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Biosorption of Copper by Immobilized Biomass of *Pseudomonas stutzeri*

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Abstract The kinetics of copper ion biosorption by *Pseudomonas stutzeri* cells immobilized in alginate was investigated. During the first few minutes of the metal uptake, the copper biosorption was rapid, and then became progressively slower until an equilibrium was reached. At a biomass concentration of 100 g/l, the copper biosorption reaction reached approximately 90% of the equilibrium position within 30 min. A Freundich-type adsorption isotherm model was constructed based on kinetics with different amounts of biomass. When using this model, the experimental values only agreed well with the predicted values in a solution containing less than 200 mg/l Cu(II). Desorption of the bound copper ions was achieved using electrolytic solutions of HCl, H_2SO_4, EDTA, and NTA (0.1 or 0.5 M). Metal desorption with 0.1 M NTA allowed the reuse of the biosorbent for at least ten consecutive biosorption/desorption cycles, without an apparent decrease in its metal biosorption capability. A packed-bed column reactor of the immobilized biomass removed approximately 95% of the metal in the first 30 liter of wastewater [containing 100 mg/l Cu(II)] delivered at a rate of 20 L/day, and, thereafter, the rate gradually decreased.

Key words: *Pseudomonas stutzeri*, biosorbent, immobilized biomass, biosorption/desorption cycles, copper, NTA

The contamination of wastewater by toxic metal ions is a worldwide environmental problem. Increasingly stringent controls are being imposed on those industries that discharge such metals in their effluent. As a result, improved technologies are being sought to achieve low residual metal concentrations in final effluents. The biosorption of heavy metals is one of the most promising technologies involved in the removal of toxic metal ions from industrial wastewater. Although other conventional processes, such as chemical precipitation, ion exchange, reverse osmosis, and solvent extraction, remove toxic heavy metals from industrial effluents, biosorption employs inexhaustible, inexpensive, nonhazardous materials, and generates low volumes of nonhazardous waste [10, 13]. Recently, many studies have been carried out on the development of highly efficient biosorbents and biosorption-based treatment processes for the removal of toxic metal ions from contaminated wastewater [3, 13], and some systems have since become commercially available in Europe and U.S.A. [1, 3].

The majority of the commercially oriented research in the field of decontamination of heavy metals has concentrated on the use of an immobilized cell system, instead of free living or dead biological material. In particular, microbial cells, or cell derivatives, have been incorporated into polyacrylamide, alginate, carrageenan, agar, or fibrin beads for the use in packed bed columns [7, 12, 15, 16, 22, 27]. The main reasons for using this form of reactor are that immobilized cell systems can be conventionally modeled, the biosorbent beads are robust, thereby permitting the biomass to be reused, and the use of beads simplifies the phase separation after the metal uptake [8].

The principal mechanism of metallic cation sequestration involves the formation of complexes between a metal ion and the functional groups (carboxyl, carbonyl, amino, amido, sulfonate, phosphate, and others) present on the surface or inside the porous structure of the biological material. Recently, it was demonstrated that carboxyl groups of alginate play a major role in the complexation of heavy metals when using the dead biomass of the brown seaweed *Sargassum fluitants* [15]. Alginate is a common term for a family of linear polysaccharides containing 1,4-linked β-D-mannuronic and α-L-guluronic acid residues arranged in a nonregular, blockwise fashion along the chain. Calcium-alginate immobilized *Zoogloea ramigera* exhibits a high absorption efficiency, as much as 96% for

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Cd, Zn, Mn, Cu, and Sr in synthetic wastewater, when using an air-bubble column reactor [14]. Accordingly, an attempt was made to apply *Pseudomonas stutzeri*, isolated in a previous study [5], as an efficient copper biosorbent in a sodium-alginate immobilized cell system. The biosorption kinetics of the immobilized cell system were investigated and an isotherm curve constructed to evaluate the copper biosorption performance of the immobilized cell system for copper removal. The reutilization of the immobilized cell was also tested using electrolyte solutions and a packed-bed column reactor.

MATERIALS AND METHODS

Biological Materials

The biomass used was a strain of *Pseudomonas stutzeri*, previously isolated from the drainage of a copper mine in Korea. The biomass had already been shown to be effective at biosorbing copper ions [5]. The bacteria were inoculated into a jar fermentor (Bioengineering 11523, Switzerland) containing 15 l of a basal medium (1% glucose, 10% polypeptone, 0.5% yeast extract, 0.5% NaCl, pH 6.0) without copper and incubated at 30°C and 200 rpm (D.O. 5 mg/l) for 48 h. The biomass was harvested by centrifugation at 8,000 rpm (Centrikon T-324, Germany) and then washed with distilled water three times.

