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Metal recovery by aged beads prepared using cell-suspension from the waste of beer fermentation broth

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Abstract−Lead, copper, and cadmium were adsorbed onto aged calcium alginate beads containing cell-suspension from the waste of beer fermentation broth. Beads prepared by adding 0.6% (w/v) sodium alginate into the cell suspension from the waste of beer fermentation broth and making the cell suspension drop into the 1% (w/v) calcium alginate solution were stored in the 1% (w/v) calcium chloride solution for 1 year. The specific metal uptake of the aged cell-suspension immobilized bead was 312 mg Pb^{2+}, 158 mg Cu^{2+}, and 112 mg Cd^{2+}/g bead dry weight at pH 7.5 of the metal solution, respectively. The relation between the specific metal uptake by the aged cell-suspension immobilized beads and the equilibrium metal concentration was nonlinearly regressed and well described by the Freundlich isotherm. The specific cadmium uptake capacity of aged cell-suspension immobilized beads was between the specific cadmium uptake capacities of commercial beads Duolite GT-73 and Amberite IRA-400 and higher than those of the fresh *Saccharomyces cerevisiae* ATCC 834 and *Saccharomyces cerevisiae* ATCC 24858 immobilized beads.

Key words: Biosorption of Heavy Metals, *S. cerevisiae*, Cell Immobilized Bead, Cell Suspension, Waste of Beer Fermentation Broth

INTRODUCTION

Contamination by heavy metals in wastewater has become worse with industrial development. Heavy metal ions in the industrial wastewater are conventionally removed by chemical precipitation or ion exchange. However, the ion-exchange method is only adopted when the recovered metal is valuable because of the high cost of resins. Chemical precipitation has the disadvantages of a low yield of metal recovery and a high volume of generated sludge [1]. Many microbial cells are known to adsorb metals on their cell walls [2] and some microbial cells with low viability accumulated metal ions [3]. The heavy metal removal mechanism of microorganisms can be classified as extracellular accumulation/precipitation, cell surface sorption/precipitation, and intracellular accumulation [4]. *S. cerevisiae* cells have shown the specificity for the biosorption of lead [5], copper [6], and cadmium [7]. The first step of metal ion accumulation in the *S. cerevisiae* is a rapid binding to negatively charged groups on the cell surface, and the second step is the penetration through the cell membrane and into the cytoplasm. The third step is the metal ions' accumulation in the cell cytoplasm [8].

Immobilization of the biosorbents is required in order to reduce the loss of the adsorbents during repeated cycles of adsorption/desorption and reuse the biosorbents [8]. Biosorbents have to be produced at a low cost in order to qualify for industrial application. In the previous study [9], the potential of the cell suspension discarded as a waste product from beer factories as biosorbents was investigated. Cell-suspension available from the waste of beer fermentation broth (WBFB) can reduce the manufacturing cost of biosorbents and save on the costs of industrial waste treatment. The cell-suspension of *S. cerevisiae* discarded from a brewery was immobilized in calcium alginate beads and used for the recovery of heavy metals such as lead, copper, and cadmium. *S. cerevisiae* cells in cell-suspension from WBFB were under lysis and, thus, the characteristics of the cell-suspension immobilized bead might be different from those of *S. cerevisiae* cells in the exponential growth phase. Mowll and Gadd reported that the metal uptake capacity of yeast cells increased as much as 8 times with the change of the constituents of the cell membrane [10]. In this study, the metal uptake capacity of the cell-suspension immobilized beads stored in the calcium chloride solution for 1 year was investigated with the hope that the beads could be reused without much loss of metal uptake capacity. The metal uptake capacity of the aged cell-suspension immobilized beads was compared with the cadmium uptake capacity of beads containing fresh *S. cerevisiae* ATCC 24858 that is used to produce ethanol and that of fresh *S. cerevisiae* ATCC 834 that produces L-phenylacetyl carbinol (L-PAC) [11].

EXPERIMENTAL

1. Preparation of Aged Cell-Suspension Immobilized Beads

WBFB was provided by the Chosun Brewery Inc. in Masan, Korea. The entrapment of cell-suspension from WBFB was described in detail by Park and Choi [9]. The insoluble solid portion of the cell-suspension from WBFB was harvested by centrifuging, washing the precipitate with distilled water, and then recentrifuging. 0.6% (w/v) of sodium alginate was put into the cell suspension and 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 hrs at room temperature. The immobilized cell-suspension beads were stored for 1 year at room temperature. To measure the dry density of aged beads, the aged cell-suspension immobilized beads were dried at 90 °C until their weight did not change any more.

