ELSEVIER

Journal of Hazardous Materials B134 (2006) 80–86

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Biosorption of lead, copper and cadmium by an indigenous isolate *Enterobacter* sp. J1 possessing high heavy-metal resistance

Wei-Bin Lu [a], Jun-Ji Shi [b], Ching-Hsiung Wang [c], Jo-Shu Chang [b,*]

[a] *Department of Cosmetic Science, Chung Hwa College of Medical Technology, Tainan, Taiwan*
[b] *Department of Chemical Engineering, National Cheng Kung University, Tainan, Taiwan*
[c] *Department of Biological Engineering, Yung Ta Institute of Technology and Commerce, Pingtung, Taiwan*

Received 6 April 2005; received in revised form 13 October 2005; accepted 17 October 2005
Available online 28 November 2005

Abstract

This study was undertaken to investigate biosorption kinetics and equilibria of lead (Pb), copper (Cu) and cadmium (Cd) ions using the biomass of *Enterobacter* sp. J1 isolated from a local industry wastewater treatment plant. Efficiency of metal ion recovery from metal-loaded biomass to regenerate the biosorbent was also determined. The results show that *Enterobacter* sp. J1 was able to uptake over 50 mg of Pb per gram of dry cell, while having equilibrium adsorption capacities of 32.5 and 46.2 mg/g dry cell for Cu and Cd, respectively. In general, Langmuir and Freundlich models were able to describe biosorption isotherm fairly well, except that prediction of Pb adsorption was relatively poor with Langmuir model, suggesting a different mechanism for Pb biosorption. Adjusting the pH value to 3.0 led to nearly complete desorption of Cd from metal-loaded biomass, while over 90% recovery of Pb and Cu ions was obtained at pH $\leqq$ 2. After four repeated adsorption/desorption cycles, biomass of *Enterobacter* sp. J1 retained 75, 79 and 90% of original capacity for adsorption of Pb, Cu and Cd, respectively, suggesting good reusability of the biosorbent. A combinative model was proposed to describe the kinetics of heavy-metal adsorption by *Enterobacter* sp. J1 and the model appeared to have an excellent prediction of the experimental data. The model simulation results also seemed to suggest that intracellular accumulation may occur during the uptake of Pb.

Keywords: Biosorption; *Enterobacter* sp.; Lead; Copper; Cadmium

1. Introduction

Biomass of algae, fungi and bacteria has been known to readily adsorb or accumulate metal ions [1–4]. The ability of metal uptake by those microorganisms (known as biosorption or bioaccumulation) has caught great attention due to its potential to provide an effective and economic means for heavy-metal remediation [2,5]. The uptake of heavy-metals by biomass is usually classified into three categories; namely, cell-surface binding, intracellular accumulation and extracellular accumulation [3]. Being metabolism-independent, the cell-surface binding can occur in either living or inactivated microorganisms, whereas the intracellular and extracellular accumulation of metals are usually energy-driven processes, thereby taking place only in living cells. The state of art in the field of biosorption of heavy-metals was reviewed by Volesky and Holan [3] and Volesky [4]. To enhance the applicability of biosorption in wastewater treatment, it is of importance to identify more microbial strains that could uptake metals with high efficiency and specificity as well as to design better bioprocesses that effectively remove or recover heavy-metals from aquatic systems. This motivated us to isolate new bacterial strains from metal-contaminated environment and evaluate the new strains for their ability to remove heavy-metals from the polluted environment.

In this study, we investigated the biosorption characteristics of *Enterobacter* sp. J1, which was isolated from local industrial wastewater treatment plant and exhibited high resistance to a variety of heavy-metals. The target metal pollutants in this work were lead (Pb), copper (Cu) and cadmium (Cd), which are frequently found in the industrial effluents in Taiwan. The kinetics and equilibrium of biosorption was systematically examined. A new combinative model taking into account the surface binding as well as intraparticle diffusion

* Corresponding author. Tel.: +886 6 2757575x62651; fax: +886 6 2357146.
E-mail address: changjs@mail.ncku.edu.tw (J.-S. Chang).

0304-3894/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2005.10.036

was developed to describe the transient behavior of metal adsorption by the biomass of *Enterobacter* sp. J1. SEM/EDS analysis was used to reveal the changes in morphology of cell-surface after biosorption and also to confirm the presence of heavy-metals associated with the presumably metal-loaded cells. The desorption efficiency of the metals from loaded biosorbents was also determined to further evaluate the feasibility of applying this strain in practical heavy-metal removal processes.

