Article ID: 1003－6326(2004)03－0603－06

An impedance response model for inhibitory effect of antibiotics on bacterial growth in biometallurgy①

TAO Han(陶 菡), WEI Warr-zhi(魏万之), ZHANG Shu-fen(张书芬),
ZHANG Jing-zhong(张进忠), MAO You-an(毛友安)
(State Key Laboratory of Chemo/Biosensoring and Chemometrics,
College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, China)

Abstract: An impedance response model reflecting the inhibitory effect of antibiotics on bacterial growth in biometallurgy was established. Three inhibition parameters, i.e. the maximum amount inhibitory constant of the bacterial growth (K_1), the maximum specific growth inhibitory constant(K_2) and the lag time inhibitory constant(K_3), were included in the model. The influence of these parameters on the response curve was discussed in detail. By fitting experimental data towards the proposed model, three growth parameters (A, μ_m and λ) in the presence of antibiotics were gained and compared. The results show that the growth ability of bacteria is decreased due to the influence of antibiotics. The experimental and fitted curves have goodness-of-fit in the range of 0.987－1.008. Moreover, the kinetic growth parameters obtained from this model are closed to those from the Logistics popular growth model. These results show that the proposed model is validity to reflect the inhibitory effect of antibiotics on bacteria.

Key words: impedance response model; Logistics popular growth model; inhibitory parameters; *Staphylococcus aureus*; antibiotics

CLC number: TU 991.21 **Document code:** A

1 INTRODUCTION

There are abundant metal elements in the nature, such as Co, Mg, Zn, Mn and Fe. Through microbial metabolism, all of these metal elements can be utilized and transformed, thus their material circle in the nature can be realized[1]. Since 1970's, with the development of microbial technology, microorganism has been used widely in bacterial leaching, transforming of metal and absorbance of harmful metal(especially heavy metal) in polluted environmental[2-4] due to its transforming-metal ability.

Since penicillin was found in 1929, a large amount of antibiotics was produced and used frequently in clinics. One of its results is the accumulation of antibiotics pollutant in environments. It is well known that the effect of antibiotics on microorganism is mainly expressed as inhibition function[5]. So the existence of antibiotics pollutant will result in the decrease of the ability of microorganism in transforming and absorbance of metal in environment, and it can also affect the leaching efficiency of microorganism in bacterial leaching. So establishing a quantitative model to reflect the inhibitory effect of antibiotics on the bacteria maybe provide useful information for bacterial leaching and the transforming and absorbance of metal by bacteria in the presence of antibiotics pollutant. So far, many studies have reported the inhibitory effect of antibiotics on bacteria[6, 7]. However, we are not aware of any published studies that established quantitative model to reflect the inhibitory effect of antibiotics on the bacteria. This paper is aimed at performing this aspect work.

Piezoelectric quartz crystal (PQC) sensor can be characterized by active and passive methods[8]. In the passive method (PQCI), the quartz crystal is connected externally to an impedance analyzer which uses an alternating voltage at various frequencies across the terminal of the crystal. The impedance analysis can provide multidimensional information characterizing the behavior of PQC sensor in investigated system. The technique not only is a powerful tool for studying the qualitative process, but also can be applied to quantitative determination. PQCI has been successfully applied in many fields, including the determination of enzyme activity[9], study of actomyosin deploymerization[10] and rheumatid factor[11], monitoring of environmental wasterwater[12] and mutagenic process[13].

In this work, PQCI analysis technique was combined with the growth of microorganism for investi-

① **Foundation item:** Project (20020532007) supported by Doctorate Fund of the Education Ministry of China; project(03123) supported by Sci/Tech Research Project of Education Ministry of China

Received date: 2003－06－06; **Accepted date:** 2004－01－08

Correspondence: WEI Warr-zhi, Professor; Tel: +86-731-8824704; E-mail: weiwz2003@hotmail.com

gating the inhibitory effect of antibiotics pollutant on bacteria and a new impedance response model reflecting the inhibition effect quantitatively was derived. The bacteria *S. aureus* and antibiotic penicillin were selected to develop the model.

