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Biosorption and Desorption of Copper (II) Ions by *Bacillus* sp.

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Abstract

Batch biosorption experiments were conducted to investigate the removal of Cu^{2+} ions from aqueous solutions by a series of bacterial strains isolated from a local activated sludge process. The characteristics of 12 isolates were identified and examined for their ability to bind Cu^{2+} ions from aqueous solution. Among the isolates, two species exhibited biosorption capacity >40 mg of Cu/g of dry cell. Isotherms for the biosorption of copper on bacterial cells were developed and compared, and the equilibrium data fitted well to the Langmuir and Freundlich isotherm models. The biosorption of copper increased significantly with increasing pH from 2.0 to 6.0 regardless of the species. More than 90% of copper sorbed on the cells of *Bacillus* sp. could be recovered by washing with 0.1 *M* HNO_3 for 5 min. The performance of two different desorption processes was also tested and compared. The results show that five biosorption and desorption cycles are a better operation process than five successive biosorptions followed by one desorption to remove and recover copper from aqueous solution. The biosorbent could be used for at least five biosorptions and desorption cycles without loss of copper removal capacity. It can be concluded that the activated sludge or sludge-isolated bacteria could be a potential biosorbent for copper removal.

Index Entries: Activated sludge; bacteria; bioremediation; copper; desorption; heavy metal; metal removal; wastewater treatment process.

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Introduction

Heavy metals, namely copper, zinc, nickel, chromium, cobalt, and silver, are commonly found in the effluents from electroplating and metal-processing industries. Heavy metals are also the major waste constituents from the manufacturing of paints, plastics, batteries, alloys, and scientific instruments. It is well documented that are at high concentrations, heavy metals toxic to living organisms, particularly to those in aquatic environments. In the past few decades, metal-laden effluent discharges into municipal sewers without treatment have been strictly prohibited. In recent years, more stringent industrial effluent discharge standards have been promulgated. In Hong Kong, effluents from industrial sources are required to be pretreated to substantially reduce the heavy-metal contents to an acceptable level before discharging into municipal sewers, especially for Cu *(1)*.

Conventional methods employed for the removal of Cu ions from industrial effluents include chemical precipitation, filtration, electrochemical treatment, and ion exchange. Most of these methods are expensive and incapable of removing trace levels of Cu ions. The chemical precipitation method produces a large amount of sludge for disposal. In addition to physical and chemical methods, the potential of biosorption has been demonstrated *(2,3)*.

Biomass of bacteria has been known to readily adsorb or accumulate metal ions. Bacteria's ability to uptake metals has attracted much attention because of its potential use as an effective and economic means for the remediation of wastewater polluted by heavy metals *(4)*. Microbial cells can be supplied as waste in industrial fermentation process and biologic wastewater treatment processes *(5–7)*. Hence, biosorption may provide an economical and effective alternative to the conventional techniques for Cu removal.

It is important 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. To optimize design and operation of a biosorbent system for Cu removal, a thorough understanding of biosorption behavior and desorption kinetic characteristics of microbial cells is needed. This motivated us to evaluate the feasibility and ability of the activated sludge or sludge-isolated bacteria to remove Cu in wastewater. The Cu biosorption characteristics of one of the bacterial isolates, *Micrococcus* sp., have been reported previously *(8)*.

In the present study, the biosorption characteristics of other bacterial strains isolated (*Bacillus* sp. and *Pseudomonas* sp.) from activated sludge were examined and compared with *Micrococcus* sp. The effects of Cu concentration and pH on biosorption were systematically examined. The desorption kinetics, efficiency of Cu removal and recovery by repeated biosorption and desorption operations, efficiency of the desorption of metals from metal-loaded biosorbents, and reusability of *Bacillus* sp. were studied.

Materials and Methods

Isolation Procedures and Identification

Fresh activated sludge was collected from the return sludge channel at a local sewage treatment plant. The sludge was serially diluted in distilled and deionized water. Aliquots (0.1 mL) were spread on nutrient agar (Difco) and cultivated in an incubator at 30°C for 3 d. Twelve different colonies were picked up and maintained on the same medium for the following metal biosorption test. The colonial characteristics of these isolates were studied. The isolates were identified using the MIDI Sherlock Microbial Identification System and API 20 NE as well as 20 E systems.