2. Materials and methods

2.1. Bacterial strain and culture conditions

The microorganism used in this study was *Enterobacter* sp. J1, which was screened from local industrial wastewaters (including effluents from electroplate plants) according to their ability to tolerate elevated concentrations of heavy-metals, including lead, copper, cadmium, mercury, zinc, cobalt and nickel. The strains able to tolerate high concentrations of heavy-metals were isolated. One of the strains showed much higher metal resistance than other isolates and comparison of 16S rDNA sequence of that strain shows 99.9% homology with that of standard *Enterobacter* species in the NCBI gene bank. The strain is thereby designated as *Enterobacter* sp. J1, which was able to grow on LB agar (Difco) amended with 1500 mg/l of Pb^{2+}, 500 mg/l of Cu^{2+}, 300 mg/l of Cd^{2+}, 750 mg/l of Zn^{2+} and 300 mg/l of Ni^{2+}. The bacterial cultures were typically incubated in LB broth at 37 °C. For the shake-flask cultures, 200 rpm agitation was employed.

2.2. Preparation of biosorbents

The cells were harvested by centrifugation (10,000 × *g*, 8 min) from early-stationary cultures with a cell density of approximately 1–2 g/l. After twice rinsed with deionized water, the cells were re-suspended in designated heavy-metal solutions for the biosorption experiments.

2.3. Measurement of heavy-metals

The heavy-metal adsorbates used in this study were lead ($PbCl_2$), copper ($CuCl_2$) and cadmium ($CdCl_2$), which were obtained from Riedel-de Haen, Inc. Heavy-metals in solutions were measured with Polarized Zeeman Atomic Absorption Spectrometer (AAS; Hitachi Model-Z-6100). Before measured by AAS, the heavy-metal solutions were appropriately diluted with deionized water to ensure that the heavy-metal concentration in the sample was linearly dependent on the absorbance detected.

2.4. Procedures of biosorption experiments

2.4.1. Time-course of biosorption

The biosorbent was suspended in 50 ml of heavy-metal solutions (100 mg/l) in a glass container to reach a cell concentration of 1.5–2.5 g/l. The cell/metal suspension was gently agitated (100 rpm) at 25 °C. The pH of the solution was initially adjusted to 5.0, 5.0 and 6.0 for Pb, Cu and Cd, respectively, to avoid precipitation of metals in the form of metal hydroxides. Samples were taken from the solution at desired intervals and were subsequently centrifuged at 10,000 × *g* for 8 min. The heavy-metal concentration in the resulting supernatant was determined by AAS.

2.4.2. Determination of adsorption isotherms

The biosorbent was suspended in solutions containing heavy-metal concentrations of 0–500 mg/l. The cell concentration in the solution was in the range of 1.5–2.5 g/l. The adsorption conditions (temperature, pH, agitation rate) were identical to those used in time-course experiments. After 24 h of incubation (25 °C, 100 rpm), samples were taken from the solutions and the metal concentration in the supernatants was measured with AAS.

2.5. Desorption experiments

After biosorption experiments, the metal-loaded biosorbents were harvested from the cell/metal solutions. The metal-loading on the biomass was 36.4, 15.4 and 7.76 mg/g dry for Pb, Cu and Cd, respectively. The biosorbents were then rinsed and re-suspended with metal-free deionized water. Proper amounts of 0.1 M HCl [5,6] were added into solutions containing metal-loaded biomass to adjust the pH value to 1, 2, 3, 4, 5 and 6, respectively. After 24 h gentle agitation, samples were taken from the suspensions. The samples were centrifuged immediately and the metal concentration in the supernatant was determined. After desorption, the regenerated biomass was again exposed to metal solutions containing 100 mg/l of the three heavy-metals for the next metal adsorption experiment. This adsorption/desorption cycle was repeated four times. The efficiency of adsorption and desorption was determined according to the procedures mentioned earlier.