2 EXPERIMENTAL

2.1 Reagents

Penicillin was obtained from Sigma Chemical Corporation. All chemicals used were analytical reagent grade. Doubly distilled and sterilized water was used throughout the experiment. The culture medium for *S. aureus* was as follows: peptone, 5 g; glucose, 5 g; beef extract, 2 g; disodium hydrogen phosphate, 2 g; distilled water, 1 000 mL. The butter solution used was phosphate salt solution (pH= 7.2). The medium was sterilized by autoclaving at 121 ℃ for 15 min before use.

2.2 Materials and instrument

The AT-cut 9 MHz piezoelectric quartz crystals (12.5 mm in diameter) with a gold electrode (6 mm in diameter) on each side were purchased from National 707 Factory (Beijing, China). The gold-coated quartz crystal was sterilized by autoclaving at 121 ℃ for 15 min before use.

The experimental setup for impedance analysis is shown in Fig. 1. The system consists of a 4192A LF impedance analyzer, on which one side was connected to the terminal contacting liquid of PQC, and the other side was connected with a personal computer, in which a user program was written in Visual Basic 6.0 to control the analyzer and to acquire admittance data.

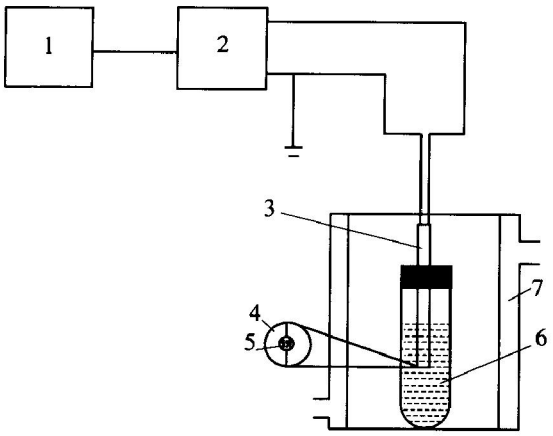

Fig. 1 Schematic diagram of experimental setup
1—Personal computer; 2—HP 4192A LF impedance analyzer; 3—PQC sensor; 4—Quartz crystal; 5—Gold electrode; 6—Detection cell; 7—Thermostatic water-jacket

2.3 Microorganism

S. aureus was obtained from College of Life Science of Hunan Normal University (Changsha, China). Four loops of *S. aureus* from an agar slant were inoculated into 100 mL sterilized conical vials containing 50 mL of sterilized culture medium, then the mixture was incubated for 16 h at 37 ℃, and preserved in the refrigerator at 4 ℃. The culture gives an approximate cell concentration of 5.1×10^7/mL by PPC method.

2.4 Procedures

The test solution was prepared by mixing 0.5 mL of bacterial solution, 5 mL of fresh culture medium and 30 μL of penicillin solution. Then the gold electrode was immersed. The detection cell was stuffed with a rubber plug and incubated at (37 ±0.1) ℃ with a thermostatic water-jacket. Then the variations of impedance parameters were monitored in a real time by HP 4192A impedance analyzer.

3 RESULTS AND DISCUSSION

3.1 Response theory of PQCI analysis

In PQCI, Muramatsu et al[14] described the relationship between the motional resistance (R_1) and viscosity-density of the liquid:

$$R_1=\frac{(2\pi f_0\rho_L\eta_L)^{1/2}A}{\kappa^2} \quad (1)$$

where κ represents the electromechanical coupling factor, ρ_L and η_L are the viscosity and density of the liquid, respectively.

Because the growth of bacteria leads to the viscosity and density variations of the test solution, the impedance model was established by examining the variation of the motional resistance (ΔR_1) in this paper.