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2. Immobilization of *Saccharomyces cerevisiae* Cells

The microorganisms *Saccharomyces cerevisiae* ATCC 834 and ATCC 24858 were received from the KCTC (Korean Collection for Type Cultures) in a freeze-dried condition. *S. cerevisiae* ATCC 834 was cultured in a medium of glucose, 10% (w/v); yeast extract, 0.6% (w/v); ammonium sulfate, 0.4% (w/v); magnesium sulfate, 0.06% (w/v); potassium phosphate dibasic, 0.1% (w/v) at 30 ºC, 200 rpm for 24 hrs. The culture medium for *S. cerevisiae* ATCC 24858 was comprised of glucose, 10% (w/v) and yeast extract, 0.85% (w/v). The cells were harvested by centrifuging the culture broth for 20 min at 3,580 g. 48.7 g of the wetted *S. cerevisiae* ATCC 24858 cells were put into 100 ml 0.6% (w/v) of sodium alginate solution. 46.7 g of the wetted *S. cerevisiae* ATCC 834 cell was put into 100 ml 0.6% (w/v) of sodium alginate solution. The sodium alginate solution containing *S. cerevisiae* cells was dropped into the stirred 1% (w/v) calcium chloride solution. The dry density of cell immobilized bead was 90.4 g/L for *S. cerevisiae* ATCC 24858 and was 110.4 g/L for *S. cerevisiae* ATCC 834, respectively. The bead size was roughly 2.46 mm in both cases.

3. Metal Adsorption

Metal uptake was carried out by both the aged cell-suspension immobilized beads and the fresh *S. cerevisiae* cell immobilized beads. 20 ml of aged cell-suspension immobilized beads (2.8 g dry weight of beads) was added to 1,000 ml of 100 mg Cd^{2+}/*l* and maintained for 5 hrs at 35 ºC while being shaken at 200 rpm. 3000 *S. cerevisiae* ATCC 24858 immobilized beads (2.2 g dry weight of beads) were added to another 1,000 ml of 100 mg Cd^{2+}/*l* and maintained for 5 hrs at 35 ºC while being shaken at 200 rpm. Thereafter, 10 ml of the metal solution was centrifuged for 20 min at 3,580 g and the cadmium concentration of the supernatant was measured by an atomic adsorption spectrophotometer (Shimadzu AA680). The cadmium uptake capacity of the cell immobilized beads was considered as the difference in the amount of cadmium between the initial solution and the supernatant. The metal uptake capacities of the free cells of *S. cerevisiae* ATCC 834 and *S. cerevisiae* ATCC 24858 were measured by the method outlined by Park et al. [8]. The cadmium uptake capacity of the aged cell-suspension immobilized beads was compared with those of cation exchange resins Duolite GT-73 and Amberlite IRA-400 (Rohm & Haas, Philadelphia, USA) calculated by using the Langmuir adsorption isotherms by Holan et al. [12].

RESULTS AND DISCUSSION

1. Metal Uptake by the Aged Cell-Suspension Immobilized Beads

The cell-suspension from WBFB shows the presence of cell debris and exposure of cytoplasm. Storing cell-suspension immobilized beads in the calcium chloride solution may change the physico-chemical properties and result in the reduction of metal uptake capacity. The dry weight and the mean diameter of aged cell-suspension immobilized beads are nearly the same as those of the fresh cell-suspension immobilized beads, 1.29 mg dry weight and 0.26 cm, respectively. The specific cadmium uptake capacity of the aged cell-suspension immobilized beads is also nearly the same as that of the fresh cell-suspension immobilized beads at pH 3 of the metal solution. The specific metal uptake capacity of the aged cell-suspension immobilized beads was measured again at pH 7.5, where the specific cadmium uptake capacity was maximized, and compared with the previous uptake characteristics of the fresh cell-suspension immobilized beads which were obtained at pH 3 [9]. The effect of pH on the biosorbents is similar to that of a weakly acidic cation exchange resin, and the specific metal uptake by biosorbents is dependent on the pH of the solution [13]. The profiles of metal uptake by the aged cell-suspension immobilized beads are shown in Fig. 1. There is no indication of bioaccumulation of metals; the metal uptake increases and reaches the saturated value. The specific cadmium uptake capacity of aged beads is 27 mg Cd^{2+}/g aged bead dry weight and much higher than 13.4 mg Cd^{2+}/g fresh bead dry weight,