2.6. Scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS) analysis

In order to observe how the sorption of metal ions on the cell-surface would alter the cell-surface morphology and to further confirm the identity of metal ions on the cell mass, SEM and EDS analysis was employed in this study. Metal-loaded and metal-free (control) biosorbents were treated with glutaraldehyde for 1 h and were dehydrated by acetone (50–100%) for 30 min. The pre-treated samples were coated with Au via vapor deposition prior to being introduced to SEM/EDS (Hitachi S4100, Japan) for analysis.

3. Result and discussion

3.1. Biosorption isotherms

The adsorption isotherms for Pb, Cu and Cd by biomass of *Enterobacter* sp. J1 are shown in Fig. 1. The equilibrium capacity of Pb appeared to be significantly higher than Cu and Cd on the

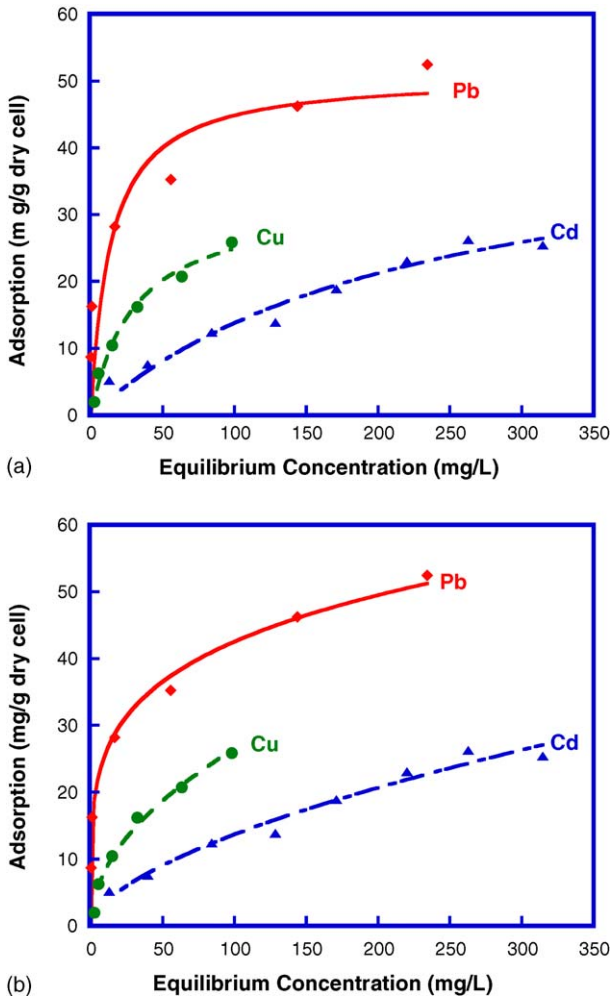

Fig. 1. Isotherms of Pb, Cu and Cd biosorption by *Enterobacter* sp. J1: (a) simulation with Langmuir isotherm model; (b) simulation with Freundlich isotherm model (symbols: experimental data; lines: model prediction).

weight basis. The biosorbent was able to uptake Pb at a capacity of up to nearly 50 mg Pb/g dry cell, while the maximum biosorption for Cu and Cd was around 30 mg/g dry cell. However, the molar adsorption capacity of *Enterobacter* sp. J1 decreased in the order of Cu (510 μmol/g dry cell) > Cd (411 μmol/g dry cell) > Pb (241 μmol/g dry cell). Langmuir isotherm and Freundlich model were used to describe the adsorption equilibrium of the three metal ions. The Langmuir model, valid for monolayer sorption onto a surface of a finite number of identical sites, is given as:

$$q = \frac{q_{\max} C_e}{K_d + C_e} \quad (1)$$

where q is the adsorption capacity (mg/g dry cell) at equilibrium, $q_{\max}$ is the maximal adsorption capacity (mg/g dry cell), C_e is the equilibrium concentration of adsorbate (mg/l) and K_d is the dissociation constant (mg/l). The empirical Freundlich equation which based on sorption on a heterogeneous surface is given as:

$$q = KC_e^{1/n} \quad (2)$$

Table 1
Adsorption constants estimated from simulations with Langmuir and Freundlich models for isotherms of Pb, Cu and Cd using *Enterobacter* sp. J1 as the biosorbent

Adsorbate	Langmuir model			Freundlich model		
	K_d (mg/l)	$q_{\max}$ (mg/g cell)	r^2	*1/n*	*K*	r^2
Pb	13.6	50.9	0.884	0.219	15.5	0.970
Cu	30.6	32.5	0.995	0.499	2.66	0.993
Cd	235	46.2	0.978	0.595	0.883	0.985

where K and n are Freundlich constants characteristic of the system. The Freundlich isotherm is widely used, but provides no information on the monolayer adsorption capacity, in contrast to the Langmuir model [7].

