Kinetic and equilibrium studies for the adsorption process of cadmium(II) and copper(II) onto *Pseudomonas aeruginosa* using square wave anodic stripping voltammetry method

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ARTICLE INFO

ABSTRACT

A novel method for the simultaneous determination of cadmium(II) and copper(II) during the adsorption process onto *Pseudomonas aeruginosa* was developed. The concentration of the free metal ions was successfully detected by square wave anodic stripping voltammetry (SWASV) on the mercaptoethane sulfonate (MES) modified gold electrode, while the *P. aeruginosa* was efficiently avoided approaching to the electrode surface by the MES monolayer. And the anodic stripping peaks of Cd^{2+} and Cu^{2+} appear at -0.13 and $0.34\,V$ respectively, at the concentration range of $5–50\,\mu M$, the peak currents of SWASV present linear relationships with the concentrations of cadmium and copper respectively. As the determination of Cd^{2+} and Cu^{2+} was in real time and without pretreatment, the kinetic characteristics of the adsorption process were studied and all the corresponding regression parameters were obtained by fitting the electrochemical experimental data to the pseudo-second-order kinetic model. Moreover, Langmuir and Freundlich models well described the biosorption isotherms. And there were some differences in the amount of metal ion adsorbed at equilibrium (q_e) and other kinetics parameters when the two ions coexisted were compared with the unaccompanied condition, which were also discussed in this paper. The proposed electrode system provides excellent platform for the simultaneous determination of trace metals in complex biosorption process.

1. Introduction

The water environment has been contaminated by persistent pollutants of agriculture and industrial origin during recent decades. Heavy metal contamination has serious impact on the environment, due to discharges from industrial wastes, agricultural and urban sewage. Heavy metals can be accumulated by organisms through a variety of pathways, including respiration, adsorption and ingestion, which are highly toxic to many organs of both humans and animals [1,2]. The conventional methods used to treat wastewater containing heavy metals consist of chemical precipitation, solvent extraction, ion exchange, membrane processes, dialysis, carbon adsorption and so on [3,4]. However, most of the methods mentioned above are fussy in operation, frequently expensive, or inefficient at trace concentration. Thus, it is very desirable to develop a rapid, simple, reliable method for treating such toxic metal ions. Biosorption using microbes is a cost-effective approach to remove heavy metals in the industrial effluents [5], The main advantages of this technique are the

reusability of biomaterial, low operating cost, improved selectivity for specific metals of interest, removal of heavy metals from effluent irrespective of toxicity, and no production of secondary compounds which might be toxic [6,7]. *Pseudomonas aeruginosa* (*P. aeruginosa*) is widely used for its advantages in high efficiency of biosorption performance and low operating cost [8,9]; while the inactive biosorption of metal by microbe is focused as well [10].

Anodic stripping voltammetry (ASV) is regarded as a highly sensitive, accurate and convenient technique for trace metal analysis, which is fit for simultaneous analysis of metal ions [11–13]. In order to avoid organic macromolecular polluting the electrode surface [14,15], which can lead to the electrode activity loss and reduced sensitivity, a porous film should be modified on the electrode surface. And the self-assembly of mercaptoethane sulfonate (MES) on the gold electrode surface was successfully used for the detection of trace metal ion in the presence of *P. aeruginosa* cells [16]. Thus, the gold electrode modified by MES can be used to directly determine the copper and cadmium ions simultaneously. Moreover, compared to the conventional techniques determining the metal ions during the biosorption process such as gravimetry, colorimetry and atomic absorption spectrophotometry [17,18], ASV not only shuns the bothers of pretreatment and time-consuming, but also can offer

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the real-time information for the study of the biosorption kinetics process.

In this paper, square wave anodic stripping voltammetry (SWASV) at MES monolayer modified electrode was first used for the simultaneous monitoring that copper(II) and cadmium(II) adsorbed onto the nonliving biomass of *P. aeruginosa*. The porous disorganized monolayer formed by MES prevents the contaminants *P. aeruginosa* fouling the gold electrode surface, while the free metal ions can penetrate through the monolayer to be determined by the voltammetric detector. The concentrations of the free Cd^{2+} and Cu^{2+} would be detected by SWASV each 2–5 min until the biosorption equilibrium approaches. Based on the electrochemical results, the adsorption kinetics and isothermal models were investigated.

2. Material and methods

2.1. Reagents

Mercaptoethane sulfonate (MES, purity ≥99%) was purchased from Sigma–Aldrich (St. Louis, MO, USA). Beef extract and peptone were obtained from Shanghai Reagent Factory (Shanghai, China). Experimental solutions of cadmium and copper ions were respectively prepared by diluting 1×10^{-3} M of cadmium and copper stock solutions, which were obtained by dissolving corresponding chlorides into doubly distilled water. All of the other reagents were of analytical reagent grade and were used without further purification. Doubly distilled water was used throughout.

