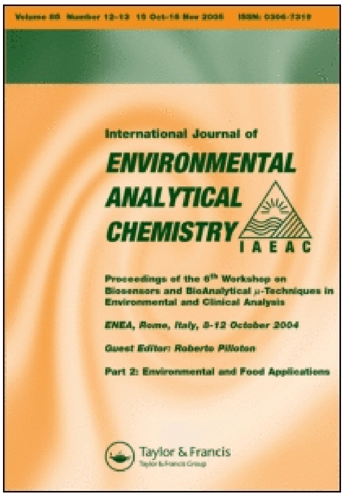

International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Monitoring Environmental WasteWater Using A Piezoelectric Impedance Microbial Sensing Technique

Shufen Zhang[a]; Wanzhi Wei[a]; Jinzhong Zhang[a]; Youan Mao[a]; Le Deng[a]
[a] College of Chemistry and Chemical Engineering, Hunan University, Changsha, P.R. China

Online publication date: 17 September 2010

Intern. J. Environ. Anal. Chem., Vol. 82, No. 3, pp. 113–122

MONITORING ENVIRONMENTAL WASTEWATER USING A PIEZOELECTRIC IMPEDANCE MICROBIAL SENSING TECHNIQUE

SHUFEN ZHANG, WANZHI WEI*, JINZHONG ZHANG, YOUAN MAO and LE DENG†

College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, P.R. China

(Received 27 March 2001; In final form 15 October 2001)

A new method was proposed for monitoring antibiotics and other pollutants based on the assessment of the growth of *Staphylococcus aureus* (*S. aureus*) with a piezoelectric impedance analysis technique. During the growth process of *S. aureus*, the motional resistance variation (ΔR_1) increases and the frequency shift (Δf) of the piezoelectric quartz crystal (PQC) decreases correspondingly. The two responses can conveniently be used in process analysis. Under the given experimental conditions, a linear relationship between the resistance detection time (RDT) and logarithm value of the concentration of penicillin was obtained in the range of 0.01–10 μg/ml with a detection limit of 4×10^{-3} μg/ml. Other antibiotics and pollutants also have the similar linear relationships. The proposed method is simple, convenient and sensitive. Particularly, it does not need the immobilization of the microorganism. This method was used for monitoring of wastewater from a hospital.

Keywords: Antibiotics; Inhibition process; Piezoelectric impedance analysis; Wastewater; Environmental monitoring

INTRODUCTION

Water pollution makes environmental quality worse all over the world[1], so it is important to monitor the sources of pollution. To date, many methods have been developed for monitoring wastewater, such as polymerase chain reaction (PCR) method[2], integral sorption methods[3], piezoelectric quartz crystal (PQC) sensors[4] and microbial sensor methods[5,6]. Biological oxygen demand (BOD) is an important index in environmental monitoring which can well reflect the polluted extent of water by common organics. BOD concerns with biochemical process of microorganisms and it presents the total polluted level. However, when the monitored water sample contains certain amount of antibiotics, the growth of microorganisms is inhibited. The dissolved oxygen consumed by bacteria is lower than that having no antibiotics. In this case, the BOD method cannot demonstrate the pollution extent accurately. In this paper we propose a new method to monitor the above wastewaters based on the inhibition of pollutants to a microorganism.

*Corresponding author. Fax: +86-731-8824525. E-mail: zhangshufen@cmmail.com
†Present address. Currently working in Hunan Normal University, Changsha, China

ISSN 0306-7319 print: ISSN 0000-000X online/01/010000-00
DOI: 0000-0000.00.000000