The results of simulation with Freundlich and Langmuir models are illustrated in Fig. 1. The adsorption constants estimated from the isotherms and the corresponding correlation coefficients are given in Table 1. Both models had a good agreement with the data for Cu and Cd biosorption, evidenced by the high r^2 values (all greater than 0.978). However, prediction of Pb adsorption by Langmuir isotherm was less precisely with a lower r^2 value of 0.883, in contrast to better fits of the Pb adsorption data by Freundlich model ($r^2 = 0.970$). This seems to suggest that Pb biosorption by *Enterobacter* sp. J1 was more likely heterogeneous surfaced adsorption, instead of monolayer sorption. Moreover, there also exists a possibility that in addition to surface binding, other mechanisms may also contribute to the uptake of Pb (e.g. intracellular uptake). This issue will be further assessed in the following sections. From simulation with Langmuir isotherm, the predicted maximum adsorption capacity ($q_{\max}$) was 50.9, 32.5 and 46.2 mg/g dry cell for Pb, Cu and Cd, respectively, (Table 1). The Pb also had the lowest K_d value of 13.6 mg/l, much lower than that of Cu (30.6 mg/l) and Cd (235 mg/l). Inspection of the two adsorption constants ($q_{\max}$ and K_d) suggests that *Enterobacter* sp. J1 was more efficient in adsorbing Pb than the other two adsorbates.

3.2. Adsorption dynamics

The pseudo-second-order adsorption and the intraparticle diffusion model were often used to describe the kinetics of biosorption. The second-order kinetic model [9] is expressed as:

$$\frac{t}{q} = \frac{1}{k_1 q_e^2} + \frac{t}{q_e} \quad (3)$$

where q_e and q are the amounts of heavy-metal adsorbed on adsorbent (mg/g) at equilibrium and at time t, respectively; k_1 (mg/g min) is the rate constant of second-order adsorption [8,9]. From Eq. (3), the amounts of heavy-metal adsorbed on adsorbent (mg/g) at time t can be re-arranged as:

$$q = \frac{t}{\frac{1}{k_1 q_e^2} + \frac{t}{q_e}} \quad (4)$$

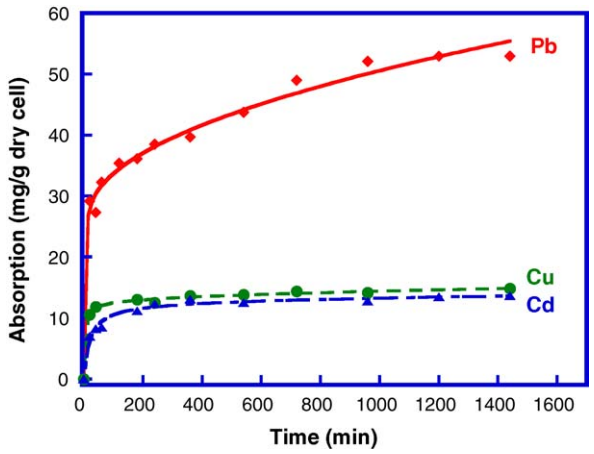

Fig. 2. Time-course adsorption of Pb, Cu and Cd by *Enterobacter* sp. J1 at an initial metal concentration of 100 mg/l (symbols: experimental data; lines: prediction by the combinative model developed in this study).

The intraparticle diffusion equation [10] can be described as:

$$q = k_i t^{1/2} \quad (5)$$

where k_i is the intraparticle diffusion rate constant (mg/g/min$^{0.5}$).