TEM and EDS Analyses

The TEM (Hitachi H-600, Japan) examination of the intracellular copper accumulation was performed as previously described [8]. The inorganic chemical composition was analyzed using an energy dispersive X-ray spectroscope (EDS, Serial II, Noran, U.S.A.) attached to the TEM.

Preparation of Alginate-Immobilized Biomass

Ten grams of the lyophilized biomass was suspended in 1 l of a 2% sodium alginate solution, and then NaCl (0.85%) was added. The obtained mixture was transferred to an injection apparatus (Fig. 1) and the ejected biomass particles (bead size: 0.5–0.7 mm) were hardened in a 1.47% $CaCl_2$ solution for 2 h. After stabilization with a 1% polyethyleneimine (PEI) solution, the beads were washed with distilled water.

Biosorption Experiments

The biosorbing beads (0.1, 0.5, 1.0, 3.0, 5.0, 10.0 g) were placed in contact with 100 ml of copper-containing solutions. The initial copper concentration was 100 mg/l. The flasks were kept at room temperature. Aliquot amounts were collected every 1 h for 6 h period to determine the residual metal concentration in the solutions. A Freundrich-type isotherm equation was constructed using the copper concentrations measured after 4 h of incubation. The constructed isotherm equation was applied to the wastewater

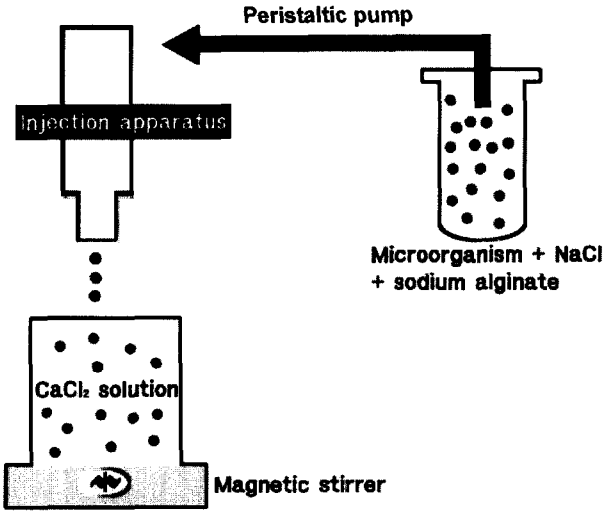

Fig. 1. Schematic diagram of apparatus preparing immobilized biomass.

contaminated with copper at initial concentrations of 50, 100, and 200 mg/l. Appropriate controls and blanks were examined throughout the sorption experiments to check the glassware sorption of metal and other potential side effects.

Reutilization of Immobilized Biomass

Electrolytic solutions of HCl, H_2SO_4, Na_2CO_3, $NaHCO_3$, NTA, and EDTA were used as the metal desorbents. Ten grams of the biomass, previously exposed to the copper-containing solution, was treated with 100 ml of the desorbents (each at 0.01 M, 0.1 M, and 0.5 M) at room temperature with stirring. The biomasses were intermittently withdrawn and centrifuged, after which the copper concentration in the supernatants was assayed.

Ten grams of the copper-loaded biomass at a level of 100 mg/l was treated with 100 ml of the desorbents (each at 0.1 M). After metal desorption, the resulting biomass was re-exposed to copper, as described above, and then the desorption treatment was repeated. The re-exposure and desorption treatment was repeated 10 times. Supernatant aliquots of each sample were withdrawn to determine the amount of copper remaining in the solution.

Packed-Bed Column Experiment

An immobilized biosorbent of lyophilized or disrupted cells with a French press of 1,800 psi was prepared as described above. The biosorbents were packed in a sorption column with a 5.0 cm I.D. and 100 cm length, as shown in Fig. 2. A solution of 100 mg/l Cu(II) was prepared by diluting copper plating industry wastewater with distilled water. The initial concentrations of metal ions in the wastewater were as follows; Cu: 4,430 mg/l, Cd: 0.1 mg/l, Pb: 0.5 mg/l, Zn: 1 mg/l, Ni: 1.2 mg/l, Cr:

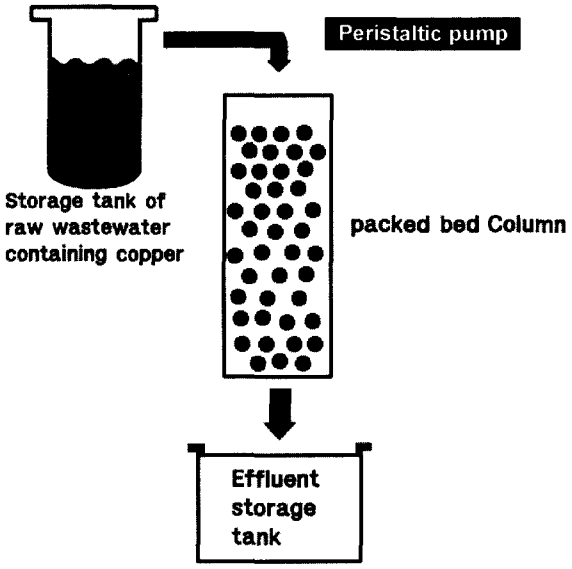

Fig. 2. Schematic flow diagram of proposed copper removal system utilizing packed-bed column reactor.