Piezoelectric impedance analysis is a practical method for studying the quartz crystal resonance and provides multidimensional information reflecting some physical and chemical properties of the investigated system[7,8]. The equivalent circuit model of the PQC sensor was described by Buttry and Ward[9]. It is composed of motional and static arms in parallel. The motional arm contains three equivalent circuit elements in series, the motional resistance (R_1), motional inductance (L_l) and motional capacitance (C_1) while the static arm only contains static capacitance (C_0) and all the four equivalent circuit parameters have distinct physical meanings[7–9]. In this paper, we only discuss the variations of R_1 and C_0. The motional resistance R_1 corresponds to the loss in mechanical energy dissipated to the surrounding medium and the supporting structures. The static capacitance C_0 originates from the two parallel plate metal electrodes on the quartz surface and stray capacity to the supporting structure. R_1 is related to the variation caused by the piezoelectric effect, while C_0 is not related to it. The piezoelectric impedance analysis technique has been successfully applied to many fields, including the determination of α-amylase[10], rheumatiod factor[11], and actomyosin depolymerization[12], monitoring of blood clotting process[13] and mutagenic process[14].

In this work, this technique was combined with the growth of a microorganism for monitoring the antibiotics and other pollutants in wastewater. The method was based on the fact that the growth of *Staphylococcus aureus* was inhibited in the presence of penicillin which is a typical inhibitor to *S. aureus*, and the physical and chemical properties of culture system would change accordingly. This causes the variations of equivalent circuit parameters of PQC. The changing process of impedance parameters could reflect the inhibition process. So antibiotics detection in wastewater could be performed by piezoelectric impedance analysis.

EXPERIMENTAL

Reagents

All chemicals used were of analytical reagent grade. Doubly distilled and sterilized water was used throughout. The composition of the culture medium for *S. aureus* was as follows: glucose, 5 g; bovine extract, 2 g; disodium hydrogen phosphate, 2 g; distilled water, 1000 ml. The pH value of the medium was adjusted to 7.4 by phosphate buffer solution. The medium was dispensed into 200 ml amounts in conical vials and sterilized by autoclaving at 121°C for 30 min. All antibiotics were purchased from the hospital of Hunan University (Changsha, China).

Materials and instrumentation

AT-cut 9 MHz PQC (12.5 mm in diameter) was purchased from state-run 707 factory (Beijing, China). The gold electrodes of the crystal which have surface areas about 0.3 cm^2 were vacuum-evaporated by using an Eiko IB-3 ion coater. The gold-coated quartz crystals were sealed with 704 silicon rubber to get one side of the crystal in contact with liquid. To insure the clarity of the gold-coated electrode, the gold electrode surfaces were first treated with $H_2SO_4 + H_2O_2$ (V/V 3 : 1) for 5 min, then cleaned with water and acetone, sequently. Before use, the electrodes were sterilized by autoclaving at 121°C for 30 min.

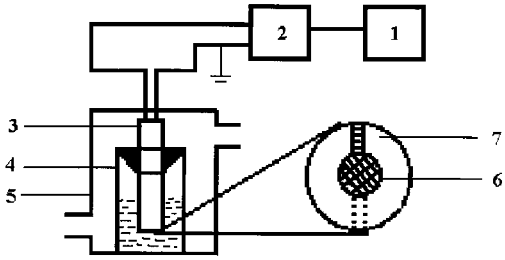

FIGURE 1 Schematic diagram of the piezoelectric impedance analysis system. (1) Personal computer; (2) HP 4192A LF Impedance analyzer; (3) PQC sensor; (4) Detection cell; (5) Thermostatic water-jacket; (6) Gold electrode; (7) Quartz crystal.

The experimental setup for piezoelectric impedance analysis is shown in Fig. 1. The system comprises of a 4192A LF Impedance Analyzer which was one side connected to the terminal contacting liquid of PQC, and the other side was connected to a personal computer in which a user program was written in Visual Basic 5.0 to control the analyzer. The equivalent circuit parameters were obtained at a time interval of ca. 3 min, and displayed on the Visual Basic form in the experiments.