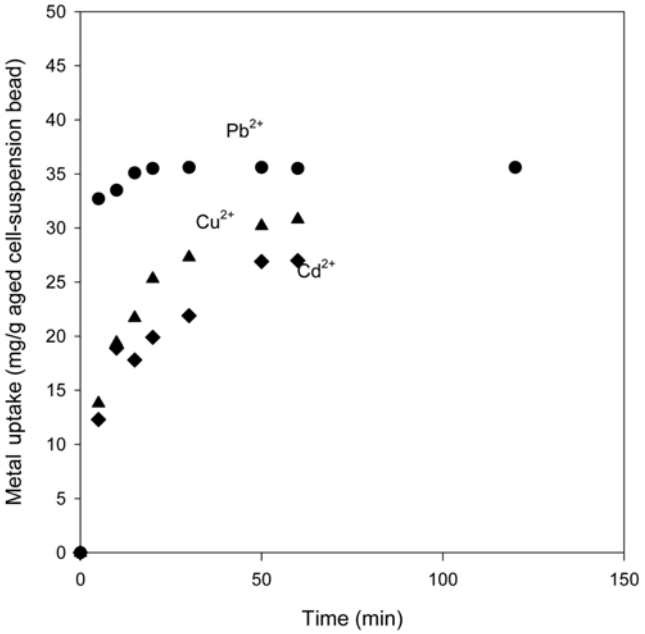

Fig. 1. Profiles of metal uptake by the aged cell-suspension immobilized beads. 20 ml (2.8 g dry weight) of the aged cell-suspension immobilized beads was added to 1,000 ml of 100 ppm metal solution and maintained at 35 ºC and pH 7.5 with shaking at 200 rpm.

Table 1. Metal uptake capacity of fresh and aged cell-suspension immobilized beads. 40 ml (3.8 g dry weight) of fresh cell-suspension discarded from a brewery was added to 1,000 ml of 100 mg metal/*l*. 3.8 g dry weight of fresh beads and 2.8 g weight of 1 year aged beads were put into individual 1,000 ml of 100 mg metal/*l*. Metal uptake was carried out for 5 h at 35 ºC and pH 3 (fresh beads, fresh cell suspension) and pH 7.5 (old beads) with shaking at 200 rpm

Adsorbent	Metal uptake capacity (mg/g adsorbent dry wt)		
	Pb	Cu	Cd
Cell suspension*	20.6	8.2	6.7
Fresh cell-immobilized Ca-alginate bead*	23.7	14.3	13.4
1 year old cell-immobilized Ca-alginate bead	35.5	30.8	27.0

*Adapted from Park and Choi [9]

as shown in Table 1. The adsorption with fresh beads was carried out at pH 3 and showed slow accumulation of cadmium by cell suspension followed rapid biosorption after 30 min [9]. This seems to be caused by the existence of cell debris and the exposure of the cytoplasm in the cell suspension. Cadmium cations are rapidly bound to the surface of the cell with the metabolism-independent biosorption at pH 7.5 and, thus, the other slow metabolism-dependent bioaccumulation in which metal ions penetrate through the cell membrane [8] becomes negligible. The lead and copper uptake capacities are 35.5 mg Pb^{2+} and 30.8 mg Cu^{2+}/g aged bead dry weight, respectively. The amount of lead and copper uptake by aged cell-suspension immobilized beads was larger than that of fresh cell-suspension immobilized beads. However, this is not remarkable because metal precipitates spontaneously at high pH of the solution, and a high recovery of metal at a high pH is partially attributable to spontaneous metal precipitation [14]. In this study, the optimum pH for cadmium uptake causes lead and copper precipitation. In the previous report [9], 13% of the total amount of copper 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 lead and copper uptake capacities of aged cell-suspension immobilized beads include spontaneous metal precipitation; thus, the actual increase in copper and lead uptake capacities is not expected. In the case of cadmium uptake, the increase of pH of the metal solution is considered to be effective for the enhancement of the cadmium uptake capacity of aged cell-suspension immobilized beads.