0.1 mg/l, and Fe: 2.4 mg/l. COD, SS, and pH of the wastewater were 1 mg/l, 1.1 mg/l, and 10.4, respectively. A solution of 100 mg/l Cu(II) was fed into the column from the top, and pumped through the packed bed at constant velocity of 20 l/day using a peristatic pump (EYELA, SMP21-2, Japan). Samples of the effluent were collected and the concentrations of Cu(II) in the samples were determined using an Inductively Coupled Plasma Spectrometer (ICP, Optima 3000DV, Perkin-Elmer, U.S.A.).

RESULTS AND DISCUSSION

Accumulation of Copper in Bacterial Cell

The copper accumulation in the cell of *P. stutzeri* and metal-induced changes in the cell morphology were shown in Fig. 3(A). When compared with a normal cell without accumulated copper, the copper-accumulated bacterial cell exhibited several electron-dense granules inside or on the surface of the cell. The EDS analysis showed that the electron-dense granules were a complex containing a high amount of copper ions [Fig. 3(B)]. A previous study by the current authors presented that the bacteria can accumulate as much as 310 mg/g copper ions in a dry cell [5].

Alginate-Immobilized Biomass

The bacterial cells of *P. stutzeri* were immobilized into sodium-alginate with the use of the injection apparatus presented in Fig. 1. Beads of the immobilized biomass were produced and the diameter of the beads ranged from 0.5 to 0.7 mm [Fig. 4(A)]. As shown in Fig. 4(B), the copper-treated beads adsorbed copper ions and became blue-colored. The TEM examination also demonstrated the biosorption of copper ions on the surface of the beads. When compared with the freshly prepared biomass [Fig. 4(C)], the copper-accumulated biomass [Fig. 4(D)] contained several electron-dense granules on the surface, which were found to be a copper complex by the EDS analysis (F).

Biosorption Kinetics of Immobilized Biomass

The metal-binding properties of the immobilized biomass for six different amounts (0.1, 0.5, 1.0, 3.0, 5.0, and 10.0 g)

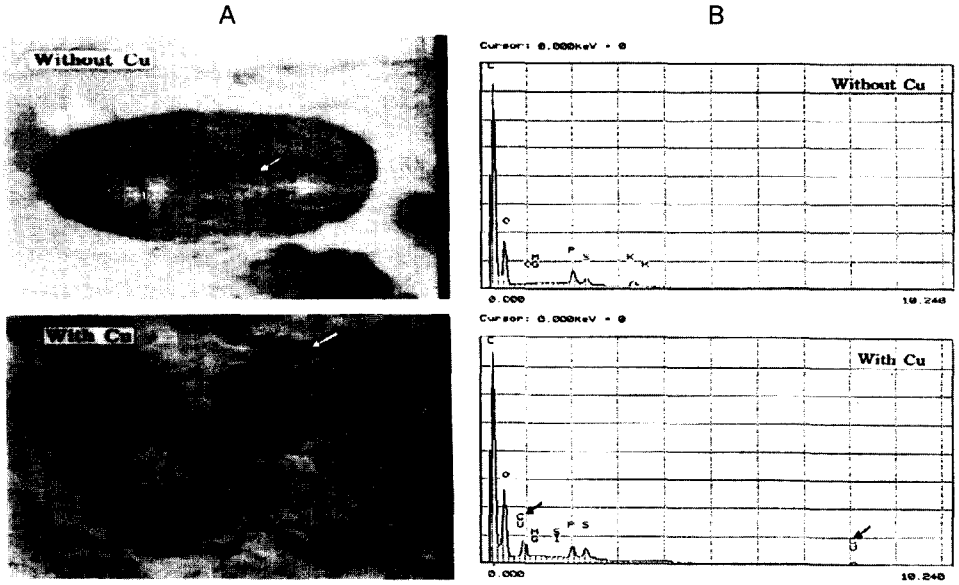

Fig. 3. (A) TEM observation (40,000×) of copper accumulation inside or on the surface of *P. stutzeri* cells.
The arrow indicates the area of the EDS analysis. (B) X-ray energy dispersion spectra (EDS) of *P. stutzeri* treated with Cu(II). Arrows indicate the copper ion peaks.