Microorganism

S. aureus was obtained from College of Life Science of Hunan Normal University (Changsha, China). Four loops of *S. aureus* on agar slant were inoculated into 100 ml sterilized conical vials which contained 50 ml sterilized culture medium. It was incubated for 16 h at 37°C, and preserved in a 4°C refrigerator, the culture gives an approximate concentration of 1.57×10^6 cells/ml by pour plate count (PPC) method.

Procedures

The experimental solution was prepared by mixing 2 ml of culture medium, 2 ml of bacterial solution and 40 μl of penicillin solution. Then the gold electrode was immersed. The detection cell was stuffed with a rubber plug and placed to incubate at 37 ± 0.1°C in the thermostatic water-jacket. Then the variations of the impedance parameters were real-time monitored by HP 4192A LF Impedance Analyzer.

For a comparison, after finishing the impedance measurement, the final bacteria number was detected by PPC method.

RESULTS AND DISCUSSION

Response theory of the piezoelectric impedance analysis

In PQC analysis, there is a linear relationship between the change (Δm) and the frequency shift (Δf)[15]:

$$\Delta f_m = -\frac{2f_0^2}{(\rho_Q \mu_Q)^{1/2}} \frac{\Delta m}{A} \quad (1)$$

Where ρ_Q and μ_Q are the density and the shear modulus of quartz, respectively. A is the area of the quartz plate.

In addition, the physical and chemical properties of liquid may also affect the frequency of PQC. The relationship between them can be described as[16]:

$$\Delta f_L = -\frac{f_0^{3/2}(\rho_L \eta_L)^{1/2}}{\pi(\rho_Q \mu_Q)^{1/2}} \tag{2}$$

where ρ_L and η_L are the viscosity and density of the liquid, respectively.

Thus, the total frequency shift (Δf_t) should be the sum of Δf_m and Δf_L

$$\Delta f_t = \Delta f_m + \Delta f_L \tag{3}$$

In piezoelectric impedance analysis, Muramatsu *et al.*[7] described the relationship between the motional resistance (R_1) and viscosity–density of the liquid.

$$R_1 = \frac{(2\pi f_0 \rho_L \eta_L)^{1/2} A}{\kappa^2} \tag{4}$$

where κ represents the electromechanical coupling factor.

If Δf_L is dominated by the net changes in the viscosity and density of liquid, as for the presently used 9 MHz crystal, the ratio of Δf_L and ΔR_1 should be 10.1 Hz Ω^{-1}[17]. In fact, $\Delta f_L / \Delta R_1$ is often greater than 10.1, because measured value of Δf may contain the contributions of both mass and viscosity–density factor. In this paper, this criterion was used to judge the variations of the viscosity and density of the culture system during the inhibition process.

Typical response curves of Δf, ΔR_1 and ΔC_0 during the normal process

The typical response curves of piezoelectric impedance parameters including Δf, ΔR_1 and ΔC_0 during the normal growth of *S. aureus* are shown in Fig. 3. It can be seen that ΔR_1 shows a constant value in the initial 1.6 h, and the first plateau appears in the ΔR_1 versus time curves, then the ΔR_1 increases greatly, finally reaches a stable level and the second plateau appears. We defined the time at which the first plateau appeared in ΔR_{1-t} curve as resistance detection time (RDT), and it related to the amount of bacteria added and the detection limit of the equipment. Compared with that of ΔR_1, Δf shows a reverse changing trend. As shown in Eqs. (2) and (4), changes in the two parameters indicate viscosity and density variations of culture system during the normal growth. It can be seen that the shape of ΔR_1 curve resembled the theoretical growth curve of bacterium, and the changing situation of ΔR_1 was different during three growth phases of the bacterium.

From Fig. 2, a decrease of ca. 1180 Hz in Δf and increase of ca. 38 Ω in ΔR_1 between the two plateaus were obtained. The slope of Δf versus ΔR_1 was calculated as ca. 31.05.Hz Ω^{-1}, this value is far more than 10.1. This fact indicates that the contributions to Δf resulted not only from an increase in viscosity and density, but also from other factors, e.g. the adsorption of the culture components onto the gold electrode. However, ΔR_1 can well reflect the changes in viscosity and density of culture system according to Eq. (4).