The time-course adsorption data (Fig. 2) show that adsorption of Pb, Cu and Cd occurred rapidly within the first 100 min. This is quite normal because biosorption is considered a spontaneous process and thereby often occurs very rapidly [4]. Adsorption of Cu and Cd leveled off after the early rapid adsorption stage, but the trend of Pb sorption seemed to have a second adsorption phase after 100 min. This suggest that adsorption of Pb may undergo different mechanisms from that of Cu and Cd adsorption. This argument is also supported by the distinct adsorption isotherm behavior for Pb observed in the model simulation results (Fig. 1 and Table 1). In light of this, it is suspected that the uptake of heavy-metals by the *Enterobacter* sp. J1 strain was probably not only due to cell-surface binding, but also via intracellular accumulation [3]. In order to take both mechanisms into account, a new model that combines the second-order kinetic model (Eq. (4)) and the intraparticle diffusion model (Eq. (5)) together is proposed as follows:

$$q = k_i t^{0.5} + \frac{t}{\frac{1}{k_1 q_e^2} + \frac{t}{q_e}} \quad (6)$$

The simulation results of this model is depicted in Fig. 2, showing that the model was able to predict the data quite well ($r^2 > 0.99$; Table 2). This suggests that the new combinative model described in Eq. (6) can be used to describe metal uptake by the bacterial biomass with the consideration of both cell-surface binding and intracellular accumulation. The model developed here is particularly suited to interpret the kinetics of metal uptake when cell-surface adsorption is not the only route. The kinetic constants obtained from the model are also indicated in Table 2. It shows that the value of intrapartical diffusion rate constant (k_i) is very small for Cu (0.0716 mg/g/min$^{0.5}$) and Cd (0.0716 mg/g/min$^{0.5}$). In contrast, the k_i value for Pb (0.763 mg/g/min$^{0.5}$) was 10-time larger than that for Cu and Cd. With a much larger k_i, the possibility of intracellular accumulation seems to be relatively higher for Pb uptake, while Cu and Cd adsorption may be due primarily to cell-surface binding. Comparing the adsorption results of the three metal ions in Fig. 2, it is also likely that intracellular accumulation played a crucial role in a higher uptake capacity of Pb.

Table 2
Comparison of the rate constants (k_1 and k_i) and equilibrium adsorption capacity (q_e) obtained from simulation of the time-course biosorption data of Pb, Cu and Cd by a new combinative model (Eq. (6)) developed in this study

Adsorbate	k_1 (kg/g/min)	q_e (g/kg)	k_i (kg/g min)	r^2
Pb	0.0238	26.5	0.763	0.994
Cu	0.0229	12.3	0.0716	0.998
Cd	0.0043	12.0	0.0473	0.992

3.3. Effect of pH on desorption efficiency

Regeneration of biosorbent for repeated uses is a critical issue in practical application of the biosorbent. The recovery of heavy-metals from metal-laden biomass has been approached by utilizing various desorption agents, including HCl, H_2SO_4, Na_2CO_3, EDTA and β-mercaptoethanol [6,11–13]. Among those approaches, decreasing pH value by HCl appeared to have the best desorption efficiency [5,6] and was thus selected as the desorption agent in the study. To determine the optimal pH for metal desorption, the amount of metals released from the acid-treated biomass at different pH was determined. Basically, metal recovery was invisible for pH > 4.0, below which the desorption efficiency increased rapidly as the pH kept decreasing (Fig. 3). Desoprtion of Cd was nearly complete for pH ≦ 3, while desorption efficiency of Cu and Pb reached 90% when pH was decreased to below 2.0. Therefore, it is

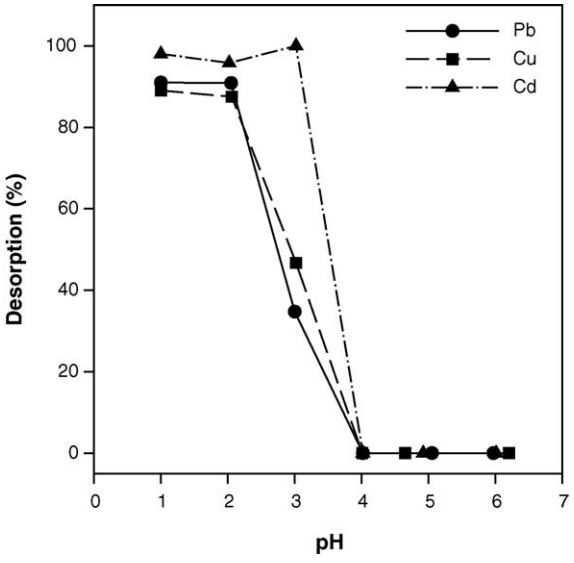

Fig. 3. The effect of pH on the desorption efficiency of Pb, Cu and Cd from metal-loaded biomass of *Enterobacter* sp. J1.

