

## Metal Recovery Using Immobilized Cell Suspension from a Brewery

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**Abstract**—Lead, copper, and cadmium were adsorbed onto calcium alginate beads containing the cell suspension discarded from a brewery. In the cell suspension, there were many cells under lysis. The cell-suspension immobilized beads were prepared by adding 0.6% (w/v) sodium alginate into the cell suspension from the brewery and then making the cell suspension fall dropwise into the swirling 1% (w/v) calcium alginate solution. The dry weight of insoluble solid in the cell suspension was 96 g dry weight/l and the dry density of the bead containing cell suspension was 140 g dry weight/l of the bead. The specific metal uptake of the cell-suspension immobilized bead was 23.7 mg Pb<sup>2+</sup>, 14.3 mg Cu<sup>2+</sup>, and 13.4 mg Cd<sup>2+</sup>/g bead dry weight, respectively. The cell-suspension immobilized beads retained the initial metal-uptake capacity after 20 repeated batches of adsorption and desorption, but the fraction of metal desorbed from the beads by 1 M HCl solution was only 70% of the adsorbed metal. The beads, which had been contained for 14 successive days in the 0.5% (w/v) CaCl<sub>2</sub> solution at 4 °C just after 20 cycles of adsorption/desorption, retained the initial metal-uptake capacity after 30 repeated cycles, and more than 90% of the copper and cadmium adsorbed on the beads was desorbed by the 1 M HCl solution.

Key words: Biosorption of Heavy Metals, *S. cerevisiae*, Brewery, Cell Suspension, Bead

### INTRODUCTION

Contamination by heavy metals in industrial wastewater has long been an important environmental issue. Heavy metal ions are removed by conventional methods such as chemical precipitation, coagulation, ion exchange, and membrane filtration techniques. However, these conventional methods are inefficient and expensive at low concentrations of metal ions in the range of 1 to 100 mg metal ions/l of solution. In order to improve the efficiency of metal recovery, new technologies have been developed using biosorbents, silica containing ion-chelating agents [Kim et al., 2000], polyethylene and polypropylene fiber [Choi and Nho, 1999], and insoluble cellulose xanthate [Kim and Lee, 1999]. Marine algae have been used for adsorption of gold [Kuyucak and Volesky, 1989] and recovery of heavy metals [Lee, 1997; Kratochvil et al., 1997]. Some kinds of microbial cells are specific to the recovery of heavy metal ions and show large capacity of metal uptake [Park et al., 1999]. Adsorption of metal ions by microbial cells is carried out quickly in some minutes and desorption of the metal ions from the biosorbent is easily accomplished in acidic solutions such as HCl solution. In order to qualify for industrial application, biosorbents have to be produced at a low cost and should be reusable. The cycle time of adsorption/desorption should be as short as possible. Additionally, the loss of adsorbents during repeated cycles of adsorption/desorption should be negligible. Immobilization of biosorbents can meet the above requirements [Volesky, 1990]. The most conventional technique of immobilization is the entrapment of microbial cells.

A huge amount of microbial cells or their extracellular exopolymers such as zooglan and pullulan is required for metal recovery at

the industrial scale. The cell suspension discarded as a waste product from the biofactory can be used as biosorbents without the manufacturing cost of biosorbents, and moreover, saving the cost of industrial waste treatment if the cell suspension would be capable of metal recovery. In this study, the cell suspension of *S. cerevisiae* discarded from a brewery was immobilized in calcium alginate beads, which were used for the recovery of heavy metals such as lead, copper, and cadmium. *S. cerevisiae* cells have shown the specificity for the biosorption of lead [Ahn and Suh, 1996], copper [Stoll and Duncan, 1997], and cadmium [Volesky et al., 1992]. Suh et al. [1998] reported that *S. cerevisiae* cells uptook lead by complex mechanisms; cells with low viability could accumulate lead after 24 h of biosorption and it was difficult to discern organelles in the cell membrane because of the lead uptaken during 5 days of metal recovery. It has also been reported [Mowll and Gadd, 1983] that the metal uptake capacity of yeast cells increased as much as 8 times with the change of the constituents of the cell membrane.