3.2 Typical ΔR_1 response curve of bacterial growth in the absence or presence of antibiotics

In Fig. 2, line 1 shows the response curve of ΔR_1 that indicates the growth situation of *S. aureus* in normal case. It can be seen that ΔR_1 almost does not change during the initial 5 h and the first plateau is formed. Then, ΔR_1 increases continuously for about 11 h, and then reaches a stable level to form the second plateau. The above response curve can be explained by the bacterial growth theory. Since bacterial growth lies in the lag phase in the initial time, the growth of bacteria is very slow and the viscosity-density of solution nearly does not change. Hence ΔR_1 nearly does not change and the first plateau appears. When the growth of *S. aureus* enters logarithm growth period, the bacterial number increases rapidly, leading to a quick increase in viscosity-density of the solution, which produces an increase of ΔR_1. Since the limitation of nutrients and other fac-

tors, the growth of *S. aureus* reaches the saturation phase and the bacteria grows slowly or even does not grow any more. As a result, ΔR_1 nearly does not change again and the second plateau appears.

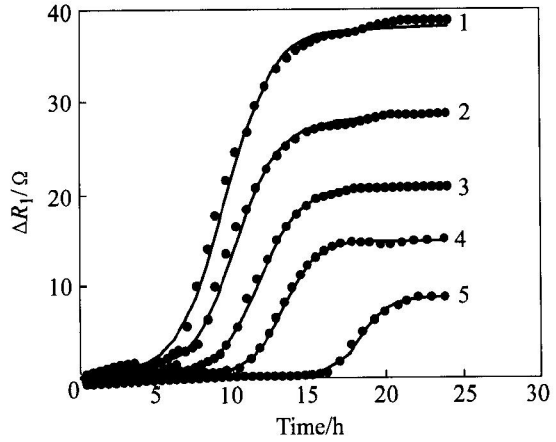

Fig. 2 Time courses of ΔR_1 values experimentally obtained and fitted in the presence of different concentration of antibiotics
(The lines represent the fitted results and the symbols represent the experimental results;
The concentration of penicillin in solution(μg/mL):
1—0; 2—0.05; 3—0.10; 4—0.15; 5—0.25)

The shape of ΔR_1 is a sigmoid curve just like the theoretical growth curve of bacteria described by Logistics model. The first plateau phase corresponds to the lag phase of the growth of bacteria, the quickly increasing phase corresponds to the exponential growth phase and the second plateau phase corresponds to the bacterial growth saturation phase.

In Fig. 2, lines 2 – 5 show the response curves of ΔR_1 in the presence of different concentration of antibiotics. Compared with line 1 in Fig. 2, lines 2 – 5 display the similar change trends. All of them are sigmoid curves and can be divided into three-change phase, i. e. the first plateau phase, the quickly increasing phase and the second plateau phase. However, we can also see the difference between them from the following aspects. Firstly, the time that the first plateau lasted is different. In the presence of antibiotic, the lasting time is longer than that in normal case. Secondly, the signal size of ΔR_1 between the two plateaus is different. ΔR_1 is about 38 Ω in normal growth case, but in the presence of 0.25 μg/mL penicillin, ΔR_1 is only about 9 Ω.

Since the ΔR_1 response curve corresponds to the theoretical bacterial growth curve, the above differences indicate that the lag time is prolonged; the maximum number of bacteria is reduced due to the effect of antibiotic. The effect is more serious with the increase of penicillin concentration.

3.3 Establishment of impedance response model

A typical bacterial growth curve is well described by Logistics popular growth model[15]:

$$\ln\frac{N}{N_0}=\frac{A}{1+\exp\left|\frac{4\mu_m}{A}(\lambda-t)+2\right|} \tag{2}$$

where the asymptote A is the relative maximum value of $\ln(N/N_0)$; the maximum specific growth rate μ_m is defined as the tangent in the inflection point; the lag time λ is defined as the x-axis intercept of this tangent. Each of them has its specific biological meaning and changes with the change of the growth situation of microorganism.