Preparation of Biosorbent

The bacterial cells of each isolate were grown in 1-L conical flasks containing 100 mL of nutrient broth at 30°C with shaking at 200 rpm. The 72-h cultivated cells were harvested by centrifuging (Beckman J21-21 Model) at 10,000*g* for 30 min. After rinsing twice with distilled and deionized water, the cells were then suspended in a designated volume of distilled and deionized water for preparing the biomass stock solution. The concentration of biomass stock solution was determined by oven drying at 105°C for 24 h after filtration before and after biosorption experiments.

Biosorption

The biosorbent at a final concentration of 1 to 2 g of cell/L was suspended in a 100-mL solution containing 100 mg of Cu/L in a polypropylene bottle, which was gently agitated (250 rpm) at 25°C. The pH of the metal solution was adjusted to 5.5 by 0.1 *M* NaOH and 0.1 M HNO_3 just before experimentation and at 9 h during the experiment. Samples of 5 mL were taken from the solution at 3 and 12 h and subsequently centrifuged at 10,000*g* for 10 min. The concentration of each heavy metal in the supernatants was determined using a Perkin-Elmer atomic absorption spectrophotometer model 100. To determine the effect of pH on Cu biosorption, the metal solutions and the bacterial suspensions were adjusted separately to the desirable pH (2.0–6.0) by the addition of 0.1 *M* NaOH and 0.1 *M* HNO_3 and mixed. The mixture was then incubated at an initial Cu concentration of 100 mg/L at 25°C on an orbital shaker for 12 h.

Desorption Kinetics

After the biosorption experiment on one selected isolate, *Bacillus* sp., the metal-loaded bacterial cells were harvested from the cell and metal suspensions initially containing 0, 2, and 50 mg/L of Cu, respectively. The bacterial cells were then rinsed with distilled and deionized water and resuspended in 0.1 *M* HNO_3 solution. After gentle shaking, samples were taken from the suspensions at designated time intervals. The samples were centrifuged immediately, and the metal concentration in the supernatant was determined.

Biosorption and Desorption Cycles

Bacterial cells with a final concentration of 1 to 2 g of cell/L were suspended in metal solutions containing 0, 2, and 50 mg/L of Cu in centrifuge tubes at pH 5.0. The tubes were shaken at 25°C for 12 h. After 12 h, the metal-sorbed cells were centrifuged, rinsed with distilled and deionized water, and resuspended in 35 mL of 0.1 *M* HNO_3 for 3 h in order to recover the metal ions from the cells. The regenerated biosorbents were again suspended in metal solutions for the next biosorption run. The biosorption and desorption steps were repeated five times. The metal concentrations in the supernatants were determined after biosorption and desorption.

Successive Biosorption and Desorption

Bacterial cells at a final concentration of 1 to 2 g of cell/L were suspended in solutions containing 0, 2, and 50 mg/L in centrifuge tubes. The pH of the cells and metal solutions was adjusted to 5.0 by adding 0.1 *M* HNO_3 and NaOH. The tubes were shaken at 25°C for 12 h. The tubes were then centrifuged, and the supernatant was decanted and analyzed for unsorbed Cu. The centrifuged cells were resuspended in 35 mL of fresh Cu solution and Cu sorption took place for a further 12 h. This procedure was repeated five times. After the fifth sorption cycle, Cu from the cells was desorbed by extraction with 0.1 *M* HNO_3. The amount of sorbed or desorbed Cu was calculated by measuring the decrease or increase in Cu in the contacting solution.

Results and Discussion

Isolation and Identification

The isolation procedure yielded about 12 strains. All the heterotrophic aerobic isolates from the activated sludge showed a wide variety of species including Gram-negative and -positive bacteria. Most of them were rods and a few bacteria were filamentous and coccus. These bacterial isolates were identified; their identities are listed in Table 1.

Biosorption

Table 1 shows that the Cu removal data of bacterial isolates are characterized by large variations in biosorption capacity. The amount of biosorption ranged between 2.9 and 42 mg/g. Metal binding on the cell surface is possible through phosphoryl, carboxyl, sulfydryl, and hydroxyl groups of cell envelope *(2)*. These different sorption capacities would be a consequence of the structure and chemistry in the bacterial envelope. Three isolates sorbed Cu more than 20 mg/g: *Bacillus* sp. (21 mg/g), *Pseudomonas* sp. (37 mg/g), and *Micrococcus* sp. (42 mg/g). These three isolates were chosen for biosorption isotherm study on the basis of their higher efficiency in removing Cu from solution than the other isolates.