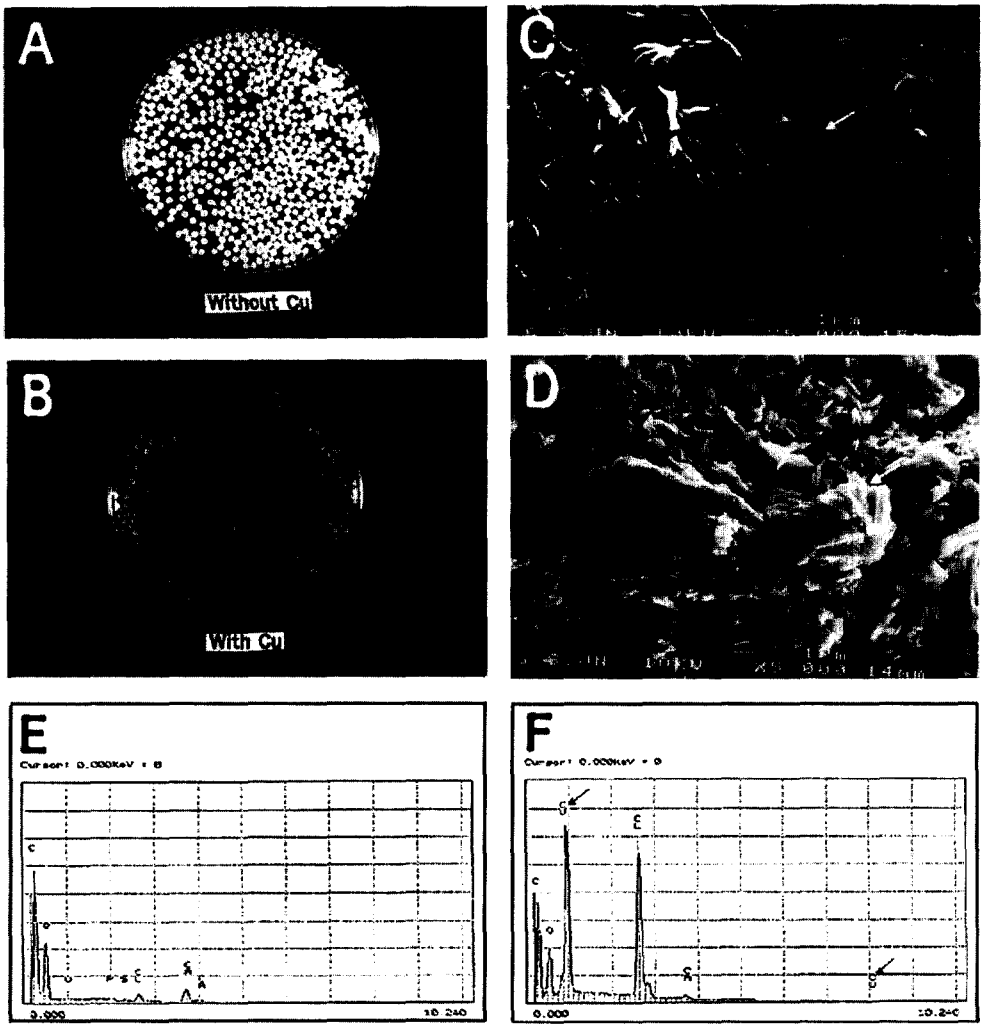

Fig. 4. TEM observation of biomass of *P. stutzeri* cells immobilized in alginate.
(A) Freshly prepared biomass. As the biomass accumulated copper ions, it became blue-colored. (B). Copper crystals were found on the surface of the copper accumulated biomass (D), when compared with the freshly prepared biomass (C). The arrows in (C) and (D) indicate the area of the EDS analysis presented in (E) and (F), respectively. X-ray energy dispersion spectra of the freshly prepared (E) and copper accumulated (F) immobilized biomass. Arrows indicate the copper ion peaks.

were investigated in a solution containing 100 mg/l Cu(II) over a 6 h incubation time, and the results are summarized in Fig. 5. The concentrations of copper ions in the solution were decreased with increasing amounts of the biomass added. However, addition of 10 g of the immobilized biomass only achieved the equilibrium of the copper adsorption within 1 h. This indicates that certain amounts of the immobilized biomass could be required for rapid and successful removal of the metal ions in the solution. After 4 h of incubation time, the amount of copper ions removed per gram weight of biomass decreased with an increasing amount of the biomass added (X/M values in Table 1). This result suggests that an increase in the biomass added to the limited volume of the solution reduced the amount of copper ions remaining and adsorbed. The decrease in the copper concentrations in the solution induced a mass transfer of the metal ions, which were more favorable to the solution. Similar results have already been reported for *Rhizopus arrhizus* [26] and *Actinomyces levoris* and *Streptomyces viridochromogenes* [9], where an increase in the biomass added caused an increase in the total amount of metals adsorbed, but decreasing amount of metal ions adsorbed per cellular weight.