We found through microscopic analysis that some of the *S. cerevisiae* cells in cell suspension discarded from the brewery were under lysis. Therefore, the characteristics of metal uptake by the cell-suspension immobilized bead may be different from those of *S. cerevisiae* cells in the exponential growth phase. We investigated the characteristics of metal uptake of the cell suspension and the cell-suspension immobilized beads, hoping that the beads could be reused without loss of metal uptake capacity even though it had been reported that the maximum cell loading of entrapped beads was limited to 25% (w/v) because of weak mechanical strength [Buchholz, 1979].

### EXPERIMENTAL

#### 1. Entrapment of Cell Suspension in Calcium Alginate Beads

The cell suspension at the state of discard was provided by Cho-

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sun Brewery Inc. in Masan, Korea. The insoluble solid portion of the cell suspension was harvested by centrifuging 10 ml of the cell suspension for 20 min at 3,580 g, washing the precipitate with distilled water, and then recentrifuging for 20 min at 3,580 g. The dry weight of the insoluble solid was obtained by drying at 80 °C until its weight reached constant value. 0.6% (w/v) of sodium alginate was put into the cell suspension and was dissolved overnight at room temperature. The cell suspension containing sodium alginate was added dropwise into the stirred 1% (w/v) calcium chloride solution and maintained for 2 h at room temperature. Cell-free calcium alginate beads were also prepared by the same method mentioned above, except without cell suspension. In order to measure the dry density of beads, the cell-suspension immobilized beads were dried at 90 °C until their weight did not change any more. The dry weight of cell-suspension immobilized beads was 1.29 mg dry weight per bead and the mean diameter of a bead was 0.26 cm.

## 2. Metal Adsorption

Metal uptake was carried out by both the cell suspension discarded from the brewery and the cell-suspension immobilized beads. 10 ml of cell suspension was added to 200 ml of 100 mg Pb<sup>2+</sup>/l and maintained for 5 h at 35 °C with shaking at 200 rpm. Thereafter, 10 ml of the metal solution was centrifuged for 20 min at 3,580 g and the lead concentration of the supernatant was measured by an atomic adsorption spectrophotometer (Shimadzu AA680). The lead uptake capacity of the cell suspension was considered as the difference in the amount of lead between the initial solution and the supernatant. The metal uptake capacity of the cell suspension for copper and cadmium was measured by the aforementioned method. The solution of lead, copper, and cadmium was prepared by dissolving CdCl<sub>2</sub>·2.5H<sub>2</sub>O, CuCl<sub>2</sub>·2.5H<sub>2</sub>O, and Pb(NO<sub>3</sub>)<sub>2</sub>, respectively, in the distilled water. The pH of the metal solution was controlled by adding 1 M HCl or 1 M NaOH.

600 cell-suspension immobilized beads were put into 200 ml of 100 mg Pb<sup>2+</sup>/l and maintained for 2 h at 35 °C with shaking at 200 rpm, and the lead concentration of the solution was measured by an atomic adsorption spectrophotometer. Lead uptake by beads was considered as the decrease of the lead amount in the solution during biosorption. Copper and cadmium uptake of beads was measured by the same method. The cadmium uptake capacity of the cell-suspension immobilized beads was compared with that of cation exchange resins Duolite GT-73 and Amberlite IRA-400 (Rohm & Haas, Philadelphia, USA) calculated by using the Langmuir adsorption isotherms suggested by Holan et al. [1993]. Metals adsorbed on the cell-suspension immobilized beads were desorbed by putting beads into 200 ml of 1 M HCl and maintaining for 20 min at 35 °C with shaking at 200 rpm. Beads used for adsorption/desorption of metals were regenerated for the next cycle of adsorption/desorption by putting beads into 0.5% (w/v) CaCl<sub>2</sub> solution and maintaining for 10 min at 35 °C without shaking.

## RESULTS AND DISCUSSION

### 1. Metal Uptake by Cell Suspension

The state of cell suspension discarded from a brewery was investigated through microscopic analysis. As shown in Fig. 1, some cells were under lysis like the state of cells used for the production of L-phenylacetyl carbinol from benzaldehyde [Park and Lee, 2001].

