematical models, resulting in poor fitting of the experimental data.

In our previous study, a semiempirical model for bacterial growth and bioleaching of the indigenous *A. thiooxidans* was successfully developed based on the concept of transport

Abbreviations: DCW, dry cell weight (g/l); MD, mean deviation; SA, sensitivity analysis; UA, uncertainty analysis

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Nomenclature

A_L	constant for X_L estimation
A_T	constant for X_T estimation
B_L	constant for X_L estimation (day^{-1})
B_T	constant for X_T estimation (day^{-1})
C_L	constant for X_L estimation
C_T	constant for X_T estimation
D_{EST}	the estimated values according to the kinetic models
D_{EXP}	experimental data
h_T	transfer coefficient constant for X_T estimation (m^3/cells day)
K_L	constant, maximum free bacteria concentration (cells/m^3)
K_T	constant, maximum total bacteria concentration (cells/m^3)
N	number of experimental runs
R^2	standard deviation
S	the sensitivity measure, standard deviation
S^0	the concentration of elemental sulfur (g/l)
t	time (day)
x^*	the four-dimensional factor vector, dimensionless
X_A	bacteria adsorbed per unit surface of substrate (cells/m^2)
X_L	concentration of free bacteria in liquid phase (cells/m^3)
X_T	concentration of total bacteria in solid–liquid mixture (cells/m^3)
X_1	the first cultivation factor, concentration of elemental sulfur (g/l)
X_2	the second cultivation factor, initial pH
X_3	the third cultivation factor, concentration of $CaCl_2$ (g/l)
X_4	the forth cultivation factor, concentration of $MnSO_4$ (g/l)

Greek letters

δ	the Euclidean distance from the origin in the (μ, S) space
Δ	the selected increment for the component of x in the x^* vector
μ	the sensitivity measure, mean

phenomena and driving force [17]. Only one mathematical equation and three parameters (K_L, B_L, and C_L) are required to describe the complicated bacterial growth and bioleaching behaviors. This model fits the experimental data explicitly well, even better than the previously developed theoretical models [12–16]. However, neither the effects of these parameters on the predicted bacterial growth nor the correlations between some important cultivation factors and these parameters is available, resulting in the limited application of the semiempirical model. To better understand the effects of these parameters on this model and the correlations between these parameters and some important cultivation factors, sensitivity analysis of the semiempirical model was further conducted in this study.

Usually, uncertainty and sensitivity analyses are both important components in the development of mathematical models [18]. The values of model parameters and the input values of variables are subject to many sources of uncertainty. A better understanding of the sensitivity of the model outputs to the uncertainty in the values of the input variables and parameters is necessary in developing confidence in the model and its prediction. Usually, uncertainty analysis (UA) involves determining the uncertainty in model predictions that results from imprecisely known input variables; while sensitivity analysis (SA) aims to quantify the relative importance of each input variable and model parameter in determining the predicted value of a model state variable. So far, SA is one of the most powerful tools performed on mathematical models to determine the relative contribution of the input variables and parameter values to the observed variations in the model outputs. These computational experiments can determine, within reasonable limits, which parameters or initial variable values may have negligible, significant, linear, or nonlinear effects on the model outputs [19]. Many different methods have been developed for performing SA and a variety of techniques are currently available [20].

The sensitivity analysis performed in this study is threefold: (1) to apply a well known screening test, Morris method [18,21,22], a relatively inexpensive and useful tool, to pinpoint the parameters most likely to be influential for the semiempirical model; (2) to investigate the effects of four cultivation factors (the concentrations of elemental sulfur (S^0), $CaCl_2$, and $MnSO_4$, and the initial pH) on the three parameters (K_L, B_L, and C_L) of the semiemperical model; (3) to develop the regression equations for predicting the values of these three parameters with the concentration of elemental sulfur as the sole information available, which in turn can be fed into the semiempirical model for predicting the bacterial growth with high accuracy.

