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The Recovery of Heavy Metals Using Encapsulated Microbial Cells

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We prepared capsules containing *Saccharomyces cerevisiae* and *Zoogloea ramigera* cells for the removal of lead (II) and cadmium ions. Microbial cells were encapsulated and cultured in the growth medium. The *S. cerevisiae* cells grown in the capsule did not leak through the capsule membrane. The dried cell density reached to 250 g/l on the basis of the inner volume of the 2.0 mm diameter capsule after 36 hour cultivation. The dry whole cell exopolymer density of encapsulated *Z. ramigera* reached to 200 g/L. The capsule was crosslinked with triethylene tetramine and glutaric dialdehyde solutions. The cadmium uptake of encapsulated whole cell exopolymer of *Z. ramigera* was 55 mg Cd/g biosorbent. The adsorption line followed well Langmuir isotherm. The lead uptake of the encapsulated *S. cerevisiae* was about 30 mg Pb/g biomass. The optimum pH of the lead uptake using encapsulated *S. cerevisiae* was found to be 6. Freundlich model showed a little better fit to the adsorption data than Langmuir model. 95 percent of the lead adsorbed on the encapsulated biosorbents was desorbed by the 1 M HCl solution. The capsule was reused 50 batches without loosing the metal uptake capacity. And the mechanical strength of the crosslinked capsule was retained after 50 trials.

Key words: biosorption, capsule, lead, cadmium, *Zoogleoa ramigera, Saccharomyces cerevisiae*

INTRODUCTION

Toxic heavy metal ions have been removed conventionally by a chemical precipitation or an ion exchange technique. These methods are inefficient or expensive when the concentration of metal ion is low in the order of 1 to 100 ppm. The chemical precipitation method is generally used for the merits of easy installation, cheap maintenance cost and little energy requirement in spite of low yield of metal recovery and much amount of sludge. The metal recovered by the ion exchange method should be of great value because the cost of resin is very expensive.

Some kinds of microbial cells adsorb metals on the wall of themselves. The exopolymers produced by the microbial cells such as *Zoogloea ramigera* and *Aureobasidium pullulans* can also adsorb metals. The concept of biosorption was introduced by the recovery of ^{239}Pu from sea water using activated sludge. *Sargassum natans*, marine algae, could adsorb gold selectively [1]. To be used on the industrial scale, the biosorbents should satisfy the conditions such as the cheap production cost of biosorbents, efficient and fast adsorption and desorption and easy removal of biosorbents from solution. Immobilized biosorbents can be reused and satisfy most of above requirements. The conventional immobilization technique is bead entrapment. But the general bead entrapment method has a limit of cell loading. The fraction of cell in the bead should be less than 25% by volume because of mechanical strength [2]. For large beads, the cell usually concentrated on the periphery of the bead [3, 4] because of the mass transfer resistance of oxygen and nutrients in the gel matrix of the bead.

The capsule entrapment method of the microbial cells was developed [5-7] using one-step microencapsulation method [8]. Microbial cells are inoculated in the capsule when the capsule is formed, and the encapsulated cells are cultured in the growth medium. *Saccharomyces cerevisiae* and *Corynebacterium glutamicum* were inoculated in 2 mm calcium alginate capsule [5-7]. Dry cell weight of encapsulated *Saccharomyces cerevisiae* cells reached to 310 g/L based on the inner volume of the capsule. The dry cell weight of encapsulated *Corynebacterium glutamicum* was 200 g/L depending on the concentration of yeast extract of the medium.

It may be possible to recover heavy metals from solution using the encapsulated biosorbents such as microbial cell itself or exopolymer produced by the cells. In this study, we prepare the encapsulated *Saccharomyces cerevisiae* and whole cell biosorbents using exopolysaccharide of *Zoogloea ramigera*. The effect of fermentation conditions on the metal uptake of the free-cultured cells and encapsulated biomass will be investigated. We investigate adsorption characteristics of the encapsulated biosorbents. The durability of encapsulated biosorbents will be challenged by investigating how many cycles of adsorption-desorption process can be successfully carried out.

MATERIALS AND METHODS

Cell Line and Medium

The cell used for the lead uptake on the cell wall itself was *Saccharomyces cerevisiae* (ATCC 24858). The cell line utilized to produce exopolysaccharide which was able to adsorb cadmium was *Zoogloea ramigera*