Table 1
Identification and Cu^{2+} Removal Capacity of Isolates

Isolates	Cu removal capacity (mg Cu/g dry cell)
Bacillus sp.	21.0
Pseudomonas sp.	37.0
Micrococcus sp.	42.0
Neisseria sicca	5.6
Aeromonas hydrophila	2.9
Pseudomonas sp.	3.8
Xanthomonas maltophilia	3.1
Bacillus lentimorbus	2.9
Pseudomonas sp.	3.9
Bacillus subtilis	8.4
Gordona bronchialis	7.5
Kocuria varians	5.5

Effects of pH on Biosorption

Cu uptake was negligible at pH 2.0 and then increased rapidly as the pH was increased from 3.0 to 5.0 (Fig. 1). The pH of the solution obviously affected Cu removal by the three selected isolates. As suggested in previous studies *(6,9)*, the biosorption process of metal is analogous to the ion-exchange process. Therefore, metal cations and protons compete for binding sites on the cell walls as pH decreases, lowering uptake of metal. Additional support for this assumption was the finding that Cu could easily be desorbed from the cells by lowering the pH in the following desorption study. This finding also indicated that the Cu removed was mainly bound to cell walls and external surfaces of the biomass.

Biosorption Isotherms

Biosorption isotherms for Cu are presented in Fig. 2. The estimated model parameter values and the correlation coefficients for the Langmuir and the Freundlich isotherms are given in Table 2. The goodness of fit was satisfactory over the range of the experiments. Values of Langmuir maximum biosorption capacity, q_{max}, for the different materials ranged from 21 to 47 mg/g. The Freundlich coefficient k ranged from 4.9 to 15 mL/g. These values varied among the tested biosorbents, indicating large difference in their Cu sorption behavior.

Based on the Langmuir biosorption maximum, q_{max}, the species of *Micrococcus* and *Pseudomonas* showed the highest biosorption capacity. However, the corresponding Freundlich coefficient, k, was similar among the three species. The parameters k and q_{max} reflect different characteristics. The Freundlich k represents the amount of Cu sorbed when the solution

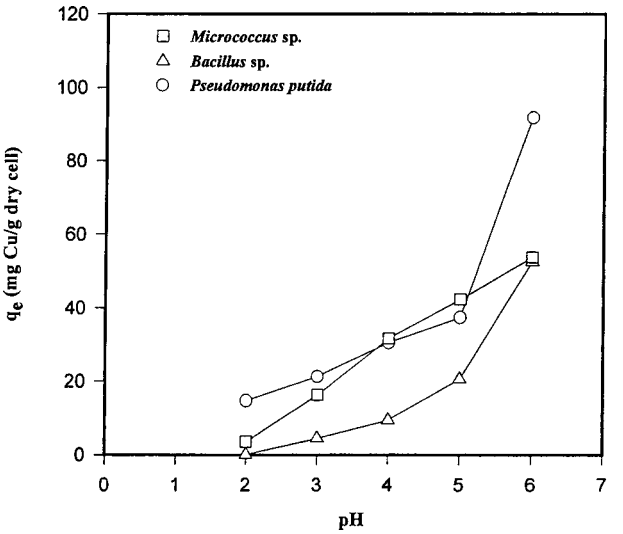

Fig. 1. Effects of pH of solution on Cu removal capacity of three isolates at initial concentration of 100 mg of Cu/L.

concentration in the equilibrium is unity. On the other hand, q_{max} represents the saturation level of sorbed Cu at high solution concentrations. Similarly, the parameters n and b are also not directly comparable. The parameter n measures the extent of impact on biosorption of a change in residual solution concentration from unity (a high value for n implies a relatively large change in sorbed Cu when the residual Cu concentration deviates from unity, either above or below it). By contrast, the parameter b measures the affinity of the biosorbent for the solute (a high value of b means a high sorption level at low solution concentration). Thus, the Freundlich parameter k and the Langmuir parameter b both measure, in a sense, the effectiveness of Cu biosorption at low Cu concentration in solution.