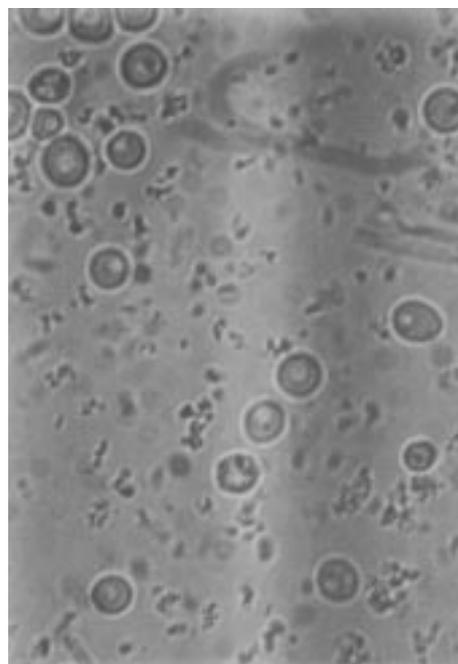


Fig. 1. The state of *S. cerevisiae* cells in the cell suspension discarded from a brewery. There are some cells under lysis.

The presence of cell debris and exposure of cytoplasm in the cell suspension suggests that the mechanism of metal uptake by cell suspension from brewery is different from that of *S. cerevisiae* cells from the exponential growth phase. Generally, there are two mechanisms of metal uptake by microbial cells. One is a metabolism-independent biosorption in which metal cations are rapidly bound to the outer surface of the cell. The other is a slow metabolism-dependent bioaccumulation in which metal ions penetrate through the cell membrane and are accumulated inside the cell. However, the mechanism of metal ion accumulation differs according to microorganisms and metal ions used [Suh et al., 1998]. UO<sub>2</sub><sup>2+</sup> accumulated as needlelike fibrils in a layer approximately 0.2 μm thick on the surfaces of *S. cerevisiae* cells and little or no UO<sub>2</sub><sup>2+</sup> accumulated inside these cells without visible UO<sub>2</sub><sup>2+</sup> deposition [Strandberg et al., 1998]. In contrast, Volesky and May-Phillips [1995] insisted that UO<sub>2</sub><sup>2+</sup> was deposited on the cell wall and the cell membrane as well as throughout the cytoplasm of *S. cerevisiae* cells according to their observation by TEM. Cell suspension from a brewery is composed of live cells, dead cells, cell debris, and cytoplasm (Golgi complex, lysosome, mitochondria, lysosome, glycogen, vacuoles, and so on). To date, no report has been found about the exact mechanism of metal uptake by the constituents of cytoplasm. If the constituents of the cytoplasm and the inner surface of the cell wall are able to adsorb metal ions, the exposure of the cytoplasm and inner cell wall of the cell debris may increase the rate of biosorption and reduce the fraction of metal uptake capacity due to the bioaccumulation mechanism.

The profiles of metal uptake by cell suspension are shown in Fig. 2. There is no indication of bioaccumulation of copper; the copper uptake of cell suspension increased with time and reached a plateau value after 30 min. However, slow accumulation of cadmium by cell suspension followed rapid biosorption after 30 min. There

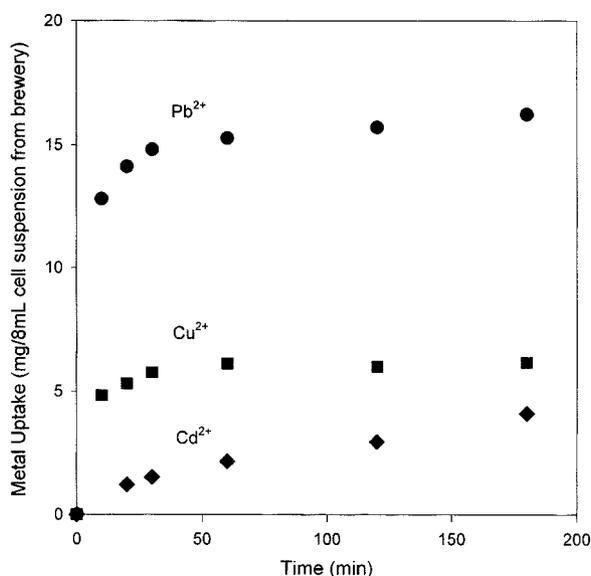


Fig. 2. Profiles of metal uptake by the cell suspension from brewery. 8 ml of the cell suspension was added to 200 ml of metal solution and maintained at 35 °C and pH 3 with shaking at 200 rpm.