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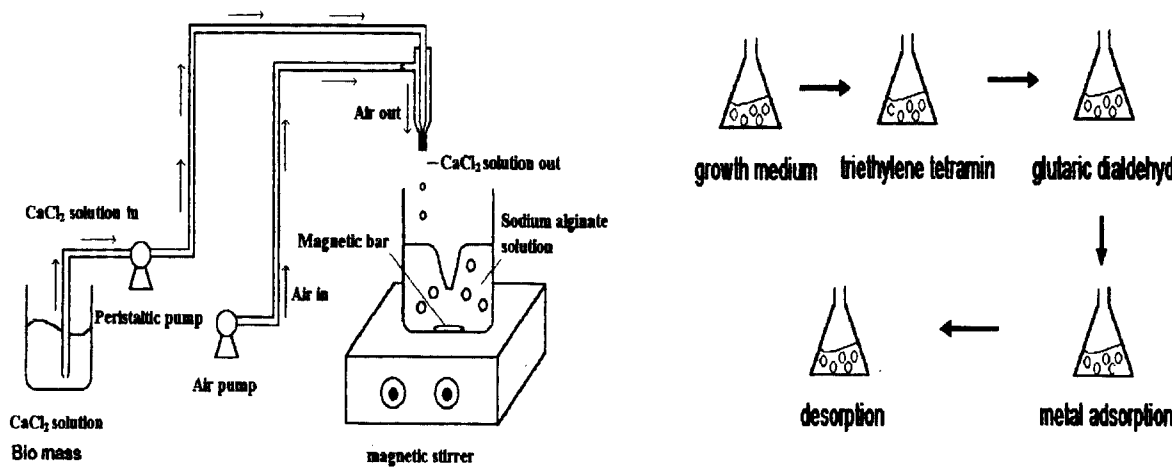

Fig. 1. Schematic representation of the formation of encapsulated whole cell biosorbent.

(KCTC 2582). The composition of growth medium of *S. cerevisiae* was 20 g glucose, 3 g yeast extract, 5 g bacto peptone, 3 g malt extract per one liter of medium. The composition of growth medium of *Z. ramigera* was 0.5 g arginine hydrochloride, 1.0 g alanine, 1.5×10^{-6} g Vitamin B_{12}, 20 g glucose, 2 g K_2HPO_4, 1 g KH_2PO_4, 0.2 g $MgSO_47H_2O$ per one liter of medium and that of the production medium was 30 g glucose, 1 g K_2HPO_4, 0.5 g KH_2PO_4, 0.5 g $MgSO_47H_2O$ per one liter of medium.

Preparation of Encapsulated Biosorbents

Calcium alginate capsule was prepared as shown in Fig. 1. The cell was inoculated in the 100 ml of sterilized growth medium. We harvested cells by centrifuging 20 ml of the broth solution after cultivating cells for 24 hours at 27°C and at 200 rpm in a shaking incubator. Collected cells were mixed with 100 ml of 1.3% (w/v) $CaCl_2$ solution containing 13 g $CaCl_2$, 0.234 g xanthan gum and 0.5 g nonionic surfactant per liter of solution. $CaCl_2$ solution dropped into the 0.6% (w/v) alginate solution circulating in the beaker and the cell inoculated capsule was made [5, 7]. Three or four hundred inoculated capsules were poured into 100 ml of growth medium and cultivated for 4 days at 27°C and at 200 rpm in a shaking incubator. For the *Zoogloea ramigera*, the medium was refreshed everyday and *Saccharomyces cerevisiae* was cultivated in the growth medium for 36 hours without changing the medium. Biosorbent immobilized capsules were dried at 95°C in an oven until the weight of capsule did not decrease. The difference between dried weights of capsule containing cell and vacant capsule was defined as dry biosorbent weight immobilized in a capsule.

Crosslinking and Metal Detection

To prevent swelling and dissolution, the capsules containing whole cell biosorbent were crosslinked with 1% triethylene tetramine solution and 1% (w/v) glutaric dialdehyde solution for 30 hours. The concentration of lead and cadimium in the solution was measured with AAS (Atomic Adsorption System, Shimadzu AA 680).

RESULTS AND DISCUSSION

Cell Loading

We inoculated microbial cells by pouring 10 ml of the broth solution into 100 ml of growth medium and cultured at 27°C and 200 rpm in a shaking incubator. We harvested whole cell biosorbents by centrifuging broth solutions for 5 minutes. The glucose consumption and *Saccharomyces cerevisiae* cell growth rate were measured. After 8 hours cultivation, glucose of the medium was nearly exhausted and dry cell weight reached to 3 g/L. The density of dry whole cell exopolymer of *Z. ramigera* was 12.5 g/L after 120 hours cultivation.

Three or four hundred capsules, in which microbial cells had been inoculated, were cultured in 100 ml of the growth medium at 27°C and at 200 rpm in a shaking incubator. The *S. cerevisiae* cells grew in the capsule and the dry cell weight reached to 250 g/L based on the inner volume of the capsule after 36 hours cultivation and was about 70 times higher than that of free culture. For the culture of *Z. ramigera*, the growth medium was refreshed every day. The pH of the growth medium decreased from 5.2 to 4 after 24 hours. Calcium alginate capsule dissolved during cultivation because of HPO_4^{-2} and $H_2PO_4^{-1}$ ions in the growth medium. The mechanical strength of the capsule was maintained by adding 50 g $CaCO_3$ and 10 g $CaCl_2$ to one liter of arginine growth medium. The dry weights of cell and exopolymer immobilized in the capsules was 190 g/L based on the inner volume of the capsule and was 15 times heavier than that of free culture. *Z. ramigera* is capable of energy generation by respiratory processes. We supplied air to the medium in the air lift reactor at the rate of 5 l/min. The encapsulated dry cell weight increased about 10% as the volumetric oxygen transfer rate k_La increased from 2.55/hr to 500/hr like Fig. 2.