2.2. Apparatus

The electrochemical measurements were carried out in a conventional three-electrode detected cell on a CHI 660 A electrochemical workstation (Shanghai Chenhua Apparatus Co., Shanghai, China) with CHI software, connecting to a personal computer. An MES-modified gold electrode (2 mm in diameter) was used as the working electrode, a platinum wire auxiliary electrode, and a saturated calomel electrode (SCE) was used as the reference electrode. All potentials were vs. the reference electrode. A magnetic stirrer (Model 79-1, Shanghai Hexin Technique and Education Equipment Co., Shanghai, China) was employed to stir the testing solution during the biosorption process of Cd^{2+} and Cu^{2+} onto *P. aeruginosa*.

2.3. Bacterial solution and preparation of biosorbent

P. aeruginosa was purchased from Medical School of Central South University (Changsha, China). The composition of the broth medium for *P. aeruginosa* was as follows: beef extract, 5.0 g; peptone, 10.0 g; sodium chloride, 5.0 g; distilled water, 1000 ml. Then 0.1 M NaOH use used to adjust the value of pH to 7.2. The medium was sterilized by autoclaving at 121 °C for 15 min. Inoculate four loops of *P. aeruginosa* from an agar slant medium into a 250 ml sterilized conical glass flasks containing 100 ml sterilized broth culture medium, incubated at 37 °C with gentle oscillation for 24 h. After that, the cells were gathered by centrifugation at the rotating speed of 11,000 rpm for 20 min and the cells were washed twice with double distilled water. Then the cells were dried at 80 °C for 24 h. In the phase of homogenizing, the dried cells were homogeneously dispersed in double-distilled and sterilized water by a homogenizer at 2500 rpm for 30 min, and then stored in the refrigerator at 4 °C for further use.

2.4. Modification of electrode

2.4.1. Pretreatment of the gold electrode

Before modification, the gold electrode was required to be polished carefully with 0.3 μm and 0.05 μm α-alumina powder slurries on a 1200 grit Carbimet disk until a mirror shiny surface was obtained, and then immersed in an ultrasonic bath sequentially with ethanol double distilled water for 3 min to remove traces of alumina and possible contamination, and finally rinsed with double distilled water. Then the undefiled electrode was cycled from 0 to 1.5 V in 0.5 M H_2SO_4 solution until stable cyclic voltammograms were obtained, and then rinsed adequately with double distilled water and dried with N_2.

2.4.2. Self-assembling of MES

After the pretreatment, the electrode was modified by immersing in a solution mixed with 5 mM MES and 0.1 M perchloric acid at open circuit for 10 min [19]. Finally, the electrode was carefully rinsed with abundant amount of double distilled water, dried in N_2 stream, and then immediately fixed into an electrochemical cell.

2.5. Testing procedure

The detector was constructed in the 10 ml testing cell with 0.1 M NaCl solution as the electrolyte and the prepared electrodes as the working electrodes. The biosorption experiments were performed by adding 50 mg l^{-1} (final concentration) of *P. aeruginosa* cells suspensions into the testing cell containing given amount of cadmium and copper ions. For the biosorption process, the free metal ions in the solution decreased which would be determined directly by SWASV, until the adsorption reached the equilibrium. The peak currents of cadmium and copper were recorded every 2–5 min. And the experimental electrochemical parameters were optimized and the optimal parameters were used as follows: increase potential, 10 mV; amplitude, 25 mV; frequency, 15 Hz; initial potential, −0.3 V; final potential, 0.6 V; deposition potential, −0.3 V; deposition time, 10 s; quiet time, 2 s. All the electrochemical experiments were performed at room temperature (20 °C).

3. Results and discussion

3.1. The SWASV characterization of Cd^{2+} and Cu^{2+} on the MES-modified electrode and the calibration plots

MES has only a two-carbon short chain and a highly charged group at one end (sulfonate group in solution). Such a structure can diminish the affinity between the alkyls border upon and augment the electrostatic repulsion between the endmost chain groups [20]. In this way, it is easy to form a disorganized and porous MES monolayer at the surface of the gold electrode [21], which can let small particulates such as metal ions permeate through while macromolecules like microbial cells and protein molecules would be held out due to their large size. Thus, the free ions Cd^{2+} and Cu^{2+} can be detected by the sensor and the electrode can not be polluted by *P. aeruginosa* cells. Besides, because MES monolayer film is electronegative, it can attract more metal ions onto the electrode surface in the anodic stripping voltammograms.