2. Adsorption Isotherms for the Metal Uptake by Aged Cell-Suspension Immobilized Beads

The concentration of H^+ changes during biosorption. A modified Langmuir equation, which was well fitted to experimental values obtained at various pH, was developed by Yu and Kaewsarn [15] although the adsorption isotherm for metal uptake by marine algae at a fixed pH was well described by the Langmuir adsorption isotherm [16]. The relation between the specific metal uptake by the aged cell-suspension immobilized beads, obtained experimentally at pH 7.5 using Tris-HCl buffer solution, and the equilibrium metal concentration of the solution was nonlinearly regressed, as shown in Table 2. Such regression for lead uptake is well described by both Langmuir and Freundlich isotherms, although the Freundlich isotherm is preferred for copper and cadmium uptake based on regression coefficient (R^2). In the Langmuir isotherm regression for the lead uptake by the aged cell-suspension immobilized beads, q_m was nearly the same as that of the fresh beads, but the value of b was 33 times larger. The exposure of cytoplasm and cell debris in the cell suspension increases the value of q_m [9]. The large specific lead uptake capacity of the aged cell-suspension immobilized beads seems to not be influenced by the change in pH because the specific lead uptake (mg metal/g bead dry weight) increases and reaches the maximum uptake q_m of the Langmuir isotherm as the plateau value as the liquid phase equilibrium concentration of metal C_f, increases. However, there is weak evidence that a large fraction of the uptake capacity of aged cell-suspension immobilized beads is contributed due to the spontaneous precipitation because a large value of b means that the specific cadmium uptake capacity reaches q_m at the low value of C_f. Cadmium uptake by the aged cell-suspension immobilized beads can be described not by the Langmuir isotherm but by the Freundlich isotherm as shown in Table 2. It may be speculated that a large amount of the potential binding sites in cell debris and cytoplasm such as carboxylate and hydroxyl groups become active at pH 7.5 based on the large values of K and 1/n. The specific cad-

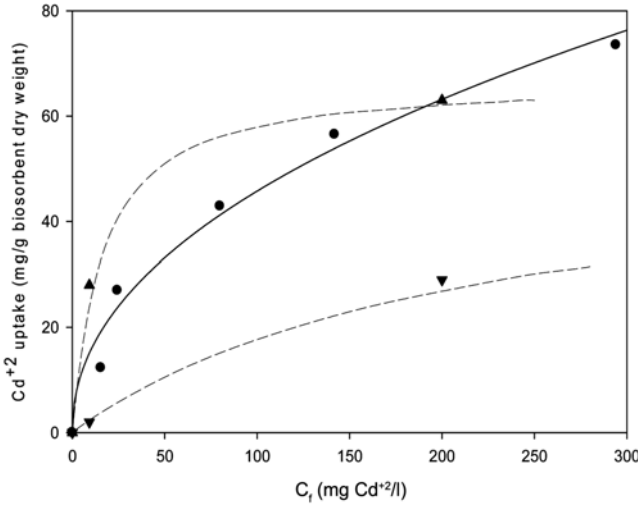

Fig. 2. Freundlich isotherm of cadmium uptake by the cell-suspension immobilized beads. Experimental data for the aged cell-suspension immobilized beads (●); and Langmuir isotherms suggested by Holan et al. [12], (dashed line) for (▲) cation ion-exchange resin, Duolite GT73; (▼) cation ion-exchange resin, Amberite IRA400.

Table 2. Freundlich isotherm constants and Langmuir isotherm constants for metal uptake by the 1 yr old cell-suspension immobilized beads (at pH 7.5) and fresh cell-suspension immobilized beads (at pH 3). 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^{2+}			Cu^{2+}			Cd^{2+}		
$q=K_fC_f^{1/n}$	k	1/n	R^2	k	1/n	R^2	k	1/n	R^2
Fresh cell-suspension Immobilized beads*	4.65	0.58	0.99	2.92	0.54	0.97	4.22	0.42	0.99
1 year old cell-suspension immobilized beads	33.7	0.49	0.94	9.08	0.45	0.99	5.36	0.47	0.98
$q=q_mbC_f/1+bC_f$	q_m	b	R^2	q_m	b	R^2	q_m	b	R^2
Fresh cell-suspension Immobilized beads*	350	0.0023	0.99	155	0.0029	0.99	82	0.006	0.97
1 year old cell-suspension immobilized beads	323	0.077	0.96	37.8	0.039	0.87	23	0.027	0.87

*Adapted from Park and Choi [9]

Table 3. The amount of cadmium uptaken by microorganisms and microorganism immobilized cells. Biosorptions was carried out in 100 ml of 100 ppm cadmium solution at pH 7.5 and 30 °C for 1 hr in the shaking incubator rotating at 200 rpm