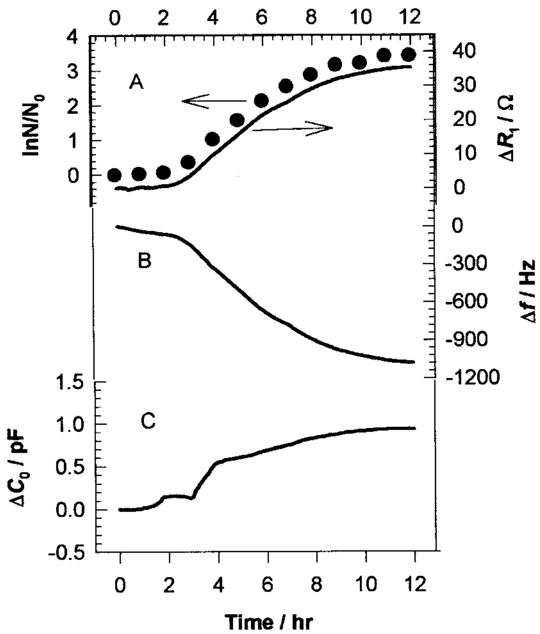

FIGURE 2 Time courses of simultaneous responses of ΔR_1, Δf and ΔC_0 during the normal growth of *S. aureus*. (A) Typical response curve ΔR_1(–); Change in bacteria concentration with time which was obtained with the PPC method (●); (B) Typical response curve of Δf; (C) Typical response curve of ΔC_0. The volume of the bacteria solution is 2 ml, and the volume of the medium is 2 ml.

It can also be found from Fig. 2 that ΔC_0 increased ca. 0.9 pF. This result indicates that dielectric constant of the crystal increased during the normal growth of the bacterium.

Typical response curves of Δf, ΔR_1 and ΔC_0 during the inhibition process

The typical response curves of piezoelectric impedance parameters, including Δf, ΔR_1 and ΔC_0 during the inhibition process are shown in Fig. 3. It can be seen that RDT is 4 h which is larger than that at the normal growth situation. ΔR_1 also has a great increasing phase and a second plateau. As shown in Fig. 3, a decrease of ca. 600 Hz in Δf and increase of ca. 16 Ω in ΔR_1 from 4 to 12 h were obtained. The slope of Δf versus ΔR_1 was calculated, as 37.5 Hz Ω^{-1}.

Comparing with Fig. 2, the results of Fig. 3 show the similar changing trend of the two parameters. But we could investigate the difference between the two figures from two aspects through the ΔR_1 and Δf curves. Firstly, the time that the first plateau keeps is different. The lasting time which is similar to the lag time in Gompertz population growth model[17] is longer than that in the normal growth case, because the bacteria will decrease to a minimum value after the addition of penicillin, and then the surviving bacteria begins to recover their growth again. In this case, it prolongs the time at which *S. aureus* gets into exponential phase and the variations of impedance parameters need a long time to be detected by impedance analyzer. Secondly, the signal size of ΔR_1 response is different, and ΔR_1 value which is similar to asymptote A in Gompertz model in Fig. 2 was greater than that in Fig. 3, because a part of *S. aureus* died or