Fig. 1 shows the SWASV of cadmium and copper in the concentration range from 5 to 50 μM on the MES-modified gold electrode. The detection of metal ions is negligibly affected by the presence of *P. aeruginosa* in the solution [22]. The sustaining electrolyte is 0.1 M NaCl solution, and the stripping peaks of Cd^{2+} and Cu^{2+} appear at −0.13 and 0.34 V respectively. At the concentration range of 5–50 μM, the peak currents of SWASV present linear relationships with the concentrations (*C*) of cadmium and copper respectively. The linear regression equation is as follows:

$$i_p = aC + b \tag{1}$$

By fitting the experimentally obtained maximum of i_p shown as drops, the results are given as follows: for Cd^{2+}: $a = 0.118 \, \mu A \, \mu M^{-1}$,
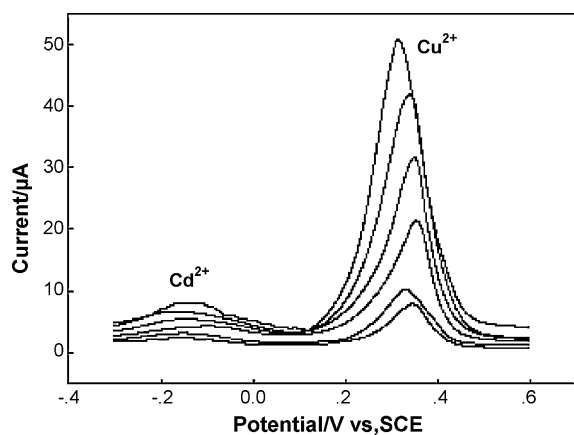
Fig. 1. Square wave anodic stripping voltammograms (SWASV) of cadmium and copper in 0.1 M NaCl at mercaptoethane sulfonate-modified gold electrodes. The concentrations of cadmium and copper are 5, 10, 20, 30, 40 and 50 μM from bottom to top.

$b = 0.0110\,\mu A$, $R^2 = 0.9979$; for Cu^{2+}: $a = 1.0066\,\mu A\,\mu M^{-1}$, $b = -0.5418\,\mu A$, $R^2 = 0.9945$. R^2 is the correlative coefficient, and the values of R^2 here imply that the response model can well describe the relationship between i_p and the concentrations of Cd^{2+} and Cu^{2+}.

3.2. Comparison of the proposed method with atomic absorption spectrometry

To verify the accuracy of the proposed method, the results obtained by this method were compared with that by atomic absorption spectrometry (AAS). 5 μM Cd^{2+} and Cu^{2+} were added to 0.1 M NaCl solution contained 25 mg l^{-1} P. aeruginosa cells. The concentration of metal ions in the simulated sample was determined by the approach described above and AAS. The samples used in the latter case were pretreated to remove the P. aeruginosa cells. The results obtained were shown in Table 1. The concentrations of metal ions detected by this method were greater than those of AAS. This may be because some metal ions were absorbed by P. aeruginosa cells during the pretreatment. The result in Table 1 suggested the accuracy and reliability of this method. Moreover, this method avoided cumbersome operations, and could provide real-time information for monitoring of the biosorption process.

3.3. The dynamics model for the biosorption process of Cd^{2+} and Cu^{2+} onto P. aeruginosa

After P. aeruginosa cells were injected, Cd^{2+} and Cu^{2+} were adsorbed onto the nonliving microorganisms by virtue of the electrostatic interaction between metal ions and the anionic ligands in the lipopolysaccharide and other surface polymers [22]. To describe the characteristic for the efficiency of biosorption, a dynamics model should be discussed. The pseudo-second-order model was

used well in adsorption studies, even in the study of biosorption process [16]. The equation of the pseudo-second-order kinetic model is expressed as follows:

$$\frac{dq_t}{dt} = k_2(q_e - q_t)^2 \tag{2}$$

where k_2 is the rate constant of second-order adsorption ($mg\,\mu mol^{-1}\,min^{-1}$), q_t ($\mu mol\,mg^{-1}$) is the amount of metal ions adsorbed at time t, and q_e ($\mu mol\,mg^{-1}$) is the amount of metal ion adsorbed at equilibrium.

The relationship of q_t, t, metal ions concentration C (μM) and the amount of biomass X ($mg\,l^{-1}$) is shown in Eq. (3).

$$q_t = \frac{C_0 - C_t}{X} \tag{3}$$

C_0 and C_t (μM) are the metal concentrations in the solution at initial and at time t, respectively. When t is equal to the equilibrium contact time, the concentration of the metal ions is equal to the concentration at equilibrium (C_e), viz. $C_t = C_e$, and $q_t = q_e$.