2. Materials and methods

2.1. Microorganisms

The indigenous *A. thiooxidans* used throughout this study was obtained from the sewerage samples from a sulphate-contaminated site near Keelung, Taiwan [23]. The growth medium (N:P = 5:1; compositions (g/l): KH_2PO_4 = 1.0, $(NH_4)_2SO_4$ = 2.54, $MnSO_4$ = 0.1, $MgSO_4$ = 0.02, $CaCl_2$ = 0.03, $FeCl_3$ = 0.02, powdered S^0 = 5.0, nystatin = 0.1; pH 4.0) was used to cultivate this microorganism in a water-bath shaker (110 rpm) at 30 °C. In addition, the optimal medium (compositions (g/l): KH_2PO_4 = 3.5, $(NH_4)_2SO_4$ = 4.9, $MnSO_4$ = 0.1, $MgSO_4$ = 0.74, $CaCl_2$ = 0.03, $FeCl_3$ = 0.02, powdered S^0 = 23.7, nystatin = 0.1; pH 4.0) obtained from our previous study using response surface methodology (RSM) [23] was also used to validate the regression equations developed in this study. Biomass concentrations and pH level were periodically measured over the entire cultivation time.

2.2. Analytical methods

Aliquots of 10 ml were taken from the culture and filtered through a general grade filter paper (Advantec, Tokyo, 90 mm) to eliminate residual sulfur. Spectrophotometer at 620 nm was performed against the general gravimetric results to obtain a calibration curve [24]. By reading the turbidity of a given sample culture, the corresponding amount of biomass was obtained (1.00 $OD_{620\,nm} \cong 0.98 \pm 0.08$ g/l dry cell weight). The absorbance was measured by DR/2000 spectrophotometer (HACH, Loveland, CO). Using pH 4.0 and 10.0 standard buffers (Fisher Scientific, Tokyo, Japan) for calibration, standard measurement of pH was undertaken by using pH electrode and meter (Cole-Parmer, Vernon Hills, IL) with an accuracy of 0.1 pH unit.

2.3. Experimental design by Morris method

The guiding philosophy in the computational experiment suggested by Morris [21] is that a major role of a preliminary computational experiment is to determine, within reasonable uncertainties, which input parameters may be considered to have effects which are negligible, linear and additive, and nonlinear or involved in interactions with the other inputs in the system. Morris method is based on experimental plans that are composed of individually randomized one-factor-at-a-time designs in the input factors. For this reason, data analysis can be based on examination of changes in the output that are unambiguously attributed to changes in individual inputs. The randomly chosen starting vector of $x^* = (1, 0, 3, 1)$, (2, 1, 0, 2), (0, 2, 1, 0), and (3, 3, 2, 3) and Δ (the selected increment for the component of x in vector x^*) = 1 were taken to the formula of Morris method [21,18]. The test factors chosen in this study were the concentration of elemental sulfur (S^0) (X_1), the initial pH (X_2), and the concentrations of $CaCl_2$ (X_3) and $MnSO_4$ (X_4). The levels and prescription of these factors investigated in this study are summarized in Table 1. The values of the three parameters (K_L, B_L, and C_L) were obtained by fitting the experimental data to the C^{++} program provided with the semiempirical model. Then, the quantitative assessment of the relative influences of these four factors on these three parameters can be obtained from the plot of Morris method.

Table 1
Full factorial central composite design matrix of four cultivation factors in coded and natural units along with the observed responses

Obs. number	X_1	X_2	X_3	X_4	S^0 (g/l)	pH	$CaCl_2$ (g/l)	$MnSO_4$ (g/l)
1	1	1	3	2	9.7	1.5	0.03	0.58
2	2	1	3	2	14.4	1.5	0.03	0.58
3	2	0	3	2	14.4	1	0.03	0.58
4	2	0	4	2	14.4	1	0.03	0.74
5	2	0	4	1	14.4	1	0.02	0.74
6	2	2	0	3	14.4	2	0.04	0.1
7	3	2	0	3	19	2	0.04	0.1
18	3	1	0	3	19	1.5	0.04	0.1
9	3	1	1	3	19	1.5	0.04	0.26
10	3	1	1	2	19	1.5	0.03	0.26
11	0	3	1	1	5	2.5	0.02	0.26
12	1	3	1	1	9.7	2.5	0.02	0.26
13	1	2	1	1	9.7	2	0.02	0.26
14	1	2	2	1	9.7	2	0.02	0.42
15	1	2	2	0	9.7	2	0.01	0.42
16	3	4	2	4	19	3	0.05	0.42
17	4	4	2	4	23.7	3	0.05	0.42
18	4	3	2	4	23.7	2.5	0.05	0.42
19	4	3	3	4	23.7	2.5	0.05	0.58
20	4	3	3	3	23.7	2.5	0.04	0.58

Obs.: observations; $X_1 = S^0$; X_2 = pH; $X_3 = MnSO_4$; $X_4 = CaCl_2$.