is weak evidence for lead accumulation by cell suspension. The total amount of metal uptake during 6 h by cell suspension was 21 mg (0.1 mmol) Pb<sup>2+</sup>, 8.2 mg (0.13 mmol) Cu<sup>2+</sup>, and 6.7 mg (0.06 mmol) Cd<sup>2+</sup>/g cell-suspension dry weight, respectively. The amount of metal uptake by cell suspension is larger than (or nearly the same as) that reported in the literature. The specific amount of lead uptaken by 0.36 g dry weight of *S. cerevisiae* (ATCC 24858) cells in 100 ml of 100 mg Pb<sup>2+</sup>/l was only 12 mg Pb<sup>2+</sup>/g cell dry weight [Jin and Park, 1998; Park et al., 1997] and the specific cadmium uptake by brewers yeast harvested in the exponential growth phase was 7 mg Cd<sup>2+</sup>/g cell dry weight [Volesky et al., 1993]. In case of lead uptake, the

exposure of cytoplasm to the cell suspension caused by cell lysis might be considered to be effective for the enhancement of the lead uptake capacity of cell suspension. This phenomenon is partly coincident with reports that the specific lead uptake by dead *S. cerevisiae* cells was 2.4 times larger than that by living cells [Ahn and Suh, 1996], a finding which was confirmed by Stroll and Duncan [1997], and the zinc uptake capacity of yeast cells can increase 8 times with changes of the constituents of the cell membrane [Mowll and Gadd, 1983]. However, the specific uptakes by cell suspension for Pb<sup>2+</sup>, Cu<sup>2+</sup>, and Cd<sup>2+</sup> based on the mol of uptaken metal are different. This also confirms that the metal uptake by cell suspension is carried out not by simple biosorption, but by a complex mechanism of biosorption and bioaccumulation. When the metal was uptaken only by biosorption, specific copper uptake and specific lead uptake by marine algae *Laminaria japonica* were the same on the basis of mol of metal uptaken [Lee and Suh, 2000].

## 2. Metal Uptake by the Cell-Suspension Immobilized Beads

The metal uptake capacity of biosorbents depends on the pH of the metal solution. The optimum pH for metal recovery by cell-suspension immobilized beads was nearly the same as that of free cell suspension: pH 4.7 for lead, pH 6.4 for copper, and 7.5 for cadmium. Optimum pH for copper recovery by the cell-suspension immobilized beads was considered to be shifted to a high value because of cell lysis, compared with the optimum pH value of 3-5 for the copper uptake of immobilized *S. cerevisiae*, as reported by Wilhelmi and Duncan [1996]. Alginate is a linear polymer of β-(1-4)-D-mannosyluronic acid and α-(1-4)-L-gulosyluronic acid residues, the relative proportions of which vary with the botanical source and state of maturation of the plant. The potential binding sites in biopolymer, alginate, are carboxylate and hydroxyl groups. The surface complex formation is the major mechanism for metal ion uptake by the calcium alginate beads. The optimum pH for copper recovery by cell-free calcium alginate beads has been reported as 3.5-6.5 [Chen et al., 1997]. However, it should not be overlooked that metal precipitates spontaneously at high pH of the solution,

Table 1. Spontaneous metal precipitate and the metal uptake by calcium alginate beads containing *S. cerevisiae* cell suspension discarded from a brewery. 2000 capsules were put into 1 liter of 100 mg Cu<sup>2+</sup>/l and maintained at 35 °C with shaking at 200 rpm. Copper uptake by beads was obtained by subtracting the amount of spontaneous precipitate from total amount of removed copper

pH	0.7	1.9	2.7	0.7	4.4	5.4	6.4	7.3	8.4
Total removed copper (mg Cu <sup>2+</sup> /l)	8.3	11.7	23.5	28.7	31.3	51.1	69.1	100	100
Spontaneous precipitate (mg Cu <sup>2+</sup> /l)	0	2.0	3.0	3.0	4.5	7.0	16.0	70	100
Uptake by beads (mg Cu <sup>2+</sup> /l)	8.3	9.7	20.5	25.7	26.8	44.1	53.1	30	0