Integrating Eqs. (1) and (3), a new equation is obtained, given by Eq. (4):

$$q_t = -\kappa \Delta i_p \tag{4}$$

where Δi_p (μA) is defined as the discrepant value of i_p at time t vs. initial. κ ($\mu mol\,mg^{-1}\,\mu A^{-1}$) is a coefficient. When $t \to \infty$, the adsorption dynamics go close to the equilibrium, $i_p \to i_{p,max}$, $q_t \to q_e$, Eq. (4) can be replaced by Eq. (5):

$$q_e = -\kappa \Delta i_{p,max} \tag{5}$$

From Eqs. (2), (4) and (5), Eq. (6) is obtained:

$$-\frac{d\Delta i_p}{dt} = \kappa k_2(\Delta i_p - \Delta i_{p,max})^2$$

$$= k_2'(\Delta i_p - \Delta i_{p,max})^2 \tag{6}$$

Integrating Eq. (6), a new equation used to describe the relation between Δi_p and biosorption kinetics parameters is expressed as follows:
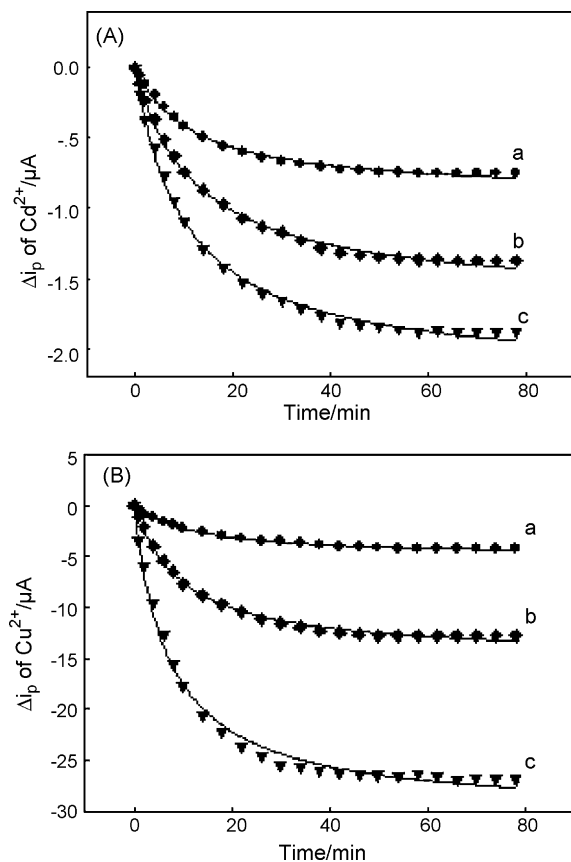
$$\Delta i_p = \frac{k_2' \Delta i_{p,max}^2 t}{k_2' \Delta i_{p,max} t - 1} \tag{7}$$

Eq. (7) is the right kinetics model for the biosorption process of Cd^{2+} and Cu^{2+} onto P. aeruginosa.

3.4. The Δi_p response of Cd^{2+} and Cu^{2+} on the MES-modified electrode during the biosorption process in the presence of either metal ions

After P. aeruginosa biomass solution was added into the electrochemical cell containing only one sort of detected ion such as Cd^{2+}, the typical Δi_p responses with time during the binding process of Cd^{2+} onto P. aeruginosa at different initial cadmium concentrations are shown in Fig. 2(A). From the adsorption curve in Fig. 2(A), it can be noted that Δi_p decreases rapidly during the initial 20 min, which corresponds to the fast adsorption period of P. aeruginosa, the value of Δi_p in this period can reach a 80 percent of the total. Then the declining rate of Δi_p slows and finally reaches a stable level forming a roof, and the adsorption process almost finishes. It can also be regarded that the biosorption course is rapid, and the nonliving P. aeruginosa cells must be a highly efficient sorbent.

In accordance with Cd^{2+}, the Δi_p response of Cu^{2+} adsorbed onto P. aeruginosa cells has the same changing trend, shown in Fig. 2(B).

Table 1
Comparison of this method (the sample without pretreatment) and atomic absorption spectrometry (the sample with pretreatment) for determination of Cu^{2+} and Cd^{2+} in the simulated sample contained 25 mg l^{-1} P. aeruginosa cells.

Ion	Detected by this method		Detected by AAS[b]	
	C (μM)[a]	RSD[c] ($n = 5$)	C (μM)[a]	RSD[c] ($n = 5$)
Cu^{2+}	4.43	4.94%	4.06	4.42%
Cd^{2+}	4.14	4.45%	3.63	4.58%

[a] Average of five determinations.
[b] AAS is atomic absorption spectrometry.
[c] RSD is relative standard derivation.

Fig. 2. (A) The Δi_p response with time for the binding process of cadmium to *P. aeruginosa* at different initial cadmium concentrations: (a) 10 μM; (b) 20 μM; (c) 40 μM. (B) The Δi_p response with time for the absorption process of copper to *P. aeruginosa* at different initial copper concentrations: (a) 10 μM; (b) 20 μM; (c) 40 μM.

3.5. The synchronous detection of cadmium and copper on the MES-modified electrode during the adsorption process

Inject *P. aeruginosa* solution (the final concentration is still 50 mg l⁻¹) into the electrochemical cell containing the detecting ions Cd^{2+} and Cu^{2+}. In the same experiment, the initial concentrations of Cd^{2+} and Cu^{2+} are the same. Fig. 3(A) and (B) respectively displays the Δi_p response with time during the binding process of Cd^{2+} and Cu^{2+} onto *P. aeruginosa* at the initial concentrations of 10, 20 and 40 μM from top to bottom.