Table 3
Efficiency of biosorbent regeneration and metal recovery during repeated adsorption/desoprtion (A/D) cycles

Metal	A/D cycle	Biosorbent regeneration efficiency (%)[a]	Metal recovery efficiency (%)[b]
Pb	1	92	97
	2	83	95
	3	80	92
	4	75	90
Cu	1	94	98
	2	88	97
	3	83	94
	4	79	92
Cd	1	96	99
	2	93	94
	3	91	95
	4	90	93

[a] Bosorbent regeneration efficiency = (regenerated adsorption capacity/original adsorption capacity) × 100%.
[b] Metal recovery efficiency = (amount of metal recovered/amount of metal adsorbed) × 100%.

concluded that the optimal pH for metal recovery is 2.0 for Pb- and Cu-loaded biosorbent and 3.0 for biomass containing Cd. Besides, the results in Fig. 3 also suggest that the binding strength of Cd on cell mass seems to be weaker than that of Pb and Cu so that Cd could be completely recovered at a higher pH.

3.4. Adsorption/desorption (A/D) cycles

Repeated adsorption/desorption operations were performed to examine the reusability and metal recovery efficiency of the biomass. As shown in Table 3, with four A/D cycles, the Pb uptake capacity decreased slightly to attain 75% of the original capacity and for all the cycles over 90% of adsorbed Pb was recovered with HCl-driven desorption (pH 2.0). Biomass of *Enterobacter* sp. J1 exhibited similar biosorbent regeneration and metal recovery efficiencies for Cu and Pb during A/D cycles. The capacity of Cu uptake maintained more than 79% of the original capacity after four A/D cycles, while the recovery of Cu was around 92–98% (Table 3). Compared to Pb and Cu results, the regeneration efficiency for Cd adsorption was found to be higher (90–96%) at each A/D cycles (Table 3). The recovery efficiency of Cd was also around 93–99%. These results indicate that biomass of *Enterobacter* sp. J1 could be used repeatedly with high regeneration and metal recovery efficiencies.

3.5. SEM/EDS analysis

The SEM micrographs of heavy-metal-free and metal-loaded *Enterobacter* sp. J1 are shown in Fig. 4. It is observed that the cell-surface morphology considerably changed after metal biosorption. Fig. 4(b)–(d) show that the surface of metal-loaded cells looked vague and distorted and seemed to be damaged by the heavy-metal ions. The alteration in morphology may also result from secretion of extracellular polymeric substance during metal biosorption, as reported by Chen et al. [14] who utilized *Desulfovibrio desulfuricans* to adsorb zinc and copper. Moreover, the EDS analysis (Fig. 5) confirmed the presence of metal adsorbates on the cell mass, giving a direct detection of metals on cells. Note that most biosorption studies determined metal sorption by measuring the residual metal concentrations in the supernatant, but did not directly prove the presence of the metals on the biomass.