Adsorption Isotherm Equation

The metal biosorption by the immobilized biomass with different amounts was rapid, and plateaus were reached within approximately 4 h, as shown in Fig. 5. As such, the biosorption of copper ions by the immobilized biomass was approximated by a Freundrich-type adsorption isotherm model, based on measuring the amount of metal sorbed on the biomass and remaining in the solution at equilibrium.

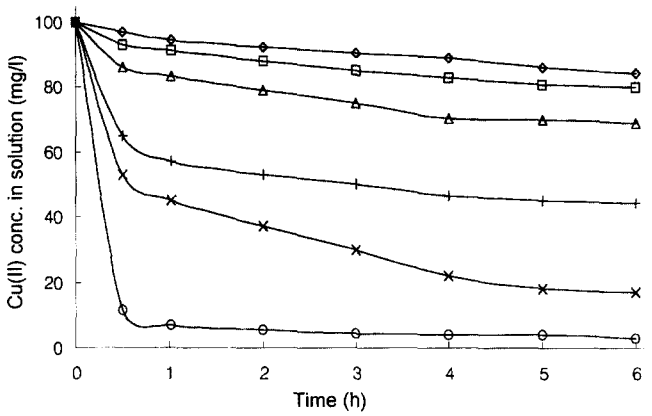

Fig. 5. Copper removal from solution by biomass of *P. stutzeri* cells immobilized in alginate, starting from an initial copper concentration of 100 mg/l.
The following amounts of biosorbent were used: 0.1 g (◇); 0.5 g (□); 1.0 g (△); 3.0 g (+); 5.0 g (×); 10.0 g (○).

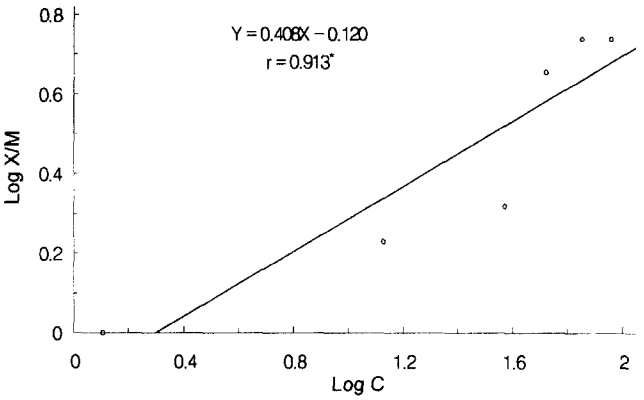

Fig. 6. Freundrich-type adsorption isotherm curve constructed using the values listed in Table 1.

$$X/M=KC^{1/n} \quad (1)$$

where X/M is the amount of the metal accumulated (mg g^{-1}), C is the concentration of the metal remaining in the solution at equilibrium (mg l^{-1}), K is the empirical constant of the adsorption rate, and 1/n is the empirical constant of the adsorption intensity.

The log transformation of Eq. (1) resulted in the following:

$$\log X/M=\log K+1/n \log C \quad (2)$$

By measuring the copper ions accumulated in the biomass and those remaining in the solution at equilibrium, the data in Table 1 were obtained. As shown in Fig. 6, a significant and positive correlation was found between the amount of the metal accumulated (log X/M) and that remaining in the solution (log C) at equilibrium. The copper binding constant, K, and binding intensity constant, 1/n, were determined from the equation in Fig. 6, using the values listed in Table 1. The constants obtained for K and 1/n were 0.759 and 0.408, respectively. Many attempts using isothermal models have already been made to approximate the removal of metal ions in wastewater when using bacterial cells or a bacterial exopolymer [2, 17, 21, 25]. It is generally agreed upon that there are three consecutive steps that describe the process of adsorption from a solution by an adsorbent particle. These three steps, as adapted to apply to metal ion biosorption by a biosorbent particle, are as follows: 1. External mass transfer of the metal ions from the solution bulk to the surface of the biosorbent particle; 2. Diffusion of the metal ions within the adsorbent particle to the sorption sites; and 3. Final uptake of the metal ions at the sorption sites, which is immeasurably fast. In the current study, the metal biosorption by the immobilized biomass was rapid, and an equilibrium position was reached within approximately 4 h. Therefore, the Freundrich-type adsorption isotherm model constructed in the current study mainly explained the early process of the copper ion biosorption described above.

Evaluation of Immobilized Biomass as a Copper Biosorbent

The Freundrich-type adsorption isotherm model constructed in the present study was tested using wastewater containing 50, 100, and 200 mg/l Cu(II). As shown in Fig. 7, the experimental values agreed well with the predicted values at lower concentrations of 50 and 100 mg/l

Table 1. Biosorption parameters for Freundrich-type adsorption isotherm model.