Table 2. Metal uptake capacity of different biosorbents. 8 ml of cell suspension discarded from a brewery was added to 200 ml of 100 mg metal/l; 600 beads were put into 200 ml of 100 mg metal/l. Metal uptake was carried out for 5 h at 35 °C and pH 3 with shaking at 200 rpm

Adsorbent	Adsorbent density	Metal uptake capacity					
		mg/g adsorbent dry wt			mg/l wet bead mg/l cell suspension		
		Pb	Cu	Cd	Pb	Cu	Cd
Cell suspension	96 g dry weight/l cell suspension	20.6	8.2	6.7	1980	790	645
Cell immobilized Ca-alginate bead	140 g dry weight/l wet bead	23.7	14.3	13.4	3320	1900	1870
Cell free Ca-alginate bead	12 g dry weight/l wet bead	260	102	100	3120	1440	1220

and a high recovery of metal at a high pH is partially attributable to spontaneous metal precipitation [Harris and Ramelow, 1990; Zhou and Kiff, 1991]. In this study, the increase in pH of the metal solution caused metal precipitation. As shown in Table 1, 13% of the total amount of metal recovery was attributed to metal precipitation at pH 2.7; moreover, the fraction increased with the pH of the solution and reached 100% at pH 8.4. The metal uptake by the cell-suspension immobilized beads was determined by subtracting the amount of precipitated metal from total amount of removed metal in solution.

The experimental values of metal uptake by the cell-suspension immobilized beads, cell suspension, and the cell-free beads are shown in Table 2. The specific metal uptake by cell-free beads on the basis of the dry weight of the biosorbent was highest. However, in an actual system, wet biosorbent is used for metal recovery, so the specific metal uptake by biosorbent needs to be considered on the basis of the volume of wet biosorbent. As shown in Table 2, the specific metal uptake by each kind of biosorbent based on the volume of wet biosorbent is the same order of magnitude. The specific metal uptake by the cell-suspension immobilized beads was larger than that by cell suspension, but equal (for cadmium) to or smaller (for copper and lead) than the added value of both the cell suspension and cell-free beads. For lead uptake, it is hypothesized that the adsorption sites of biosorbents placed in the central part of the bead may be useless because of the hindrance of metal diffusion caused by the metal deposition on the biosorbents in the periphery of the bead as reported for the metal uptake by the encapsulated biosorbents [Park et al., 2001]. However, the metal (copper and cadmium) uptake capacity of calcium alginate bead can be much increased by immobilizing a by-product of fermentation process, that is, the waste cell suspension from a brewery.

### 3. Adsorption Isotherms for the Metal Uptake by Cell-Suspension Beads

The concentration of  $H^+$  (or pH) changes during biosorption, and the effect of pH on the biosorbents are similar to that of a weakly acidic cation exchange resin [Schiever and Volesky, 1995]. There are many other reports that the specific metal uptake by biosorbents is dependent on the pH of the solution. Stoll and Duncan [1997] reported that a packed bed containing non-viable *S. cerevisiae* cells had a high breakthrough volume at a high pH. A modified Langmuir equation, which was well fitted to experimental values obtained at various pH, was developed by Yu and Kaewsam [1999], although the adsorption isotherm for metal uptake by marine algae at a fixed pH was well described by Langmuir adsorption isotherm [Matheickal, 1998].