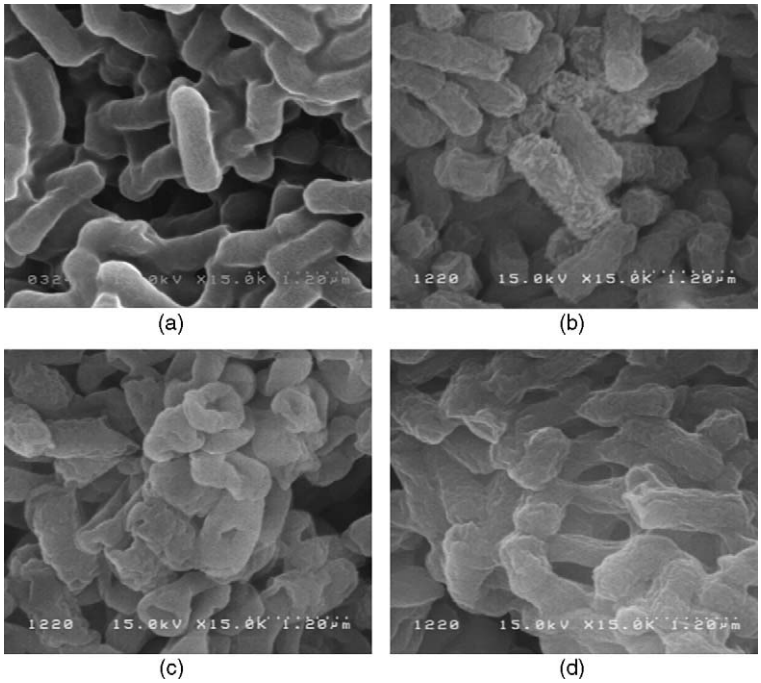

Fig. 4. SEM micrograph of *Enterobacter* sp. J1: (a) control (before biosorption); (b) after Pb biosorption; (c) after Cu biosorption; (d) after Cd biosorption.

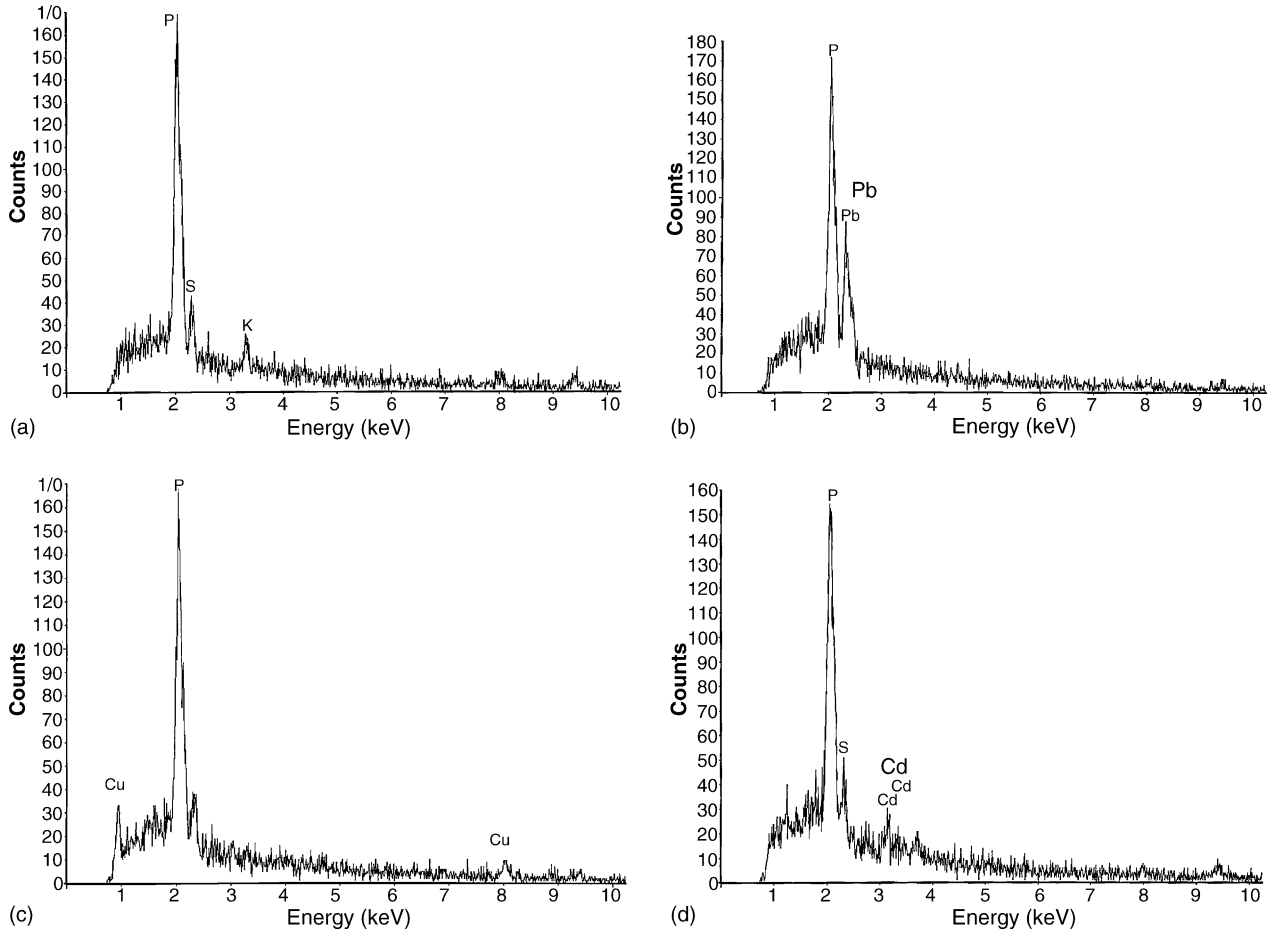

Fig. 5. EDS analysis of *Enterobacter* sp. J1: (a) control (before biosorption); (b) after Pb biosorption; (c) after Cu biosorption; (d) after Cd biosorption.