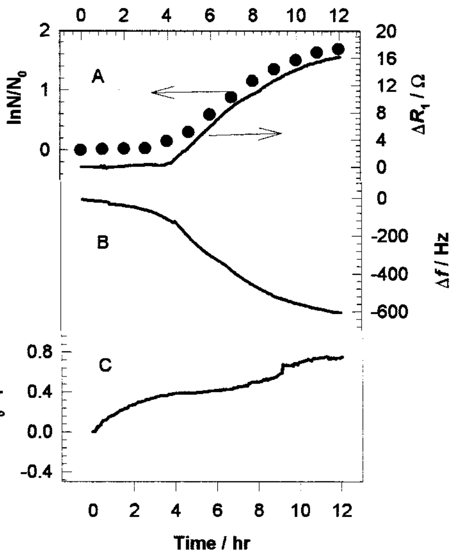

FIGURE 3 Time courses of simultaneous response of ΔR_1, Δf and ΔC_0 during the growth of the inhibitory process; (A) Typical response curve ΔR_1 (–); Change in bacteria concentration with time, which was obtained with the PPC method (●); (B) Typical response curve of Δf.; (C) Typical response curve of ΔC_0. The volume of the bacteria solution is 2 ml, and the volume of the medium is 2 ml, the volume of the penicillin (10 μg/ml) added is 40 μl.

lead to deformation and could not propagate after addition of penicillin. There are little amount of bacteria that get into the exponential phase and lead to a small change of viscosity–density in the culture system. The antibiotics have the same effect on Δf.

The tangent of the response curves in the great increase phase which is similar to the maximum specific growth rate μ_m in Gompertz model has no obvious discrimination in both cases. It is that penicillin mainly affects A and λ and has a little effect on μ_m. In addition, the ratio of Δf and ΔR_1 is more than 10.1. This result indicates that a part of the frequency shift is not caused by viscosity–density in both the cases. We obtained these differences through multiple experiments.

From Fig. 3, it can also be found that C_0 increases during the whole process.

The effect of penicillin concentration on RDT during inhibition process

Under the same experimental conditions, different amounts of penicillin were added into the culture medium and resistance response curves were obtained (as shown in Fig. 4). In the inhibition case, the RDT value is related to the amount of penicillin added except for the amount of bacteria and detection limit of equipment. The difference values of RDT versus the logarithm values of the concentration of penicillin were drawn in Fig. 4 and the calibration curve was acquired, as shown in Fig. 5. It can be found that the different values of RDT are proportional to the logarithm values of the concentration of penicillin. The results illustrate that the greater RDT values can be obtained when the amount of penicillin is higher. When the concentration of penicillin is in the range 0.01–10 μg/ml, there is a linear relationship between the difference

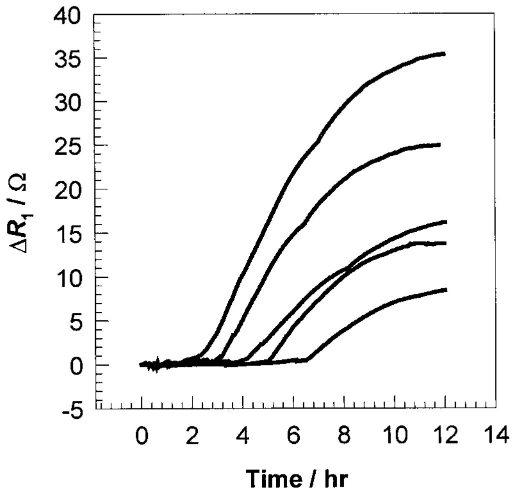

FIGURE 4 Frequency response curves of the growth of *S. aureus* in the presence of different concentrations of penicillin. The concentrations of penicillin in solution (μg/ml): (1) 0, (2) 0.01, (3) 0.1, (4) 1, (5) 10.

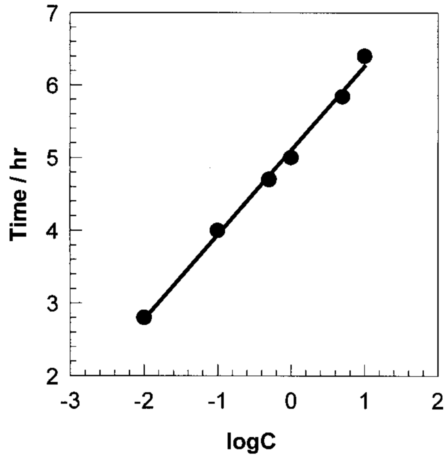

FIGURE 5 Calibration curve for the determination of penicillin. The concentration of *S. aureus* is 1.57×10^6 cells/ml.

values of RDT and the logarithm values of the concentration of penicillin. The regression equation is:

$$\mathrm{RDT} = 1.16 \log C + 5.10 \tag{5}$$

where C is the concentration of penicillin, and the correlation coefficient is 0.995 ($n = 6$), detection limit is 4×10^{-3} μg/ml.