The relation between the specific metal uptake by the cell-sus-

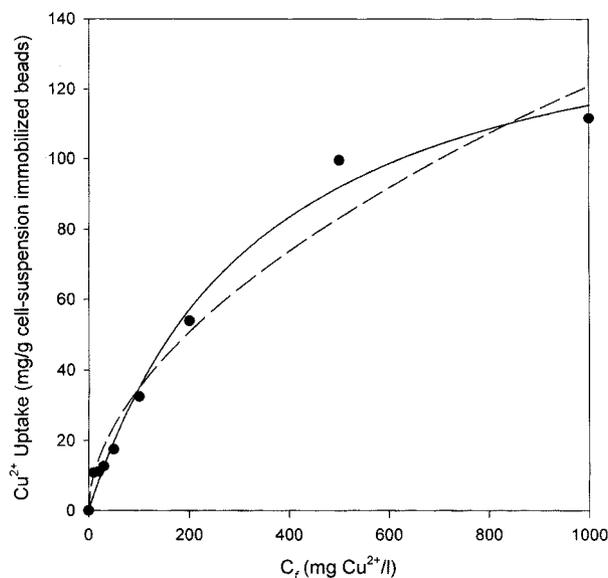
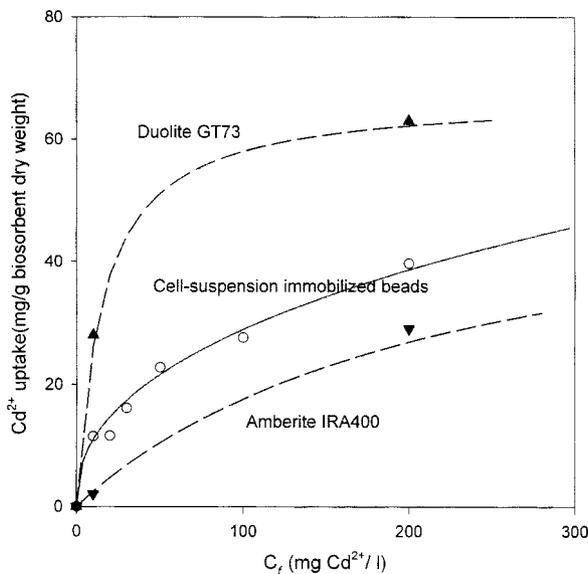


Fig. 3. Isotherm of copper uptake by the cell-suspension immobilized beads. 2000 beads were put into 1 liter of copper solution and maintained for 2 h at 35 °C and pH 3 with shaking at 200 rpm. — Langmuir isotherm, --- Freundlich isotherm, ● experimental data.

pension immobilized beads, obtained experimentally at pH 3, and the equilibrium metal concentration of the solution was nonlinearly regressed, as shown in Fig. 3 and Table 3. Such regression is well described by both Langmuir and Freundlich isotherms, although the Langmuir isotherm is preferred for lead and copper uptake and the Freundlich isotherm is preferred for cadmium uptake because of regression coefficient ( $R^2$ ). In the Langmuir isotherm, the specific cadmium uptake (mg metal/g bead dry weight) increases and reaches the maximum uptake,  $q_m$ , as the plateau value as the liquid phase equilibrium concentration of metal,  $C_f$ , increases. A large value of  $b$  means that the specific cadmium uptake,  $q$  is reached at the low value of  $C_f$ . In the Langmuir isotherm regression,  $q_m$  for the lead uptake by the cell-suspension immobilized beads was 4.5 times larger than that of the lead uptake of encapsulated *S. cerevisiae* (ATCC 24858) cells [Jin and Park, 1998], although the value of  $b$  was only 60%. The exposure of cytoplasm and cell debris in the cell suspension might increase the value of  $q_m$ , but lower value of  $b$  mean that lead affinity of cell suspension was relatively low at the low liquid phase concentration of lead. However, cadmium affinity of the cell-suspension immobilized beads at the low liquid phase concentration is relatively higher than that for copper, although

Table 3. Freundlich isotherm constants and Langmuir isotherm constants for metal uptake by the cell-suspension immobilized beads.  $R^2$ ; nonlinear regression coefficient for the relationship between the specific metal uptake of the cell-suspension immobilized beads ( $q$ ; mg metal/g bead dry weight), obtained experimentally, and the liquid phase equilibrium concentration ( $C_f$ ; mg metal/l)