Compared to the curves in Fig. 2, the ones in Fig. 3 display the same trend. The adsorption processes of Cd^{2+} and Cu^{2+} onto *P. aeruginosa* act simultaneously, it is seen to have no obvious dif-
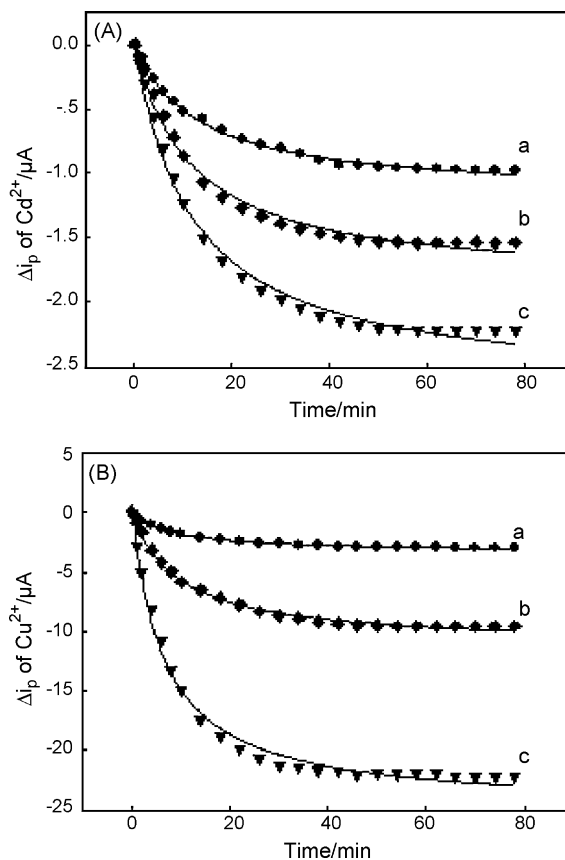
Fig. 3. (A) and (B) respectively shows the peak current with time response for the binding process of cadmium and copper to *P. aeruginosa* at different initial and cadmium copper concentrations: (a) 10 μM; (b) 20 μM; (c) 40 μM.

ferentiation in time. However, there are some differences in the maximum of Δi_p and other factors in the two conditions.

3.6. Estimation of biosorption kinetics parameters

Taking k_2' and $\Delta i_{p,\max}$ as estimation parameters, the Δi_p in Figs. 2 and 3 is fitted by using the non-linear fitting program embedded in Sigmaplot Scientific Software Version 10.0. By fitting the experimentally obtained values to the electrochemical response model in Eq. (7), the kinetic parameters are obtained and listed in Tables 2–5, respectively. The correlation coefficients R^2 in the four tables are all close to 1, which indicate that the metal ions in the condition of singleness and concomitance adsorbed onto *P. aeruginosa* can be described well by the pseudo-second-order kinetics

Table 2
Kinetic parameters and regression coefficients (R^2) for the biosorption of cadmium onto *P. aeruginosa* at various initial cadmium concentrations (C_0) obtained by fitting the experimental values in Fig. 2(A) to Eq. (7) ($\kappa = 0.01987$ μmol mg⁻¹ μA⁻¹).

C_0 (μM)	k_2' (μA⁻¹ min⁻¹)	$-\Delta i_{p,\max}$ (μA)	k_2 (mg μmol⁻¹ min⁻¹)	q_e (μmol mg⁻¹)	R^2
10	0.0928	0.903	0.5475	0.1137	0.9945
20	0.0488	1.653	0.2879	0.2553	0.9970
40	0.0447	2.199	0.2637	0.3230	0.9975

Table 3
Kinetic parameters and regression coefficients (R^2) for the biosorption of copper onto *P. aeruginosa* at various initial copper concentrations (C_0) obtained by fitting the experimental values in Fig. 2(B) to Eq. (7) ($\kappa = 0.01987$ μmol m⁻¹ μA⁻¹).

C_0 (μM)	k_2' (μA⁻¹ min⁻¹)	$-\Delta i_{p,\max}$ (μA)	k_2 (mg μmol⁻¹ min⁻¹)	q_e (μmol mg⁻¹)	R^2
10	0.0153	5.101	0.7700	0.1024	0.9974
20	0.00716	14.937	0.3603	0.2712	0.9960
40	0.00451	30.334	0.2270	0.5328	0.9937

Table 4
Kinetic parameters and regression coefficients (R^2) for the biosorption of cadmium onto *P. aeruginosa* at various initial cadmium concentrations (C_0) obtained by fitting the experimental values in Fig. 3(B) to Eq. (7) ($\kappa = 0.01987\,\mu\text{mol}\,\text{m}^{-1}\,\mu\text{A}^{-1}$).