In order to derive the inhibition model, we modified all parameters (A, μ_m, λ) in the Logistics model.

A can be modified as

$$A = A_0\exp(-K_1C) \tag{3}$$

where A_0 is the asymptote without antibiotics; C is the concentration of antibiotic, K_1 represents the inhibition effect of unit concentration antibiotic on A with dimension of C^{-1} and is defined as the maximum amount inhibitory constant. It can be seen that A is equal to A_0 when no antibiotics are present. When the antibiotics concentration is very large, A approaches zero.

Similarly, μ_m and λ can be modified as

$$\mu_m = \mu_{m_0}\exp(-K_2C) \tag{4}$$

$$\lambda = \lambda_0\exp(K_3C) \tag{5}$$

where μ_{m_0} and λ_0 are the maximum specific growth rate and the lag time in the absence of antibiotics, respectively; K_2 represents the inhibition effect of unit concentration antibiotics on μ_m and is defined as the maximum specific growth inhibitory constant; K_3 represents the inhibition effect of unit concentration antibiotics on λ and is defined as the lag time inhibitory constant. K_1, K_2, K_3 have the dimension of C^{-1}.

According to the bacterial growth model, the inhibition growth model can be defined as

$$\ln\frac{N}{N_0}=\frac{A_0\exp(-K_1C)}{1+\exp\left|\frac{4\mu_{m_0}\exp(-K_2C)\cdot(\lambda_0\exp(K_3C)-t)}{A_0\exp(-K_1C)}+2\right|} \tag{6}$$

The change of motional resistance (ΔR_1) and the number of bacteria were determined in order to establish the relationship between them. Experiments show that a linear relationship exists between ΔR_1 and the logarithm of the relative population size,

$$\Delta R_1 = k\ln\frac{N}{N_0} \tag{7}$$

where k is a coefficient. In this study, we detected the relative number of bacteria using the PPC method and obtained the value of k as 4.58 Ω.

From Eqn. (6) and Eqn. (7), a new impedance

response model which reflects the inhibitory effect of antibiotics on bacterial growth can be obtained as

$$\Delta R_1 = 4.58 \times \frac{A_0 \exp(-K_1 C)}{1+\exp\left[\dfrac{4\mu_{m_0}\exp(-K_2 C)\cdot(\lambda_0 \exp(K_3 C)-t)}{A_0\exp(-K_1 C)}+2\right]} \quad (8)$$

Taking K_1, K_2 and K_3 as estimation parameters, the fitted ones of ΔR_1 and the growth kinetic parameters (A, μ_m and λ) can be obtained by fitting the experimentally obtained values of ΔR_1 in Fig. 2 to the derived model. The results are shown in Table 1. Compared with the growth in the absence of antibiotics, the growth in the presence of antibiotics yields lower values of A, μ_m and longer λ. That is to say, the bacterial growth ability is inhibited due to the effect of antibiotics.

In order to assess the adequacy of the fitted results, the F-statistical test was used as the criterion:

$$F^2 = \frac{\sum_1^n (\Delta R_{fit} - \overline{\Delta R}_{exp})^2}{\sum_1^n (\Delta R_{exp} - \overline{\Delta R}_{exp})^2} \quad (10)$$

where $\overline{\Delta R}_{exp} = \frac{1}{n}\sum_1^n \Delta R_{exp}$; F is the goodness-of-fit.

3.4 Sensitivity of parameters to response curve

In order to examine the influence of the inhibitory parameters(K_1, K_2, K_3) on the $\Delta R_1 - t$ curve, the curves with different parameter values were obtained and discussed.

The effect of K_1 on the $\Delta R_1 - t$ curves is shown in Fig. 3. It can be seen that K_1 obviously influences the height of curves. ΔR_1 varies increasingly with K_1 decreasing. At the same time, since the denominator of Eqn. (6) also contains K_1 and the other growth parameters (μ_m and λ) are also affected by K_1, these lead to the difference at the initial part of the curves. However, K_1 mainly affects the growth parameters A.