S. cerevisiae (ATCC 834)		*S. cerevisiae* (ATCC 24858)		Cell-suspension from waste of beer fermentation broth	
Amount of adsorbent (g)	Specific uptake (mg/g dry weight)	Amount of adsorbent (g)	Specific uptake (mg/g dry weight)	Amount of adsorbent (g)	Specific uptake (mg/g dry weight)
Free cell		Free cell			
0.11	60.5	0.17	27.7		
0.17	46.6	0.22	22.0		
0.23	35.0	0.43	13.9		
Cell immobilized beads		Cell immobilized beads		1 year old cell-suspension immobilized beads	
0.24	23.5	0.22	22.4	0.28	27
0.48	18.7	0.44	18.7		
0.72	11	0.66	11.8		
				Cell free beads	
				0.063	158

mium uptake capacity of the aged cell-suspension immobilized beads is compared with that of 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. [12]. As shown in Fig. 2, the uptake capacity by the aged cell-suspension immobilized beads is much higher than that of Amberite IRA-400 and lower than that of Duolite GT-73 at the low Cd^{2+} concentration, but higher than that of Duolite GT-73 at the large Cd^{2+} concentration.

3. Comparison with *S. cerevisiae* Cell Immobilized Beads

In this study, the Cd^{2+} uptake capacities of *S. cerevisiae* ATCC 24858 and *S. cerevisiae* ATCC 834 as a completely different yeast fungus were investigated in order to evaluate the uptake capacity of the aged cell-suspension immobilized beads. As shown in Table 3, the specific cadmium uptake capacity of *S. cerevisiae* ATCC 834 is higher than that of *S. cerevisiae* ATCC 24858 because the ratio of the thickness of the outer wall (mannan layer) to the cell width of *S. cerevisiae* ATCC 834 is higher than that ratio in ATCC 24858, although the thickness of the cell wall of ATCC 834 was thinner than those of ATCC 24858, as mentioned by Park et al. [8]. However, the specific cadmium uptake capacity of the *S. cerevisiae* ATCC 834 immobilized beads is nearly the same as that of *S. cerevisiae* ATCC 24858 with the same biosorbent density. This means that the thickness ratio of the outer wall (mannan layer) to the cell size is no longer the primary factor for biosorption of the cadmium ions by the immobilized *S. cerevisiae* cells. The specific cadmium uptake capacity of the aged cell-suspension immobilized beads is 27 mg Cd^{2+}/g bead dry weight with the biosorbent density of 2.8 g/*l* although that of *S. cerevisiae* ATCC 24858 immobilized beads is 22.4 mg Cd^{2+}/g bead dry weight. The higher specific cadmium uptake of the aged cell-suspension immobilized beads seems to be caused by the exposure of cytoplasm and existence of cell debris that can accumulate cationic metal ions. The metal ion accumulation in *S. cerevisiae* cells is composed of first binding to cell surface, penetration through the cell membrane and accumulation in the cell cytoplasm [8]. In the aged cell-suspension immobilized beads, all these three steps are accomplished spontaneously. The specific cadmium uptake capacity of the aged cell-suspension immobilized beads decreased from 27 mg Cd^{2+}/g bead dry weight to 4.5 mg Cd^{2+}/g bead dry weight as the biosorbent density increased from 2.8 g/*l* to 28 g/*l*. The specific cadmium uptake capacity of the *S. cerevisiae* ATCC 834 immobilized beads decreases like the aged cell-suspension immobilized beads as the biosorbent density increases. This dependency of cadmium uptake capacity on the amount of biosorbent is surmised to be caused by the obstruction between the heavy metal combined sites, that was mentioned by Puranik and Paknikar [17]. The specific cadmium uptake capacity of cell-free calcium alginate beads was highest as 156 mg Cd^{2+}/g bead dry weight with the biosorbent density of 0.63 g/*l*. However, the specific cadmium uptake capacity of the aged cell-suspension immobilized beads based on the volume of the wet biosorbent was 3,780 mg Cd^{2+}/*l* wet bead and higher than 1,896 mg Cd^{2+}/*l* wet bead of the cell-free beads, as Park and Choi mentioned previously that the specific metal uptake based on the volume of wet biosorbent was the same order of magnitude [9].

CONCLUSION

The exposure of cytoplasm or the presence of cell debris in the cell suspension from WBFB seemed to increase the specific metal uptake of the cell-suspension immobilized beads. The specific metal uptake of the cell-suspension immobilized beads was preserved for more than 1 year in the calcium chloride solution. The specific metal uptake capacity of the aged cell-suspension immobilized beads was larger than that of commercial ion exchange bead and the conventional *S. cerevisiae* immobilized beads. A huge amount of cell suspensions from WBFB can be used for the metal recovery from industrial waste without the manufacturing cost of biosorbents. The aged cell-suspension immobilized beads might be used for a long operational time while retaining metal uptake selectivity.

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