3. Results and discussion

3.1. The development of the semiempirical model

To quickly capture the simple ideas about how the semiempirical model for the bacterial growth of the indigenous *A. thiooxidans* was developed in our previous study [17], the derivation of the mathematical equations were summarized in this section. Based on the concept of transport phenomena, the growth rate of the indigenous *A. thiooxidans* can be considered as the product of driving force and transfer coefficient as follows:

$$\frac{dX_T}{dt} = (\text{transfer coefficient}) \times (\text{driving force}) \quad (3.1\text{-}1)$$

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. (3.1-1) 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. (3.1-1) becomes:

$$\frac{dX_T}{dt} = h_T X_T (K_T - X_T) \quad (3.1\text{-}2)$$

Eq. (3.1-2) describes the growth kinetics of the bacterial explicitly well, such that:

(1) When $X_T = 0$, then $dX_T/dt = 0$. It indicates that the growth rate equals 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:

$$\frac{dX_T}{dt} = h_T X_T (K_T - X_T) \cong h_T K_T X_T \quad (3.1\text{-}3)$$

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

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

$$\frac{d^2X_T}{dt^2} < 0 \quad \text{for } X_T > 0.5K_T \text{ and } \frac{dX}{dt} > 0 \quad (3.1\text{-}4)$$

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

phase), such as

$$\frac{dX_T}{dt} = h_T X_T(K_T - X_T) = 0 \quad \text{for } X_T = K_T \tag{3.1-5}$$

By integrating Eq. (3.1-2), an equation of total cell concentration versus time is obtained:

$$\ln\left(\frac{X_T}{K_T - X_T}\right) = K_T h_T t + C_T \tag{3.1-6}$$

$$X_T = \frac{K_T A_T \exp(B_T t)}{1 + A_T \exp(B_T t)} \tag{3.1-7}$$

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 comparing to X_L, thus X_T in Eq. (3.1-7) can be replaced by X_L as follows:

$$X_L = \frac{K_L A_L \exp(B_L t)}{1 + A_L \exp(B_L t)} \tag{3.1-8}$$

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.

The mean deviation (MD) was used to evaluate the semiempirical model and is defined as follows:

$$\text{MD} = \frac{1}{N}\sum_{1}^{N} |D_{EXP} - D_{EST}| \tag{3.1-9}$$

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

3.2. The effects of the three parameters K_L, B_L, and C_L on the semiempirical model

In this study, sensitivity analysis was conducted to evaluate the effects of the changes of the three parameters (K_L, B_L, and C_L) on the bacterial growth predicted by the semiempirical model. A set of experimental data (the cultivation of this microorganism using OGM) conducted in this study was input into the C^{++} program provided with this model and the values of K_L, B_L, and C_L were determined to be 0.55, 0.56, and −2.45, respectively. As shown in Fig. 1, the semiempirical model fits the experimental data explicitly well with MD = 0.0157. However, no information about the sensitivity of these three parameters on this model is available. In addition, no specific biological meanings of these three parameters can be obtained. Thus, it is desired to perform sensitivity analysis of these three parameters on the bacterial growth before further applications of this semiempirical model can be conducted.

The values of K_L, B_L, and C_L were increased and decreased with 10 and 20% from their respective central values obtained above (0.55, 0.56, and −2.45, respectively) to investigate their influences on the dry cell weight (DCW) during bacterial cultivation and the results are presented in Fig. 2. Such kind of analysis has been intensively adopted in the literatures [25–27]. As observed in Fig. 2a, the major effect of K_L on the growth of the indigenous *A. thiooxidans* is the maximum cell concentration, with the maximum cell concentration increased with increasing K_L. From Fig. 2b, we found that the main effect of B_L on the bacterial growth is the time required to achieve the maximum cell concentration, with the time required decreased with increasing B_L. In other words, B_L is positively correlated to the growth rate in the logarithm phase. Fig. 2c shows that the major effect of C_L on the bacterial growth occurs in the lag phase, with the length of the lag phase decreased with increasing C_L. Although the above observations provide valuable information about how these three parameters influence the bacterial growth at various stages, they do not reveal any information about the correlations between these three parameters and some important cultivation factors. Therefore, the use of the semiempirical model is still limited and difficult. In order to gain more insights into the correlations between these parameters and some important cultivation factors, Morris method was further performed to investigate the effects of the concentrations of elemental sulfur (S^0), $CaCl_2$, and $MnSO_4$, and the initial pH on the three parameters of the semiempirical model.