The influence of K_2 on the $\Delta R_1 - t$ curves is shown in Fig. 4. It can be seen that the slope of curve becomes steeper and the saturation occurs sooner with smaller K_2. It can be explained as follows: since μ_m is the maximum specific growth rate and defined as the tangent in the inflection point, according to Eqn. (4), a smaller K_2 leads to a larger μ_m, thus the initial slope of the curve becomes steeper. As a result, the growth of the population is more rapid and reaches the population saturation more quickly.

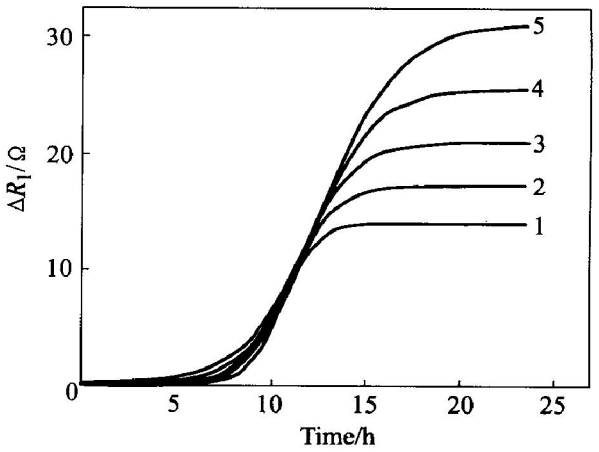

Fig. 3 Effect of K_1 on response curve
(A_0= 8.270 7; μ_{m_0}= 1.286 2; λ_0= 6.260 1, K_2= 4.1; K_3= 3.4; C= 0.1 μg/mL)
1—K_1= 10; 2—K_1= 8; 3—K_1= 6; 4—K_1= 4; 5—K_1= 2

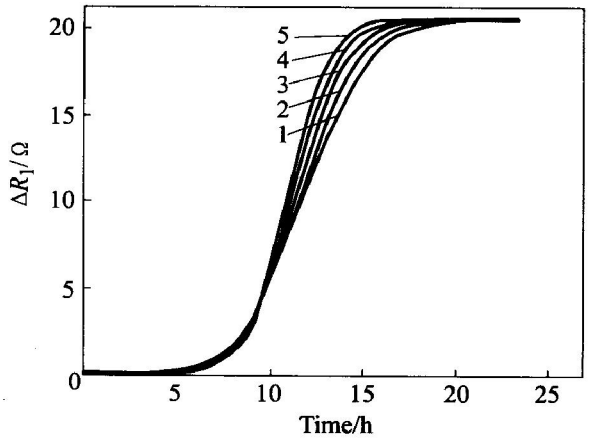

Fig. 4 Effect of K_2 on response curve
(A_0= 8.270 7; μ_{m_0}= 1.286 2; λ_0= 6.260 1, K_1= 6.1; K_3= 3.4; C= 0.1 μg/mL)
1—K_2= 6; 2—K_2= 5; 3—K_2= 4; 4—K_2= 3; 5—K_2= 2

Table 1 Parameters obtained by fitting responses of ΔR_1 in Fig. 2 according to Eqn. (8)

C/(μg·mL^{-1})	A	μ_m/h^{-1}	λ/h	K_1/(mL·μg^{-1})	K_2/(mL·μg^{-1})	K_3/(mL·μg^{-1})	F
0	8.271	1.286	6.260				0.994
0.05	6.104	1.036	7.108	6.074	4.335	2.541	0.998
0.10	4.460	0.837	8.969	6.177	4.298	3.596	0.995
0.15	3.182	0.725	10.969	6.368	3.823	3.739	1.008
0.25	1.857	0.494	16.410	5.974	3.831	3.855	0.987

It can be noted from Fig. 5 that K_3 influences the lag time of bacterial growth. As K_3 increases, it takes longer time for the culture to enter exponential phase. Since saturation values do not vary, the curves overlap after exponential phase is completed.