C_0 (μM)	k_2' (μA^{-1} min^{-1})	$-\Delta i_{p,\max}$ (μA)	k_2 (mg μmol^{-1} min^{-1})	q_e (μmol mg^{-1})	R^2
10	0.0569	1.173	0.3357	0.1522	0.9972
20	0.0468	1.852	0.2761	0.2822	0.9902
40	0.032	2.668	0.1888	0.3788	0.9927

Table 5
Kinetic parameters and regression coefficients (R^2) for the biosorption of copper onto *P. aeruginosa* at various initial copper concentrations (C_0) obtained by fitting the experimental values in Fig. 3(B) to Eq. (7) ($\kappa = 0.01987\,\mu\text{mol}\,\text{m}^{-1}\,\mu\text{A}^{-1}$).

C_0 (μM)	k_2' (μA^{-1} min^{-1})	$-\Delta i_{p,\max}$ (μA)	k_2 (mg μmol^{-1} min^{-1})	q_e (μmol mg^{-1})	R^2
10	0.0351	3.366	1.7665	0.0773	0.9987
20	0.00933	11.171	0.4696	0.2073	0.9960
40	0.0062	24.917	0.3120	0.4406	0.9927

model. From the tables, it is also seen that the amount of metal ions adsorbed on to *P. aeruginosa* at equilibrium (q_e) increases with the increasing initial metal ions concentrations.

Comparing Fig. 2(A) with Fig. 3(A) and Fig. 2(B) with Fig. 3(B), the results show that there are some differences in the value of q_e and other kinetics parameters. It is considered that there are some possible mutual influences when Cd^{2+} and Cu^{2+} as the detected metal ions are adsorbed synchronously by *P. aeruginosa*. In the concomitant condition of Cd^{2+} and Cu^{2+}, the value of $\Delta i_{p,\max}$ of Cd^{2+} is higher than that of unaccompanied with Cu^{2+}, and the corresponding q_e is also greater. As opposed to Cd^{2+}, the $\Delta i_{p,\max}$ and q_e of Cu^{2+} are lower in the concomitant condition. All mentioned above, due to the mutual influences between the two ions, the sorption of Cd^{2+} on *P. aeruginosa* is enhanced in the present of Cu^{2+}, which that of Cu^{2+} is weakened in the present of Cd^{2+}. The mutual influences here are competitive and restraining relationship. Table 6 shows the changing percentage of q_e of Cd^{2+} and Cu^{2+} in the concomitant condition vs. the unaccompanied term.

3.7. The characteristics adsorption isotherms for Cd^{2+} and Cu^{2+} onto P. aeruginosa

It is significant for studying the biosorption mechanism and designing biologic reaction to analyze the characteristics of adsorption isotherms. In this work, the adsorption characteristics of the cadmium and copper onto *P. aeruginosa* are described by the Langmuir [23] and the Freundlich [24] adsorption isothermal models, which can indicate the quantitative relation between q_e and C_e expressed respectively in Eqs. (8) and (9):

$$q_e = \frac{q_{\max} C_e}{K_d + C_e} \tag{8}$$

$$q_e = K_f C_e^{1/n} \tag{9}$$

where $q_{\max}$ (μmol mg^{-1}) is the maximum adsorption capacity and K_d (μM) is the dissociation constant depending to the stability of metal–biosorbent complexes, n is the Freundlich constant with no dimension, and K_f (l mg^{-1}) is a constant related to the adsorption capacity.

Fig. 4 shows the adsorption isotherms of Cd^{2+} onto *P. aeruginosa* in the solution with both Cd^{2+} and Cu^{2+} as the detected ions.

Table 6
The changing percentage of q_e in the solution containing the two cations compared to in the solution with either.

C_0 (μM)	Δq_e (% (Cd^{2+}))	Δq_e (% (Cu^{2+}))
10	+33.84%	−24.52%
20	+10.55%	−23.58%
40	+17.28%	−17.30%
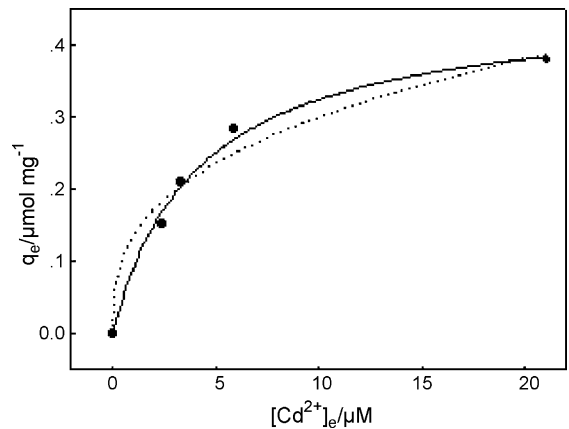
Fig. 4. Isotherms of cadmium in the competitive absorption process onto *P. aeruginosa*: (a) simulation with Langmuir isotherm model, (b) simulation with Freundlich isotherm model.