	Pb <sup>2+</sup>			Cu <sup>2+</sup>			Cd <sup>2+</sup>		
$q = K_f C_f^{1/n}$	k	1/n	R <sup>2</sup>	k	1/n	R <sup>2</sup>	k	1/n	R <sup>2</sup>
	4.65	0.58	0.989	2.92	0.54	0.966	4.22	0.42	0.990
$q = \frac{q_m b C_f}{1 + b C_f}$	$q_m$	b	R <sup>2</sup>	$q_m$	B	R <sup>2</sup>	$q_m$	b	R <sup>2</sup>
	350	0.0023	0.995	155	0.0029	0.989	82	0.006	0.973

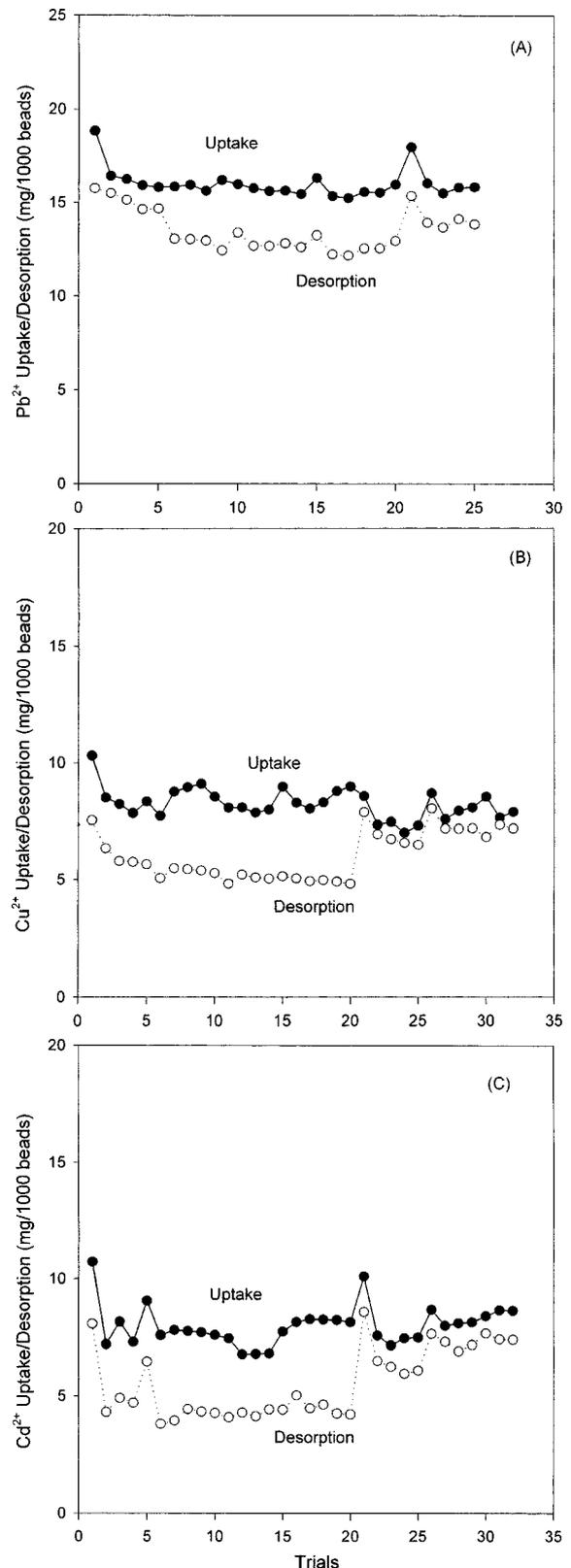


**Fig. 4.** Freundlich isotherm of cadmium uptake by the cell-suspension immobilized beads. Experimental data for the cell-suspension immobilized beads (○); and Langmuir isotherms suggested by Holan et al. [1994], (dashed line) for (▲) cation ion-exchange resin, Duolite GT73; (▼) cation ion-exchange resin, Amberite IRA400.