Micrococcus sp. showed the highest values for b and q_{max}, indicating a large capacity for Cu biosorption at all solution concentrations. The q_{max} value for *Pseudomonas* sp. was slightly higher, indicating a high saturation level at high Cu concentration. The relatively low value of k indicates that *Pseudomonas* sp. sorbed less Cu at a low solution concentration. Thus, the biosorption capacity is affected by solution Cu concentration. Despite having a lower q_{max} than the other two bacteria, *Bacillus* sp. sorbed more Cu at a low solution concentration. This indicated that *Bacillus* sp. is potentially useful for removing Cu from solutions at low concentration. Activated sludge showed moderate biosorption capacity at low or high concentrations in comparison with the other bacteria tested. The observed Cu biosorption capacities of *Pseudomonas* sp. and *Micrococcus* sp. were higher than those reported (23 mg/g dry cell) for isolated *Pseudomonas aeruginosa* PU 21 from hospital sewage *(10)* and other microorganisms such as yeast and fungal biomass *(11,12)*.

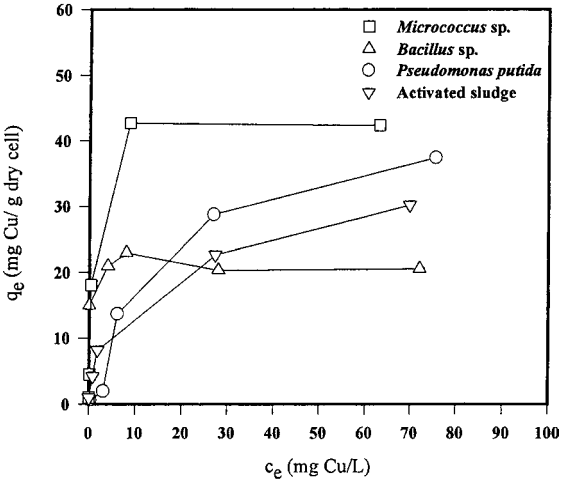

Fig. 2. Biosorption isotherms of three isolates and activated sludge at pH 5.0.

Table 2
Parameters of the Langmuir and Freundlich Isotherms
for Activated Sludge and Bacteria

	Langmuir equation			Freundlich equation		
	q_{max}	b	r^2	k	n	r^2
Activated sludge	31	0.20	0.99	4.9	0.46	0.99
Micrococcus sp.	43	2.06	0.99	14.0	0.37	0.96
Pseudomonas sp.	47	0.06	0.99	14.0	0.37	0.96
Bacillus sp.	21	1.0	0.99	15.0	0.12	0.94

Kinetics of Desorption

The kinetics of the desorption of Cu from the Cu-loaded cells of *Bacillus* sp. is demonstrated in Fig. 3. It can clearly be seen that Cu desorbed very rapidly, and the desorption reached equilibrium within 15 min regardless of nearly or partially Cu-saturated biomass. Chang et al. *(10)* also found that equilibrium was shortly reached after 5 min contact in the case of *P. aeruginosa* PU 21 for Pb, Cu, and Cd desorption. The desorption efficiency was about 95%. Diluted HNO_3 (0.1 *M*) is efficient for the recovery of Cu from *Bacillus* sp.

Biosorption and Desorption Cycles

Figures 4 and 5 illustrate the metal removal and recovery efficiencies of *Bacillus* sp. during five regeneration cycles. There was no significant difference in the Cu biosorption capacity of the biomass from cycle 1 to 5.

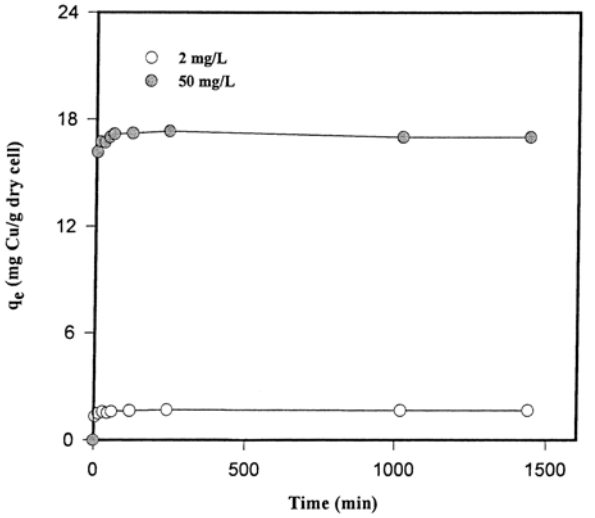

Fig. 3. Desorption kinetics on metal-loaded *Bacillus* sp. With 2 and 50 mg of Cu/L for 24 h followed by washing with 0.1 *M* HNO_3.