Biomass added (g)	Copper remained in solution (mg/l)	log C	Copper bound to the biomass[1] (mg/l)	X/M[2]	log X/M
0.1	94.4	1.97	5.6	5.60	0.75
0.5	72.2	1.86	27.3	5.46	0.74
1.0	54.4	1.74	45.6	4.56	0.66
3.0	38.1	1.58	61.9	2.06	0.31
5.0	13.1	1.12	86.9	1.74	0.24
10.0	1.3	0.11	98.7	0.98	0.00

[1]The data were measured at 4 h as shown in Fig. 5.
[2]The amount of the copper accumulated per gram weight of biomass.

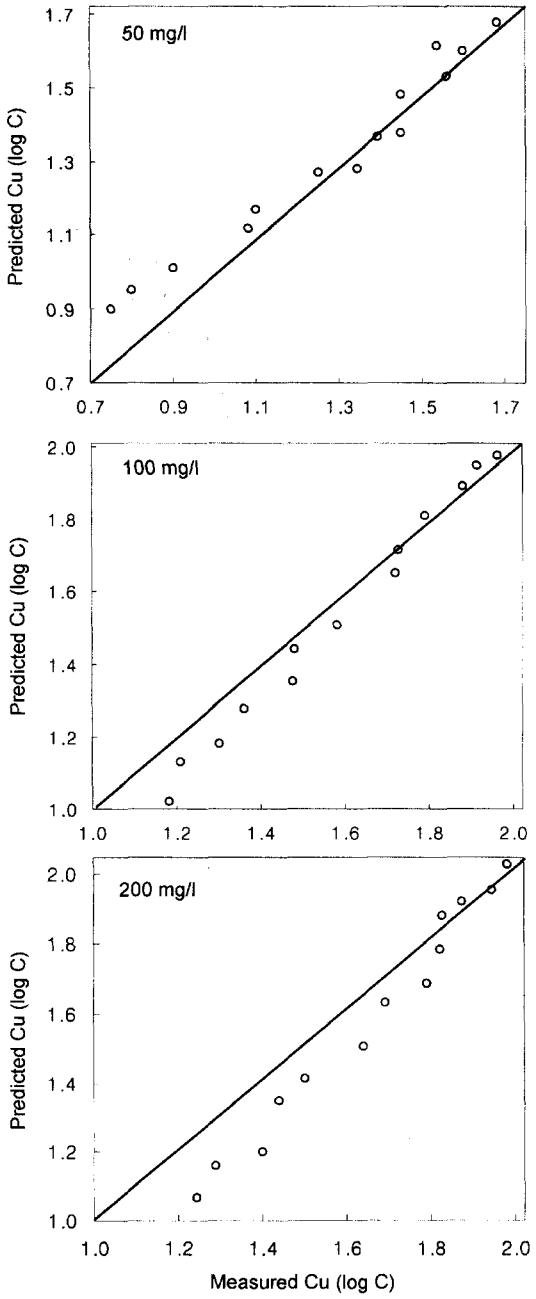

Fig. 7. Experimental copper concentration in solution vs. values predicted by the Freundrich-type adsorption isotherm curve constructed in Fig. 6, starting with initial copper concentrations of 50, 100, and 200 mg/l.

Cu(II), although, the experimental values were much lower than the predicted values at the higher concentration of 200 mg/l Cu(II). This result suggests that a higher amount of biomass than that calculated from the model was needed for the metal uptake in the wastewater containing higher concentration than 200 mg/l Cu(II). Therefore, the definition of an upper limiting concentration of the metal in the wastewater would appear to be necessary for successful biosorption.

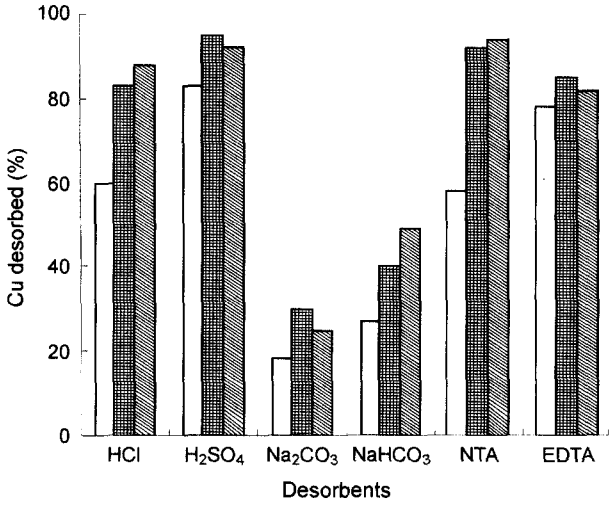

Fig. 8. Desorption of copper ions from loaded biomass of *P. stutzeri* cells immobilized in alginate by several desorbents at various concentrations.
□: 0.01 M desorbent, ▦: 0.1 M desorbent, ▧: 0.5 M desorbent.