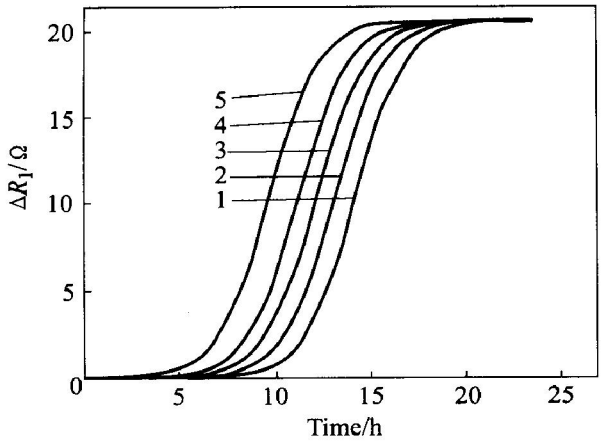

Fig. 5 Effect of K_3 on response curve
(A_0= 8.2707; μ_{m_0}= 1.286 2; λ_0= 6.260 1;
K_1= 6.1; K_2= 4.1; C= 0.1 μg/mL)
1—K_3= 10; 2—K_3= 5; 3—K_3= 3;
4—K_3= 2; 5—K_3= 1

3.5 Verifying validity of model

Fig. 2 shows $\Delta R_1 - t$ curves derived from experimentally obtained values and fitted values toward the proposed impedance response model. It can be seen that $\Delta R_1 - t$ curves derived from the fitted results are very close to the experimental results, with a goodness-of-fit of 0.987 ~ 1.008. These results show that the impedance response model can reflect the inhibitory effect of antibiotics on bacteria availably. Of course, one can obtain different values of K_1, K_2, K_3 when this model is applied to different study systems.

To check the validity of the proposed model further , the bacterial growth parameters obtained from the proposed model were compared with those from Logistic popular growth model. The fitting and experimental curves of the bacterial relative concentration in the absence or presence of different concentration penicillin are shown in Fig. 6. The fitting results of the three growth parameters obtained from two models are shown in Table 2. The results show that the bacterial growth parameters obtained from Logistic popular growth model are close to those obtained from the proposed model, which indicates the correctness of the proposed model again and shows that PQCI can be used to monitor the bacterial inhibitory growth process in real time.

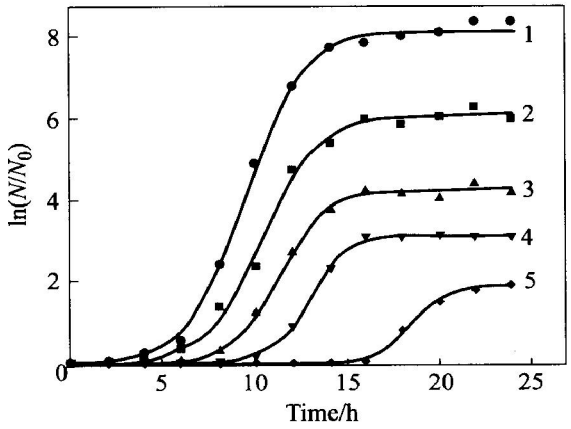

Fig. 6 Time courses of $\ln(N/N_0)$ values fitted and experimentally obtained at different concentration of antibiotics
(The lines represent the fitted results and the symbols is the experimental results;
The concentration of penicillin in solution (μg/mL):
1—0; 2—0.05; 3—0.10; 4—0.15; 5—0.25)

4 CONCLUSIONS

PQCI analysis technique has been adapted successfully to study the inhibitory effect of antibiotics pollutant on bacteria. By connecting the variation of motional resistance (ΔR_1) with Logistics population growth model, a new impedance response model was derived for the first time, which can reflect the in-