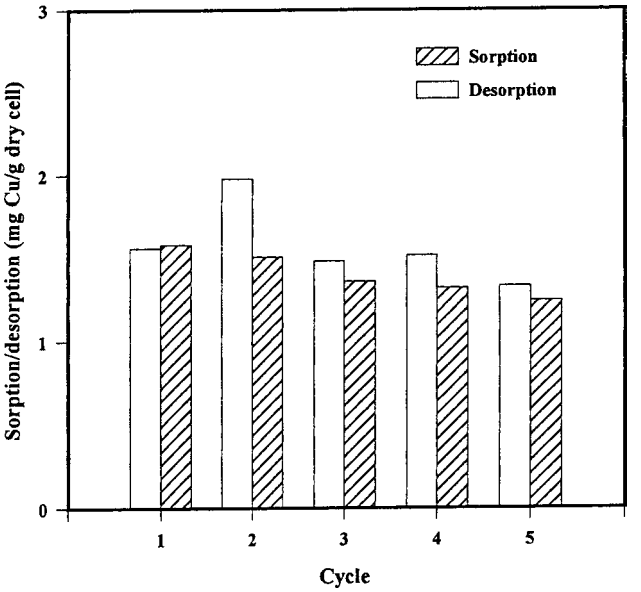

Fig. 4. Sorption/desorption cycles for Cu at pH 5.0 and 2 ppm.

For all cycles, about 90% and 95% of sorbed Cu could be recovered by 0.1 *M* HNO_3–induced desorption at low and high Cu-loaded biomass, respectively. Cu recovery from the biomass was very effective with 0.1 *M* HNO_3. In addition, the biosorption capacity of the biomass in subsequent cycles was not reduced by 0.1 *M* HNO_3.

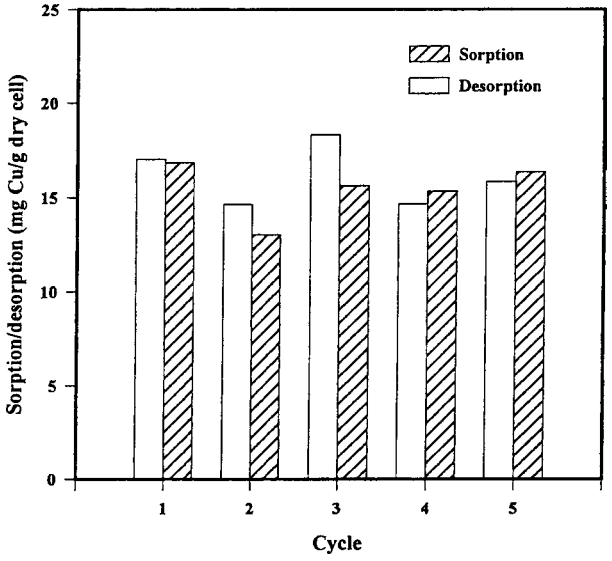

Fig. 5. Sorption/desorption cycles for Cu at pH 5.0 and 50 ppm.

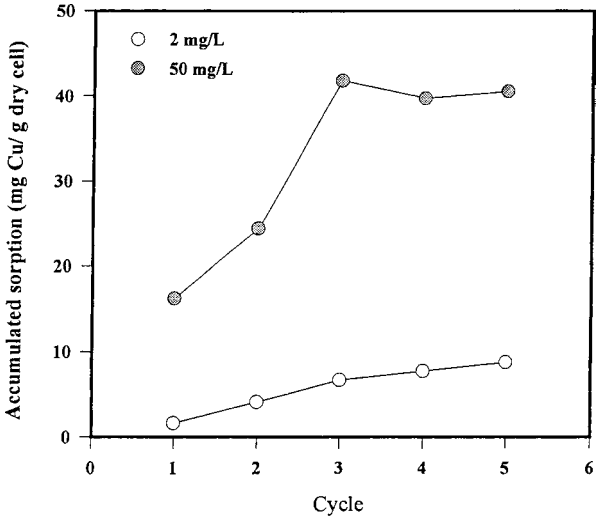

Fig. 6. Successive sorption cycles for Cu with *Bacillus* sp. At pH 5.0.