the maximum uptake capacity for cadmium is smaller than that for copper. This can be found by using the Freundlich isotherm, as shown in Table 3. Freundlich constant,  $k$  describes the specific metal uptake capacity (mg metal/g biosorbent dry weight) at the liquid phase equilibrium concentration,  $C_e$  of 1 mg metal/l. The relatively higher affinity of cadmium at low  $C_e$  is explained by  $k$  value for cadmium, which is higher than that for copper, as shown in Table 3. The specific cadmium uptake by the cell-suspension immobilized beads was compared with that by commercial ion exchange resins. Cadmium uptake by Duolite GT-73 and Amberite IRA-400 was estimated by the Langmuir adsorption isotherms as suggested by Holan et al. [1993]. As shown in Fig. 4, the uptake capacity of the cell-suspension immobilized beads was lower than that of Duolite GT-73, but was much higher than that of Amberite IRA-400 at the low  $Cd^{2+}$  concentration.

#### 4. Reusability of the Cell-Suspension Immobilized Beads

The lead uptake capacity of encapsulated *S. cerevisiae* cells was retained after 30 cycles of adsorption and desorption; however, capsules collapsed because of cell lysis during repeated batches [Jin and Park, 1998]. The cadmium uptake capacity of the whole-cell zooglanes immobilized beads decreased by 16% and the dry weight of whole-cell immobilized beads decreased by 17% after 30 batches. In this study, the loading of cell suspension in the beads was 86% on the basis of dry weight. Metals were desorbed from the cell-suspension immobilized beads by the 1 M HCl solution, and beads were regenerated by the 0.5% (w/v)  $CaCl_2$  solution between adsorption/desorption cycles. The living cells immobilized in beads may be killed by the toxicity of the 1 M HCl solution used in the desorption step. Usually, the cell death decreases the metal uptake capacity of microbial cells, but often increases the uptake capacity for some kinds of metal ions as reported by some researchers [Ahn and



**Fig. 5.** Metal uptake capacity of the cell-suspension immobilized beads. 1000 beads were put into 200 ml of 100 mg metal/l; 2 h adsorption at 35 °C and pH 3 with shaking at 200 rpm, 20 min desorption in 100 ml of 1 M HCl solution, 10 min regeneration with 0.5% (w/v)  $CaCl_2$  solution. (A) lead, (B) copper, (C) cadmium

Suh, 1996; Stroll and Duncan, 1997]. A slight decrease (10-20%) of the metal uptake capacity of the cell-suspension immobilized beads was found after the first batch of adsorption/desorption process (Fig. 5). This may be caused by the death of cells due to the toxicity of 1 M HCl and/or by the release of some portion of the immobilized cytoplasm from the bead. As shown in Fig. 5, the metal uptake capacity of the cell-suspension immobilized beads was retained after 20 cycles of adsorption/desorption; however, only 70% of the adsorbed metal was desorbed at each cycle. Beads were maintained for 2 successive weeks in 0.5% (w/v) CaCl<sub>2</sub> solution at 4 °C just after 20 cycles. Thereafter, the adsorption capacity of the beads was nearly equal to the initial value; more than 90% of adsorbed copper and cadmium was desorbed at each cycle. The increase in the desorption efficiency might be explained by the hypothesis that the strong binding sites such as phosphate in vacuoles [Volesky et al., 1993] from the cytoplasm immobilized in the calcium alginate beads are lost during long residence in the calcium chloride solution. The dry weight of beads increased by 10-20% after 30 batches of adsorption/desorption. The increase in dry weight might be attributable to the calcium ions bonded to beads during regeneration steps between cycles of adsorption/desorption.

### CONCLUSION

Some cells in the cell suspension discarded from a brewery were in lysis. The exposure of cytoplasm or the presence of cell debris in the cell suspension seemed to increase the metal uptake capacity of the cell suspension. The cell suspension could be successfully immobilized in the calcium alginate beads. Additionally, the specific metal uptake capacity of the cell-suspension immobilized beads was larger than that of cell-free beads on the basis of volume of wet biosorbents. The mechanism of metal uptake by the cell suspension from the brewery was not clear in this study, but we believe on the basis of our experimental results that the cell-suspension immobilized beads can be used repeatedly for more than 30 cycles of adsorption/desorption without any loss of metal uptake capacity and any decrease in the dry weight of beads. If there were any chelating agents in the metal solution, the application of the cross-linking method to the beads would prevent beads from dissolving during biosorption.

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