It is noticed that the values of q_e increase with the increasing C_e values. Similarly, the adsorption isotherms in other conditions also can be obtained and fitted by the two models. And the corresponding parameters and correlation coefficients are listed in Table 7, respectively.

From Table 7, it is determined that the two theoretic models can well depict the adsorption isotherms for Cd^{2+} and Cu^{2+} under all conditions in this work. And the competitive and restraining relations are also revealed obviously.

3.8. Reuse of P. aeruginosa

The reusability of the biosorbent was also investigated. For this test, *P. aeruginosa* loaded with Cd^{2+} was treated with a 1 M HNO$_3$ solution in order to desorb the Cd^{2+}. The biomass was separated by centrifugation, washed several times with deionized water and then reused for the Cd^{2+} adsorption. This process was repeated five

Table 7
Biosorption isotherm constants for the biosorption of cadmium and copper onto *P. aeruginosa* at 20 °C.

	Langmuir			Freundlich		
	$q_{\max}$ (μmol m^{-1})	K_d (μM)	R^2	K_f (l mg^{-1})	n	R^2
[a]Cd^{2+}	0.4269	6.8134	0.9673	0.0928	2.4740	0.9481
[a]Cu^{2+}	0.8975	3.0762	0.9927	0.2078	2.4093	0.9673
[b]Cd^{2+}	0.4589	4.1420	0.9944	0.1352	2.8902	0.9741
[b]Cu^{2+}	0.7541	3.4131	0.9876	0.1792	1.9707	0.9655

[a]Cd^{2+} is in the solution containing only Cd^{2+} detected. [a]Cu^{2+} is in the solution containing only Cu^{2+} detected. [b]Cd^{2+} and [b]Cu^{2+} are in the competitive absorption system.
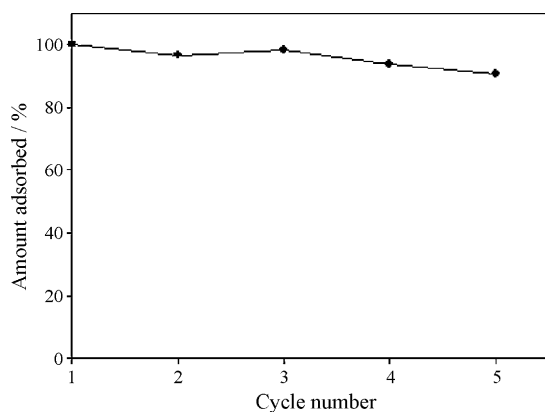
Fig. 5. Reuse of *P. aeruginosa* for sorption/desorption of Cd^{2+}. The amount of Cd^{2+} adsorbed at the first time was considered as 100%.

times and the result was shown in Fig. 5. As can be seen, this biosorbent exhibited excellent reusability. The absorption capacity had not decreased obviously after five adsorption/desorption cycles.

4. Conclusion

In this work, a sensitive and effective electrode system was successfully developed for the simultaneous determination of Cd^{2+} and Cu^{2+} during the adsorption process onto *P. aeruginosa* by SWASV. The gold electrode was modified with a selectively porous MES monolayer, and the free metal ions were detected free from macromolecule contaminants. For the real-time detection without any pretreatment, the adsorption process was successfully monitored, the adsorption kinetic parameters were obtained by fitting with the pseudo-second-order kinetic model, and the adsorption characteristics in the conditions including separate existence and co-exist of the two metal ions were also studied. Furthermore, the Langmuir and the Freundlich adsorption isothermal models well described the adsorption processes in this work due to the high correlative coefficients. By comparing the amount of metal ion adsorbed at equilibrium (q_e) and other kinetics parameters, a competitive and restraining relationship between Cd^{2+} and Cu^{2+} during the simultaneous adsorption process was revealed and discussed. The novel method used to study various metal ions adsorption system in the present work provided a new approach to simultaneous detection of trace metal ions in complex biosorption process.