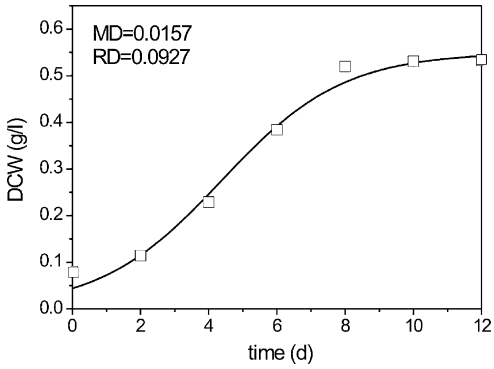

Fig. 1. Fitting of the experimental data (symbols) to the semiempirical model (solid line) during the bacterial growth of the indigenous *A. thiooxidans*. Mean deviation (MD) = 0.0157 and mean relative deviation (RD) = 0.0927 were obtained. The values of the three parameters K_L, B_L, and C_L were determined to be 0.55, 0.56, and −2.45, respectively.

3.3. Sensitivity analysis by Morris method

The experimental plan proposed by Morris is composed of individually randomized "one-factor-at-a-time" experiments, in which the impact of changing the value of each of the chosen factors is evaluated in turn [18]. The concentration of elemental sulfur (S^0) (X_1), the initial pH (X_2), and the concentrations of $CaCl_2$ (X_3) and $MnSO_4$ (X_4), considered as the four important cultivation factors for the design of the experiment based on Morris method, are summarized in Table 1. The results of this analysis indicate that these four cultivation factors have different effects on these three parameters of the semiempirical model, although not all influences are considered to be significant and crucial. Since the maximum cell concentration was obtained at

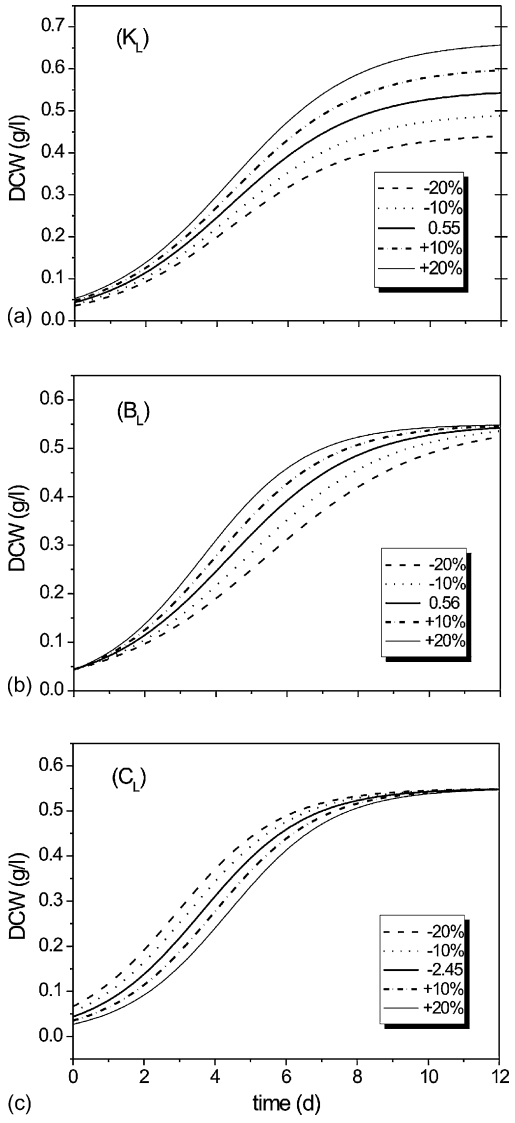

Fig. 2. Effects of the changes of: (a) K_L ($-20\% < K_L < +20\%$ with central value of $K_L = 0.55$); (b) B_L ($-20\% < B_L < +20\%$ with central value of $B_L = 0.56$); (c) C_L ($-20\% < C_L < +20\%$ with central value of $C_L = -2.45$) on the dried cell weight (DCW) predicted by the semiempirical model.

the 14th day of bacterial cultivation (data not shown) and the major effects of B_L and C_L on the DCW occur in the logarithm phase and the lag phase, respectively (described in Section 3.2), the substantially effects of these four factors on B_L and C_L can be neglected after the 14th day of cultivation. Therefore, we only focused on discussing the influences of these four factors on the parameter K_L.