Table 2 Bacterial growth parameters obtained from two models

C/(μg·mL^{-1})	A		μ_m		λ	
	Proposed	Logistics	Proposed	Logistics	Proposed	Logistics
0	8.271	8.104	1.286	1.275	6.260	6.299
0.05	6.104	6.051	1.036	0.961	7.108	7.191
0.10	4.460	4.174	0.837	0.818	8.969	8.647
0.15	3.182	3.098	0.725	0.753	10.969	10.747
0.25	1.857	1.882	0.494	0.456	16.410	16.292

hibitory effect of antibiotics on the bacteria and can be used to estimate the inhibitory parameters under different concentration of antibiotics pollutant. It maybe provides useful information for bacterial leaching and the transferring and absorbance of metal by bacteria in environment in the presence of antibiotics.

REFERENCES

[1] ZHOU De-qing. Microbiology[M]. Beijing: Higher Education Press, 2000.

[2] Texier A C, Anders Y, Cloirec P L. Selective biosorption of lanthanide (La, Eu, Yb) ions by *pseudomonas aeruginosa*[J]. Environ Sci Technol, 1999, 33: 489 - 495.

[3] Wang L, Chua H, Zhou Q. Role of cell surface components on Cu^{2+} adsorption by *Pseudomonas putida* 5-x isolated from electroplating effluent [J]. Water Res, 2003, 37: 561 - 568.

[4] Patil Y B, Paknikar K M. Removal and recovery of metal cyanides using a combination of biosorption and biodegradation processes[J]. Biotechnol Lett, 1999, 10: 913 - 919.

[5] SHEN Tong, WANG Jing-yan. Biochemistry[M]. Beijing: Higher Education Press, 1999.

[6] Fabre H, Kok W T. Detection of cephalosporins and decomposition products by liquid chromatography with indirect electrochemical detection[J]. Anal Chem, 1988, 60: 136 - 141.

[7] TAN H W, WANG R H, WANG S H, et al. Bulk acoustic biological detection of cefotaxime sodium [J]. Anal Lett, 1998, 31: 949 - 961.

[8] Tompson M, Kipling A L, Duncan-Hewitt W C. Thickness-shear-mode acoustic wave sensors in the liquid phase [J]. Analyst, 1991, 116: 881 - 890.

[9] Saum A G E, Cumming R H, Rowell F J. Use of substrate coated electrodes and AC impedance spectroscopy for the detection of enzyme activity[J]. Biosens Bioelectron, 1998, 13: 511 - 518.

[10] Kurorsawa S, Nemoto E, Muratsugu M, et al. Detection of actomyosin depolyerization with a piezoelectric quartz crystal[J]. Anal Chim Acta, 1994, 289: 307 - 313.

[11] Ghourchian H O, Kamo N, Hosokawa T, et al. Improvement of latex piezoelectric immunossay detection of rheumatoid factor[J]. Talanta, 1994, 41: 401 - 406.

[12] ZHANG S F, WEI W Z, ZHANG J Z, et al. Monitoring environmental wastewater using a piezoelectric impedance microbial sensing technique[J]. Intern J Environ Anal Chem, 2002, 82: 113.

[13] ZHANG J Z, WEI W Z, ZHOU A H, et al. Monitoring of mutagenic process with piezoelectric quartz crystal impedance analysis[J]. Talanta, 2000, 53: 525 - 533.

[14] Muramatsu H, Tamiya E, Karube I. Computation of equivalent circuit parameters of quartz crystal in contact with liquids and study of liquid properties[J]. Anal Chem, 1988, 60: 2142 - 2146.

[15] Zwietering M H, Jongenburger I, Rombouts F M, et al. Modeling of the bacterial growth curve[J]. Appl Environ Microbiol, 1990, 56: 1875 - 1881.

(Edited by YUAN Sai-qian)