Reutilization of Immobilized Biomass

Several electrolytic solutions showed different rates of copper desorption from the immobilized biomass (Fig. 8). H_2SO_4, NTA, and EDTA were found to be effective desorbents, eluting up to more than 90% of the metal bound to the biomass. The HCl solution was also effective in desorbing more than 80% of the metal. However, the solutions of Na_2CO_3 and $NaHCO_3$ showed low desorption rates of 20 to 50% of the metal bound to the biomass. In particular, the solutions of HCl and EDTA exhibited higher desorption rates in the immobilized biomass than in free cells, as reported previously [5]. This may have been due to a more rapid release of the metal bound to the immobilizing material, such as alginate, than that bound to the cell. The desorption of the metal from the immobilized biomass was rapid, and most of the metal was released into the desorbent media within the first hour. As shown in Fig. 9, plateaus were reached within approximately 1 h for more than 80% of the metal bound to the biomass.

A suitable desorbent is required to recover metals from the biomass and regenerate the biosorbent. For this purpose, different organic and inorganic acids, as well as other competing cations were used to release the metals bound to the biomass [18, 23, 24]. It was found that HCl, H_2SO_4, NTA, and EDTA were very effective desorbents for the copper ions bound to the biomass, whereas carbonate salts, such as Na_2CO_3 and $NaHCO_3$, were found to be ineffective desorbents. This suggests that acidic desorbents would seem to be more effective than alkaline desorbents for copper ions bound to *P. stutzeri* cells immobilized in alginate. This

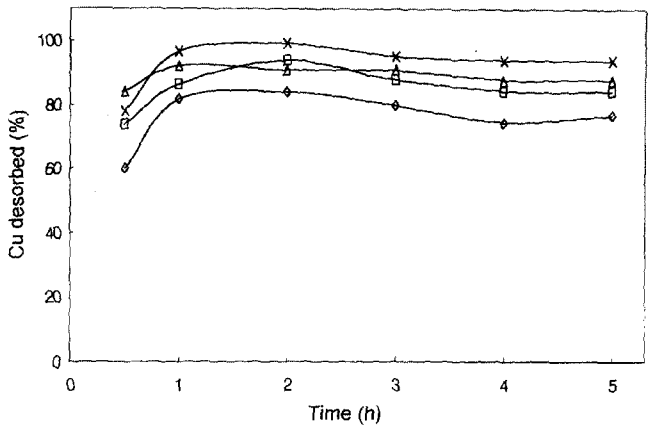

Fig. 9. Time course of copper desorption from loaded biomass of *P. stutzeri* cells immobilized in alginate by 0.1 M of HCl (◇), H_2SO_4 (×), NTA (□), and EDTA (△).

behavior could have been due to the exchange of the metal bound to the functional groups of the cells with H^+ ions rather than the competing cationic species present [4, 6, 11].

The suitablility of *P. stutzeri* as a reusable metal biosorbent was tested by using the alginate-immobilized biomass in 10 consecutive copper biosorption/desorption cycles. Figure 10 shows the proportion of the biosorbed metal and metal desorbed in each subsequent cycle. The amount of metal sorbed by the biomass in a mineral acid (HCl or H_2SO_4)-desorbent system decreased in the fourth cycle (from 90% to 60% for HCl and 90% to 40% for H_2SO_4), with little change thereafter. In contrast, the metal desorption efficiency remained relatively constant (approximately 80%) for all the cycles. This indicates that the metal biosorption was apparently diminished by the acid washing, therefore, alkaline regeneration following acid washing would be required for longer and more effective biosorption behavior. The proportion of the biosorbed metal in an EDTA-desorbent system remained at more than 80% for all the cycles, yet the metal desorption efficiency sharply decreased in the second cycle (90% to 50%) and then remained relatively constant thereafter. A high biosorption/desorption efficiency was found in an NTA-desorbent system for all cycles. Nakajima *et al.* [20] also pointed out that an immobilized biomass could be reused for several consecutive biosorption/desorption cycles, and was more suitable for a batch- or column-type reactor, which has less plugging than a free-cell biomass.

Packed-Bed Column Experiment

The copper biosorption by *P. stutzeri* cells immobilized in alginate was processed in a packed bed column reactor