The ΔR_1 between two plateaus also can reflect the amount of antibiotics in detection system, however, it needs a long detection time. We used RDT to reflect the pollution extent in this study.

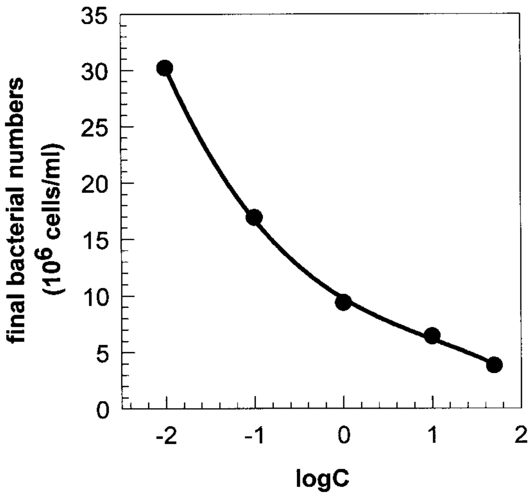

FIGURE 6 Relationship between final bacterial numbers and the logarithm values of different concentrations of penicillin.

Comparison of the piezoelectric impedance analysis method with PPC method

When the culture system was monitored by impedance microbial sensor, the number of bacteria was detected every one hour by traditional PPC method at the same conditions to evaluate the proposed method. The results in the absence and presence of penicillin are shown in Fig. 2 and 3, respectively. It can be seen that the three stages of ΔR_1 variations correspond to the three phases of bacterial growth. The results show that the situation of bacterial growth leads to the change of ΔR_1. In addition, the final bacterial numbers were detected with PPC method after completing the measurement of impedance parameters. The relationship between the final bacterial numbers and the logarithm values of the concentration of penicillin is shown in Fig. 6. It can be seen that the final bacterial number decreases with the increase of the concentration of penicillin. Experiments show that the bacterial growth is inhibited by penicillin, and the inhibition action is more serious when the concentration of penicillin is higher. This result agrees with that obtained by the proposed method.

The valence of other antibiotics to penicillin

Many antibiotics and pollutants can obviously inhibit the growth of *S. aureus* when they are present in water[18]. And hence the growth process of *S. aureus* can be conveniently applied for environmental wastewater monitoring, which come from many pollution sources, especially hospital and medical factories. Practically, the pollution level can be easily defined as the concentration of penicillin. The valence of the inhibitory capacity of 1 μg/ml penicillin was defined as 1. The inhibitory capacity of other antibiotics was thoughtfully examined. First, RDT values were obtained under the same conditions by the proposed method, then the inhibitory capacity of other antibiotics and pollutants was calculated according to Eq. (5). The results are shown in Table I.

TABLE I The valences of some antibiotics to penicillin

antibiotics	*penicillin*	*cefradine*	*streptomycin*	*erythrocin*	*Rifampicin*	*phosphonomycin*
RDT (h)	5.10	4.73	4.82	4.90	5.13	5.16
Valence	1.00	0.48	0.57	0.67	1.06	1.13

TABLE II Concentrations of antibiotics obtained by the proposed method in a hospital waste water

Date	*Monday*	*Tuesday*	*Wednesday*	*Thursday*	*Friday*
RDT (h)	3.88	3.02	3.10	3.31	2.95
The final bacterial number (10^6)	17.24	25.46	24.86	21.50	26.8
Concentration of pollutants (μg/ml)	0.148	0.016	0.019	0.029	0.014

Monitoring of a hospital wastewater

Using the proposed method, we continuously monitored the wastewater of a hospital for one week. Before detection, the water samples were filtered and sterilized by supersonic wave. Under the same experimental conditions, we obtained the detection results which are shown in Table II. The concentration of antibiotic drugs in wastewater had little change from Tuesday to Friday. But there existed an apparent change on Monday. This may be because more people visited the hospital on that day. In general, we found that the wastewater of the hospital contained some antibiotics which may affect the public health.