In general, the meaning of the term "sensitivity analysis" depends greatly on the sensitivity measure that is used. Some of the sensitivity measures that are often employed in the sensitivity analysis of a mathematical model have been well described by McRae et al. [28]. In order to compare the relative importance of these four cultivation factors: $X_1 = S^0$, $X_2 = pH$, $X_3 = CaCl_2$, and $X_4 = MnSO_4$, we have introduced a summary sensitivity measure [18]: the Euclidean distance from the origin in the (μ, S) space, denoted by δ. Using this measure, a general "order of importance" can be established for the factors examined. The values of δ for each factor are given in Table 2. The mean and standard deviation for the effect of K_L associated with these four factors are displayed in Fig. 3. These values shown in Table 2 and Fig. 3 are examined relative to each other to see which factor appears to be the most important following the criterions: (1) a value of mean which is substantially different from zero indicates an input with an important overall influence on the output; (2) a high value of the standard deviation indicates an input with a nonlinear effect on the output or an input which has a significant influence on the output being involved in interaction with other factors. Despite the factor $X_4 = MnSO_4$, which has both mean and standard deviation nearly equal to zero, none of the other three factors X_1, X_2, and X_3 has both mean and standard deviation close to zero, indicating that K_L is indeed sensitive to these three factors. Considering both the mean and standard deviation together and using the sensitivity measure δ, we found that the factor $X_3 = CaCl_2$ is the most influential one, with $\delta = 44.19$. However, it has the mean value close to zero and a large value of standard deviation, indicating the potentially extensive patterns of interaction with other factors but no direct and important overall influence on K_L. Factors $X_1 = S^0$ and $X_2 = pH$ are the second and the third influential factors, with $\delta = 34.24$ and 24.49, respectively. They are the factors showing both direct and indirect effects on K_L. The results are in good agreement with the previous findings that both S^0 and pH are the most important factors in optimizing the composition of growth medium (i.e., $KH_2PO_4 = 3.5$ g/l, $(NH_4)_2SO_4 = 4.9$ g/l, $MgSO_4 = 0.7$ g/l, and elemental sulfur = 23.7 g/l for the optimum cell concentration = 0.7 g/l) [23] and the physical parameters (i.e., temperature = 31 °C, shaking rate = 114 rpm, pH = 3.98, and inoculum

Table 2
The values of the three sensitivity measures μ, S, and δ for each of the four cultivation factors examined

Cultivation factors	μ	S	δ
$X_1 = S^0$	23.40	24.99	34.24
$X_2 = pH$	20.00	14.14	24.49
$X_3 = CaCl_2$	0	44.19	44.19
$X_4 = MnSO_4$	0	0.707	0.707

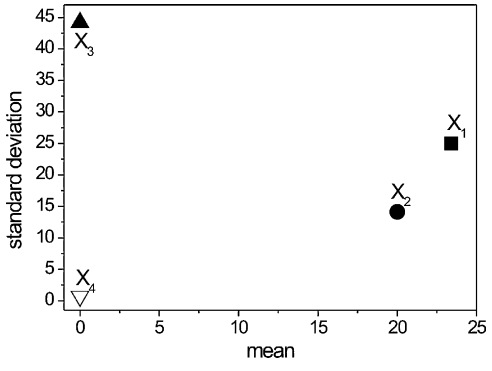

Fig. 3. Mean and standard deviation of the elementary effects for K_L associated with the four cultivation factors: $X_1 = S^0$, $X_2 = pH$, $X_3 = CaCl_2$, and $X_4 = MnSO_4$.

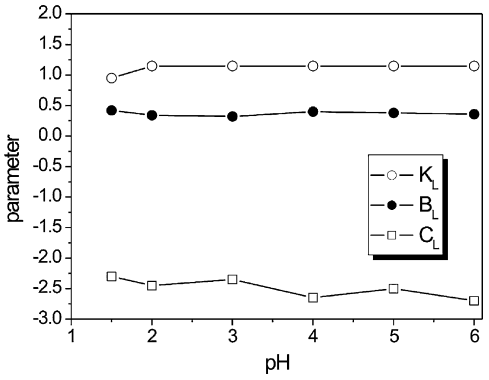

Fig. 4. The changes of the values of K_L, B_L, and C_L with respect to the change in the initial values of pH, keeping X_1 (S^0) = 23.7 g/l, X_3 ($CaCl_2$) = 0.03 g/l, and X_4 ($MnSO_4$) = 0.02 g/l as constants.