References

[1] A. Ruiz-Manriquez, P.I. Magaña, V. López, R. Guzmán, Biosorption of Cu by Thiobacillus ferrooxidans, Bioproc. Eng. 18 (1998) 113–118.
[2] J.L. Zhou, S.M. Salvador, Y.P. Liu, M. Sequeria, Heavy metals in the tissues of dolphins (Delphinus delphis) stranded on the Portuguese coast, Sci. Total. Environ. 273 (2001) 61–76.
[3] J.W. Patterson, Industrial Wastewater Treatment Technology, Butterworth Publication, Stoneham, 1985.
[4] M. Ghaedi, E. Asadpour, A. Vafaie, Simultaneous preconcentration and determination of copper, nickel, cobalt, lead, and iron content using a surfactant-coated alumina, Bull. Chem. Soc. Jpn. 79 (2006) 432–436.
[5] B. Volesky, Sorption and Biosorption, BV Sorbex Inc., St. Lambert, Quebec, Canada, 2003.
[6] H. Serencam, A. Gundogdu, Y. Uygur, B. Kemer, V.N. Bulut, C. Duran, M. Soylak, M. Tufekci, Removal of cadmium from aqueous solution by nordmann fir (*Abies nordmanniana* (Stev.) Spach. subsp. nordmanniana) leaves, Bioresour. Technol. 99 (2008) 1992–2000.
[7] O.D. Uluozlu, A. Sari, M. Tuzen, M. Soylak, Biosorption of Pb(II) and Cr(III) from aqueous solution by Lichen (*Parmelina tiliaceae*) biomass, Bioresour. Technol. 99 (2008) 2972–2980.
[8] H. Hussein, S. Farag, K. Kandil, H. Moawad, Tolerance and uptake of heavy metals by Pseudomonads, Process Biochem. 40 (2005) 955–961.
[9] K.Y. Prasanna, P. King, V.S.R.K. Prasad, Equilibrium and kinetic studies for the biosorption system of copper(II) ion from aqueous solution using Tectona grandis L.f. leaves powder, J. Hazard. Mater. 137 (2006) 1211–1217.
[10] S. Al-Asheh, Z. Duvnjak, Adsorption of copper and chromium by Aspergillus carbonarius, Biotechnol. Prog. 11 (1995) 638–642.
[11] X.H. Gao, W.Z. Wei, L. Yang, T.J. Yin, Y. Wang, Simultaneous determination of lead, copper, and mercury free from macromolecule contaminants by square wave stripping voltammetry, Anal. Lett. 38 (2005) 2327–2343.
[12] J. Xia, W. Wei, Y. Hu, H. Tao, L. Wu, A novel voltammetric method for the direct determination of copper in complex environmental samples, Anal. Sci. 20 (2004) 1037–1041.
[13] M. Ghaedi, M.K. Amini, A. Rafi, S. Gharaghani, A. Shokrollahi, Adsorptive stripping voltammetric determination of copper(II) ion using phenyl pyridyl ketoneoxime (PPKO), Ann. Chim. (Rome) 95 (2005) 457–464.
[14] G.A. Bhat, R.A. Saar, R.B. Smart, J.H. Weber, Titration of soil-derived fulvic acid by copper(II) and measurement of free copper(II) by anodic stripping voltammetry and copper(II) selective electrode, Anal. Chem. 53 (1981) 2275–2280.
[15] T. Bonfil, M. Brand, E. Kirowa-Eisner, Characteristics of subtractive anodic stripping voltammetry of Pb and Cd at silver and gold electrodes, Anal. Chim. Acta 464 (2002) 99–114.
[16] X.F. Li, W.Z. Wei, X.D. Zeng, J.X. Zeng, L. Wu, Kinetic and equilibrium studies of copper biosorption onto *Pseudomonas aeruginosa* base using direct determination of copper by a voltammetric method, World J. Microbiol. Biotechnol. 23 (2007) 1465–1471.
[17] M. Ghaedi, F. Ahmadi, H. Karimi, S. Gharaghani, Preconcentration and extraction of copper on activated carbon using 4-amino-2,3-dimethyl-1-phenyl-3-pyrazoline or 4-(4-methoxybenzylidenimin) thiophenole, J. Korean Chem. Soc. 50 (2006) 23–31.
[18] M. Ghaedi, A. Shokrollahi, A.H. Kianfar, A.S. Mirsadeghi, A. Pourfarokhi, M. Soylak, The determination of some heavy metals in food samples by flame atomic absorption spectrometry after their separation-preconcentration on bis salicyl aldehyde, 1,3 propan diimine (BSPDI) loaded on activated carbon, J. Hazard. Mater. 154 (2008) 128–134.
[19] G. Herzog, D.W.M. Arrigan, Application of disorganized monolayer films on gold electrode to the prevention of surfactant inhibition of the voltammetric detection of trace metals via anodic stripping of underpotential deposits: detection of copper, Anal. Chem. 75 (2003) 319–323.
[20] S.J. Martin, V.E. Granstaff, G.C. Frye, Characterization of a quartz crystal microbalance with simultaneous mass and liquid loading, Anal. Chem. 63 (1991) 2272–2281.
[21] I. Markovich, D. Mandler, Disorganised self-assembled monolayers (SAMs): the incorporation of amphiphilic molecules, Analyst 126 (2001) 1850–1856.
[22] P. Sar, S.K. Kazy, R.K. Asthana, S.P. Singh, Metal adsorption and desorption by lyophilized *Pseudomonas aeruginosa*, Int. Biodeterior. Biodegrad. 44 (1999) 101–110.
[23] I. Langmuir, The adsorption of gases on plane surface of glass, mica and platinum, J. Am. Chem. Soc. 40 (1918) 1361–1403.
[24] H. Freundlich, Colloid and Capillary Chemistry, Methuen, London, 1926.