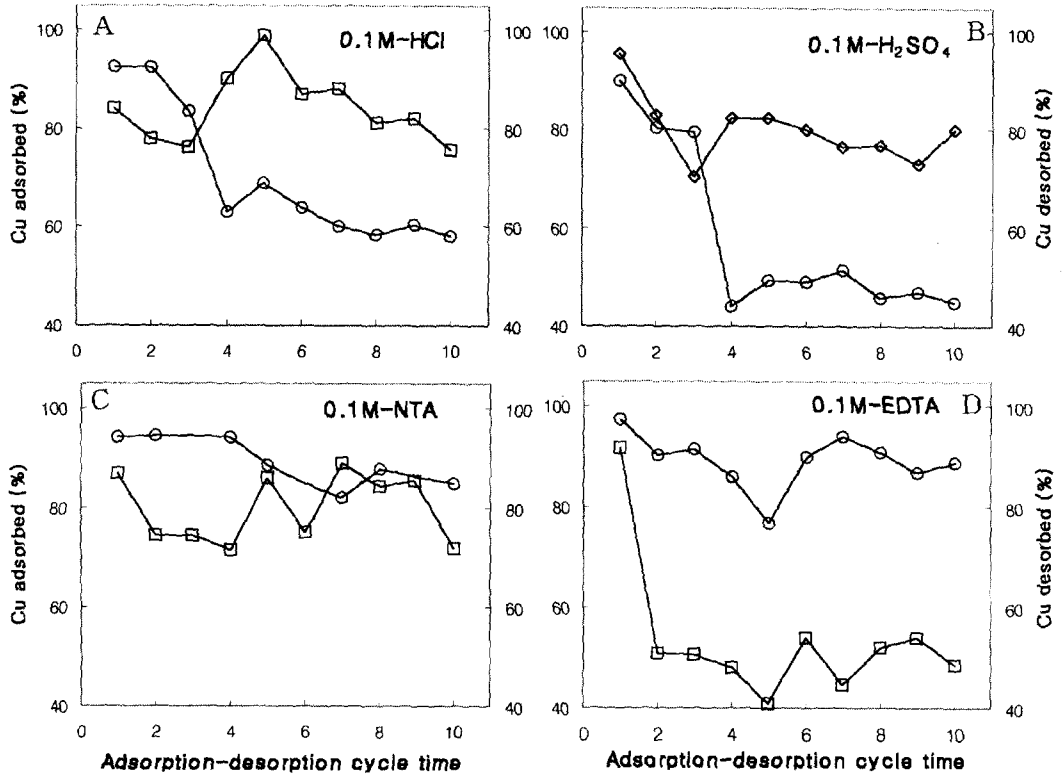

Fig. 10. Biosorption/desorption cycles with 0.1 M HCl (A), 0.1 M H_2SO_4 (B), 0.1 M NTA (C), and 0.1 M EDTA (D).
○: Adsorption; □: Desorption.

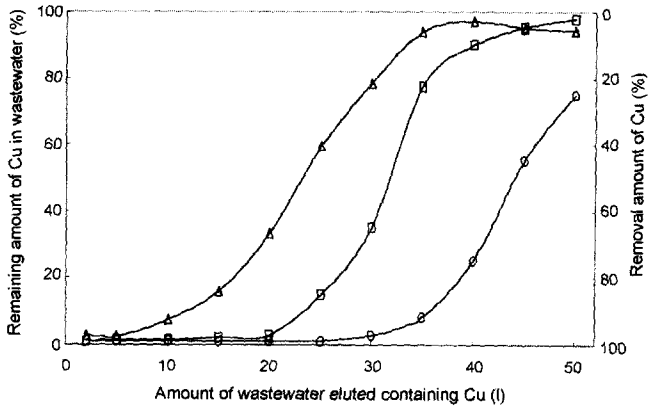

Fig. 11. Removed amounts of copper ions in wastewater containing 100 mg/l Cu(II) by a column reactor packed with the immobilized alginate (△) and immobilized biomass of intact cells (□) and disrupted cells (○).

using copper plating industry wastewater diluted to 100 mg/l Cu(II) (Fig. 11). The nonbiological sorption of copper ions into the alginate was only apparent in the first few liters of wastewater. The French press-disrupted cells immobilized in alginate maintained a high efficiency of copper biosorption for up to 30 l of wastewater and then gradually decreased, whereas the intact cells immobilized in alginate successfully removed copper ions only up to 20 l of wastewater. The enhanced copper biosorption in the immobilized biomass, prepared using disrupted cells, may have contributed to the additional release of the binding materials of the intracellular components [19].

The present paper demonstrated the suitability of *P. stutzeri* cells immobilized in alginate for reuse as a copper biosorbent in column processes, in which the biomasses are packed in metal-containing wastewater. Metal desorption with 0.1 M NTA seemed to have little effect on stability of the biomass, as judged by the retention of its metal biosorption capability after repeated biosorption/desorption cycles without apparent loss of efficiency. Accordingly, this chemical treatment would seem to be an effective method for metal desorption from a load biomass, and allows the biosorbent to be reutilized for at least ten cycles. The immobilization of the biomass enabled easy recovery and handling, as well as the possibility of using the biosorbent in a packed column reactor, which can be operated in continuous and discontinuous modes. Efforts to increase the permeability of the immobilized biomass and to construct a continuous mode for a multi-column reactor are still needed to improve metal removal by biosorbents.

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