size = 9.6% for the optimum cell concentration of 0.773 g/l) [29], respectively, using RSM. Since factor $X_4 = MnSO_4$ has the mean and standard deviation values close to zero, its influence on K_L can be neglected. From the above sensitivity analysis by Morris method, it is obvious that only the concentration of S^0 and the initial pH all exhibit significant influences on K_L, which in turn exhibits a major effect on the maximum cell concentration of the indigenous *A. thiooxidans* predicted by the semiempirical model, as described in Section 3.2.

Although the concentration of $CaCl_2$ is the most influential factor towards K_L according to sensitivity analysis, its effect is to interact with other factors but has no important and direct overall influence on K_L. Thus, we only focused on discussing the effects of the concentration of elemental sulfur and the initial pH on K_L, B_L, and C_L in details herein. Fig. 4 shows that the values of K_L, B_L, and C_L remained almost unchanged towards different initial values of pH. It indicates that although pH has been shown to exhibit significant effect on K_L both directly and indirectly by the above sensitivity analysis based on Morris method, it indeed shows no obvious influence on these three parameters when the indigenous *A. thiooxidans* was cultivated using the optimized growth medium and the optimized physical parameters obtained by adopting RSM previously [23,29]. Thus, it can be concluded that pH shows significant effect on K_L only when the cultivation of this microorganism is not conducted in its optimized growth conditions. The effects of the concentration of elemental sulfur on K_L, B_L, and C_L are shown in Fig. 5a–c, respectively. Fig. 5a shows that K_L increases with increasing the concentration of elemental sulfur. Fig. 5b shows that B_L is negatively correlated to the concentration of elemental sulfur, particularly when it is less than 15 g/l. Fig. 5c shows that C_L is positively correlated to the concentration of elemental sulfur, similar to that of K_L. The above analyses all indicate that these three parameters are very sensitive to the concentration of elemental sulfur. Therefore, the correlations between the concentration of elemental sulfur towards K_L, B_L, and C_L were further expressed in Eqs. (3.3-1)–(3.3-3), respectively, as follows:

$$K_L = 0.48084 \times S^{0.22247} \quad (R^2 = 0.9652) \tag{3.3-1}$$

$$B_L = 0.51888 \times S^{-0.071998} \quad (R^2 = 0.85508) \tag{3.3-2}$$

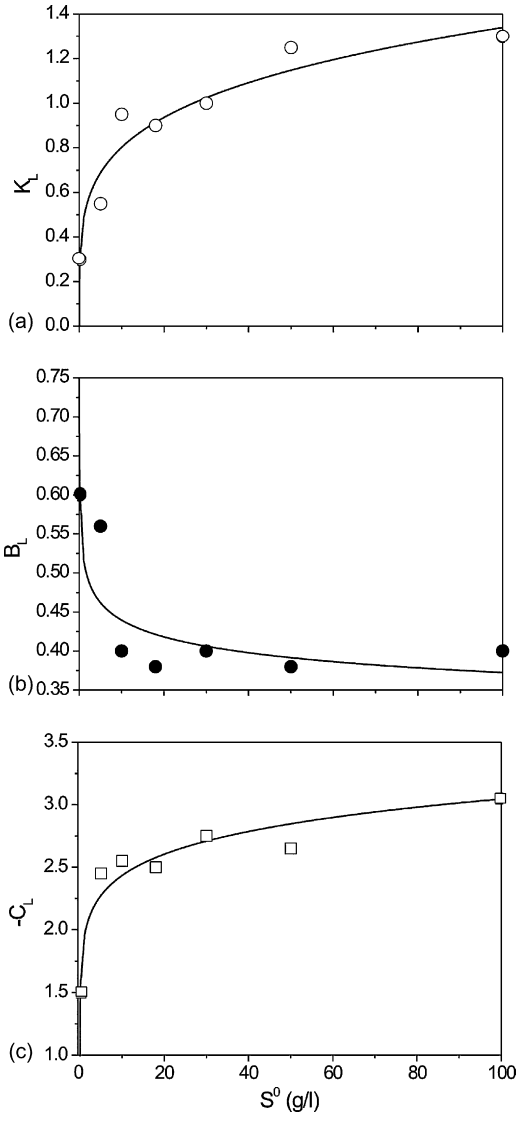

Fig. 5. The changes of the values of: (a) K_L, (b) B_L, and (c) C_L with respect to the change in the concentration of elemental sulfur (S^0), keeping X_2 (pH) = 4, X_3 ($CaCl_2$) = 0.03 g/l, and X_4 ($MnSO_4$) = 0.02 g/l as constants. The regression equations developed are: (a) $K_L = 0.48084 \times S^{0.22247}$ ($R^2 = 0.9652$), (b) $B_L = 0.51888 \times S^{-0.071998}$ ($R^2 = 0.85508$) and (c) $C_L = 1.945 \times S^{0.097353}$ ($R^2 = 0.96675$), where R^2 is the standard deviation. Please see Section 3.3 for detail.

$$-C_L = 1.945 \times S^{0.097353} \quad (R^2 = 0.96675) \tag{3.3-3}$$

where R^2 is the standard deviation. In order to test and verify the accuracies of the above regression equations, the optimal concentration of elemental sulfur = 23.7 g/l from our previous experiment using RSM [23] was used to calculate the values of K_L, B_L, and C_L, which are 0.97, 0.41, and −2.65, respectively. These values were further fed into the semiempirical model for predicting the bacterial growth of the indigenous *A. thiooxidans* and the results are shown in Fig. 6. The small value of MD = 0.0366 indicates that the semiempirical model fits the experimental data well when the values of these three parameters can be determined simply by using the three regression

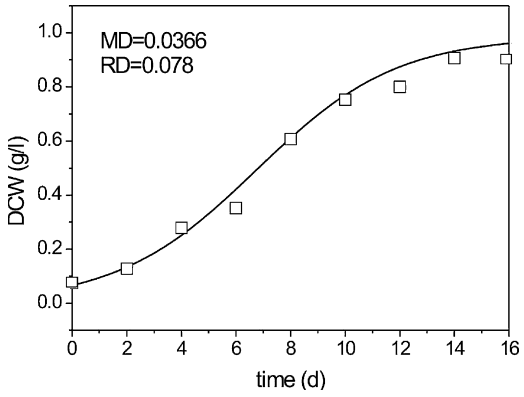

Fig. 6. The use of the semiempirical model to predict the bacterial growth with the concentration of elemental sulfur (S^0) being 23.7 g/l (obtained from the previous RSM experiments) [17]. The values of the three parameters K_L, B_L, and C_L were determined to be 0.97, 0.41, and −2.65, using the regression equations (3.3-1)–(3.3-3), respectively. MD = 0.0366 and RD = 0.078 were obtained after fitting the experimental data (symbols) to the semiempirical model (solid curve).

equations with the concentration of elemental sulfur as the sole information available.

4. Conclusions

In the present study, the experimental plan described by Morris [21] was applied to the previously developed semiempirical model for the growth of the indigenous *A. thiooxidans* [17]. The results of sensitivity analysis show that the three parameter, K_L, B_L, and C_L are closely related to the maximal cell concentration, growth rate in the exponential phase, and the residual time in the lag phase, respectively. Then, the model was further analyzed by Morris method and the results show that the concentration of $CaCl_2$ is the most influential factor, with $\delta = 44.19$. However, it has the mean value close to zero and a large value of standard deviation, indicating the potentially extensive patterns of interaction with other factors but no important overall influence on K_L. The concentration of elemental sulfur and the initial pH are the second and the third influential factors, with $\delta = 34.24$ and 24.49, respectively. They are the factors showing both direct and indirect effects on K_L. Since the concentration of elemental sulfur shows significant influences on these three parameters, three regression equations were further developed to predict the values of K_L, B_L, and C_L directly from the concentration of elemental sulfur. These parameters can be further fed into the semiempirical model for predicting the bacterial growth of the indigenous *A. thiooxidans* with high accuracy. In conclusion, sensitivity analysis based on Morris method is a powerful tool to observe the sensitivity of the three parameters (K_L, B_L, and C_L) on the bacterial growth and to determine the effects of some important cultivation factors (the concentrations of elemental sulfur, $CaCl_2$, $MnSO_4$, and the initial pH) on these parameters of the semiempirical model. With the sensitivity information available, further application of this semiempirical model for large-scale cultivation of the indigenous *A. thiooxidans* becomes possible and promising.

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