Successive Biosorption and Desorption

Bacillus sp. were further evaluated by repeatedly contacting the materials with Cu concentrations in order to gain a better insight into the long-term Cu sorption behavior. The accumulated sorption curves for *Bacillus* sp. during repeated contacting with Cu solutions over five cycles are shown in Fig. 6. Repeatedly contacting *Bacillus* sp. with a high Cu concentration solution resulted in faster attainment of steady saturation level within three

Table 3
Performance of Two Different Biosorption and Desorption Processes

	Five successive biosorption and one desorption cycles		Five biosorption and desorption cycles	
	Total sorption (mg/g)	Total desorption (mg/g)	Total sorption (mg/g)	Total desorption (mg/g)
2 mg/L	8.8	5.9	7.9	7.0
50 mg/L	42.0	37.0	81.0	77.0

cycles and much higher sorption than indicated by the Langmuir sorption maximum. At low Cu concentration contact, Cu uptake continued at a steady rate in small increments even after five cycles since the amount of Cu sorbed during repeated contacting was less than the Langmuir sorption maximum. Therefore, the biomass could be used repeatedly without desorption to remove Cu from a low Cu solution.

The total amount of Cu sorbed onto the *Bacillus* sp. in the two different biosorption and desorption processes is compared in Table 3. At high Cu concentration, the alternative biosorption and desorption process could remove more copper. More frequent regeneration of Cu sorption sites was needed to maintain the removal efficiency at high Cu concentration (higher than the Langmuir sorption maximum, q_{max}). Unlike the high concentration, the amount of Cu sorbed for reused cells and regenerated cells was quite similar at low concentration. Five successive biosorptions with one desorption process is less expensive and more environmentally friendly in the treatment of low effluent Cu. Less desorption solution will be used to regenerate the sorption sites. However, the total amount of Cu desorbed from successively reused cells without desorption was lower than that from cells regenerated by desorption during each cycle. The unrecoverable Cu in reused cells after five successive sorptions might be owing to the Cu diffusion into the interior of the cell wall.

Conclusion

Among the 12 isolates, *Micrococcus* sp., *Pseudomonas* sp., and *Bacillus* sp. exhibited Cu biosorption capacity >20 mg/g of dry cell. Copper biosorption by these bacterial strains was strongly affected by Cu solution concentration. Based on the Freundlich parameter *k*, *Bacillus* sp. was found to have a biosorption extent comparable with that of *Micrococcus* sp. at low Cu concentration. The biosorption of Cu increased significantly with increasing pH from 2.0 to 6.0 regardless of the species, thereby suggesting ion exchange as one of the dominant biosorption mechanisms. More than 90% of Cu sorbed on the cells of *Bacillus* sp. could be recovered by washing with 0.1 *M* HNO_3 for 5 min. Alternative biosorption and desorption cycles was a better operation process than successive biosorption followed by

one desorption to remove and recover Cu from aqueous solution that had a total amount of Cu higher than the Langmuir sorption maximum. *Bacillus* sp. was used for at least five sorption and desorption cycles without loss of Cu removal capacity. In conclusion, activated sludge or sludge-isolated bacteria could be a potential biosorbent for Cu removal.

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References

1. Environmental Protection Department (1991), Technical memorandum—Standards for effluents discharged into drainage and sewerage systems, inland and coastal waters, Hong Kong Government, Hong Kong SAR.
2. Volesky, B. and Holan, Z. R. (1995), *Biotechnol. Prog.* **11,** 235–250.
3. Butter, J., Evison, L. M., and Hamcock, I. C. (1998), *Water Res.* **32(2),** 400–406.
4. Lo, W., Chua, H., Wong, M. F., and Yu, P. F. (2003), *Water Sci. Technol.* **47,** 251–256.
5. Kasan, H. C. (1993), *Crit. Rev. Environ. Sci. Technol.* **23(1),** 79–117.
6. Lo, W., Chua, H., Lam, K. H., and Bi, S. P. (1999), *Chemosphere* **39(15),** 2723–2736.
7. Leung, W. C., Wong, M. F., Chua, H., Lo, W., Yu, P. H. F., and Leung, C. K. (2000), *Water Sci. Technol.* **41(12),** 233–240.
8. Wong, M. F., Chua, H., Lo, W., Leung, C. K., and Yu, P. H. F. (2001), *Appl. Biochem. Biotechnol.* **91–93,** 447–457.
9. Zhang, L., Zhao, L., Yu, Y., and Chen, C. (1998), *Water Res.* **32(5),** 1437–1444.
10. Chang, J. S., Law, R., and Chang, C. C. (1997), *Water Res.* **31(7),** 1654–1658.
11. Volesky, B. and May-Phillips, H. A. (1995), *Appl. Microbiol. Biotechnol.* **42,** 797–806.
12. Niu, H., Xu, X. S., Wang, J. H., and Volesky, B. (1993), *Biotechnol. Bioeng.* **42,** 785–787.