4. Conclusions

A local bacterial isolate *Enterobacter* sp. J1 exhibited good metal uptake capacity and high resistance to various heavy-metals. The biosorbent was able to adsorb Pb, Cu and Cd with a capacity of 50.9, 32.5 and 46.2 mg/g dry cell, respectively. According to model simulations of adsorption kinetics and isotherms, both cell-surface binding and intracellular accumulation seemed to be involved in Pb uptake. A new combinative model developed in this work successfully predicts the metal biosorption kinetics of *Enterobacter* sp. J1. The pH adjustment with HCl achieved over 90% recovery of Pb, Cu and Cd from metal-loaded biomass. The regenerated biosorbent can achieve 75–90% of its original adsorption capacity after repeated adsorption and desorption operations for four times. Hence, the biosorbent seems to have the potential to be repeatedly used for practical applications. With the advantages of high metal uptake capacity, satisfactory recovery efficiency and high metal tolerance, *Enterobacter* sp. J1 appears to have the potential to become an effective adsorbent for the removal and recovery of heavy-metals from polluted wastewater.

Acknowledgements

The authors are grateful to Prof. Wen-Ming Chen at National Kaoshung Marine University for his assistance in identifying the bacterial strain used in this work.

References

[1] D. Kratochvil, B. Volesky, Advances in the biosorption of heavy-metals, Trends Biotechnol. 16 (1998) 291–300.
[2] M. Tsezos, Biosorption of metals. The experience accumulated and the outlook for technology development, Hydrometallurgy 59 (2001) 241–243.
[3] B. Volesky, Z.R. Holan, Biosorption of heavy-metals, Biotechnol. Prog. 11 (1995) 235–250.
[4] B. Volesky, Detoxification of metal-bearing effluents: biosorption for the next century, Hydrometallurgy 59 (2001) 203–216.
[5] J.S. Chang, R. Law, C.C. Chang, Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21, Water Res. 31 (1997) 1651–1658.
[6] P.K. Wong, K.C. Lam, C.M. So, Removal and recovery of Cu(II) from industrial effluent by immobilized cells of *Pseudomonas putida* II-11, Appl. Microbiol. Biotechnol. 39 (1993) 127–131.

[7] D.G. Cetinkaya, Z. Aksu, A. Ozturk, T. Kutsal, A comparative study on heavy-metal biosorption characteristics of some algae, Process Biochem. 34 (1999) 885–892.
[8] Y.S. Ho, G. McKay, The sorption of lead (II) on peat, Water Res. 33 (1999) 578–584.
[9] Y.S. Ho, G. McKay, Pseudo-second-order model for sorption processes, Process Biochem. 34 (1999) 451–465.
[10] M.S. Chiou, H.Y. Li, Adsorption behavior of reactive dye in aqueous solution on chemical cross-linked chitosan beads, Chemosphere 50 (2003) 1095–1105.
[11] A. Nakajima, T. Sakaguchi, Accumulation of uranium by basidiomycetes, Appl. Microbiol. Biotechnol. 38 (1993) 574–578.
[12] M.P. Pons, M.C. Fuste, Uranium uptake by immobilized cells of *Pseudomonas* strain EPS 5028, Appl. Microbiol. Biotechnol. 39 (1993) 661–665.
[13] J.S. Chang, J. Hong, Biosorption of mercury by the inactivated cells of *Pseudomonas aeruginosa* PU21(Rip64), Biotechnol. Bioeng. 44 (1994) 999–1006.
[14] B.Y. Chen, V.P. Utgikar, S.M. Harmon, H.H. Tabak, D.F. Bishop, R. Govind, Studies on biosorption of zinc(II) and copper(II) on *Desulfovibrio desulfuricans*, Int. Biodeterior. Biodegrad. 46 (2000) 11–18.