CONCLUSION

As described above, the growth situations of *S. aureus* were examined in the presence and absence of antibiotics. This result is in agreement with that obtained by the PPC method, but the proposed method is simple and time saving. A relationship between RDT and the logarithm value of concentration of penicillin was also obtained. The valences of other antibiotics to penicillin were examined in this paper. Therefore, antibiotics and other pollutants can be detected using the piezoelectric impedance technique. It is expected that the proposed method can be used to detect the environmental wastewater as a composite biochemical polluted index.

Acknowledgements

This work was supported by the National Natural Science Foundation of China.

References

[1] Y. Shao and Y. Qian, *Encyclopedia of Human and Nature*. (in Chinese), pp. 143–144. Liaoning People's Press, Shenyang (1999).
[2] G. Rosina, P. Montserrat, L. Francisco, A. Aunika, W. Goran and J. Joan, *Wien. Mitt.: Wasser-Abwasser-Gewaesser*, **128**, 93–98 (1995).
[3] R.I. Ayukaev, E.V. Venetsianov, E.A. Kotanova and N.A. Tikhonov, *Khim. Tekhnol. Vody*, **19**, 115–121 (1997).

[4] J. Zhang, L. Bao, S. Yao and W. Wei, *Microchem. J.*, **62**, 405–412 (1999).
[5] I. Karube, T. Matsunaga, S. Mitsuda and S. Suzuki, *Biotechnol. Bioeng.*, **19**, 301–309 (1977).
[6] M. Yamane, A. Kawahara and S. Wakida, *Chem. Express*, **32**, 407–419 (1991).
[7] H. Muramatsu, E. Tamiya and I.K. Arube, *Anal. Chem.*, **60**, 2142–2146 (1988).
[8] S.J. Martin, V.E. Granstaff and G.C. Frye, *Anal. Chem.*, **63**, 2272–2281 (1991).
[9] D.A. Buttry and M.D. Ward, *Chem. Rev.*, **92**, 1355–1379 (1992).
[10] L. Bao, Q. Xie, Y. Xu and W. Wei, *Anal. Lett.*, **32,** 885–899 (1999).
[11] H.O. Ghourchian, N. Kamo, T. Hosokawa and T. Akitaya, *Talanta*, **41**, 401–406 (1994).
[12] S. Kurorsawa, E. Nemoto, M. Muratsugu, M. Yoshimoto, Y. Mori and N. Kamo, *Anal. Chim. Acta*, **289**, 307–311 (1994).
[13] T. Cheng, H. Chang and T. Lin, *Biosens. Bioelectron.*, **13**, 147–156 (1998).
[14] J. Zhang, W. Wei, A. Zhou, D. He, S. Yao and Q. Xie, *Talanta*, **53**, 525–533 (2000).
[15] G. Sauerbrey, *Z. Phys.*, **155**, 206–222 (1959).
[16] K.K. Kanazawa and J.G. Gordon II, *Anal. Chim. Acta*, **175**, 99–105 (1985).
[17] M.H. Zwietering, I. Jongenburger, F.M. Rombacts and K.V. Riet, *Appl. Environ. Microbiol.*, **56**, 1875–1881 (1990).
[18] H. Wu, Z. Zhu, P. Yang, G. Yang, W. Chen and W. He, *Chinese Journal of Microbiology*, **12**, 155–159 (2000).