Lead(II) Uptake by *Saccharomyces cerevisiae*

The cells which were obtained by centrifuging 100 ml of the broth solution were put into 100 ml of 100 ppm lead solution and stirred at 27°C and 200 rpm. The lead uptake by the *S. cerevisiae* cell was 12 mg/g biosorbent after 10 minutes and equaled to the saturated value. The saturated value was a little lower than that of the report of other researcher [9]. However, in our study, the metal uptake increased linearly in 10 minutes. The capsule diameter reduced from 2.2 mm to 2 mm after the cell immobilized capsules were crosslinked with triethylene tetramine and glutaric di-

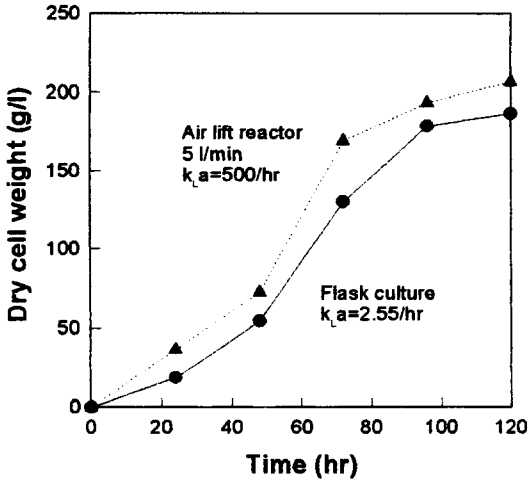

Fig. 2. Dry weight of encapsulated *Z. ramigera* according to the culturing time.

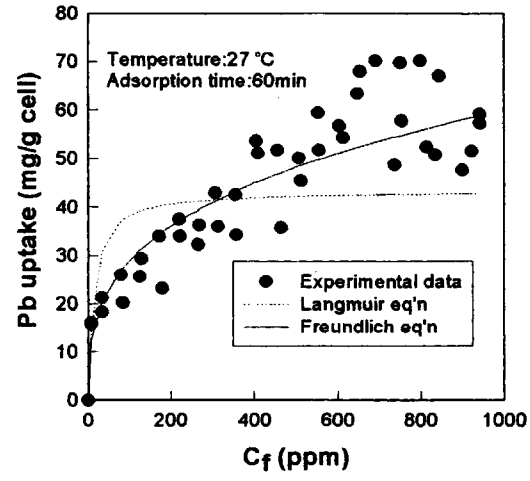

Fig. 4. Adsorption isotherm for the encapsulated *S. cerevisiae.*

aldehyde solutions. The crosslinked capsule was photographed using microscope and shown in Fig. 3. The metal uptake of the encapsulated *S. cerevisiae* was dependent on the pH of the lead solution. Lead ion was precipitated at the pH higher than 7. The maximum lead uptake was obtained at pH 6. The lead uptake of the encapsuled *S. cerevisiae* became 23 mg/g biosorbent after 60 minutes adsorption. The metal uptake by the encapsulated biosorbent was higher than that of free-cultured cell. This might be caused by the lead uptake of the calcium alginate membrane matrix. We measured the lead uptake of the vacant calcium alginate capsule. The difference between metal uptakes of cell immobilized capsule and vacant capsule was nearly the same with that of free cell. The adsorbed lead on the encapsulated biosorbent was desorbed more than 95% by the 1 M HCl solution. The lead uptake of encapsulated *S. cerevisiae* was measured at different concentrations of solution and shown in Fig. 4. The adsorption curve was well fitted with Freundlich isotherm equation.

Cadmium Uptake by *Zoogleoa ramigera*

Z. ramigera cell was cultured for 120 hours at 27°C and 200 rpm in a shaking incubator. The whole cell exopolymer which had been obtained by centrifuging 20 ml of the broth solution was put into 100 ml of 100 ppm cadmium solution and stirred at 27°C and 200 rpm. The cadmium uptake of the free cultured whole cell exopolysaccharide reached to 52 mg Cd/g biomass after 50 minutes when pH of the solution was 6.5. It took 5 minutes for the amount of cadmium adsorbed by the biosorbent to reach 70% of the maximum uptake capacity and 20 minutes to reach 90%. The cadmium uptake decreased as pH of the solution decreased and the amount was 3 mg Cd/g biomass when pH of the solution was 2.5. The maximum uptake was obtained at pH 6 or 7 as 55 mg Cd/g biomass.

The dry weight of encapsulated whole cell exopolymer was dependent on the oxygen supply and carbon/nitrogen ratio of the medium but independent on the pH control of the medium during cultivation. The specific cadmium uptake of encapsulated whole cell exopolymer, which had been cultivated in different way, was measured. The dry weight of encapsulated whole cell exopolysaccharide increased with C/N ratio and air supply, but the specific cadmium uptake was independent on the culture condition. The optimum pH of the solution for metal uptake of the encapsulated whole cell exopolymer was 6 or 7 like the case of free cell. As shown in Fig. 5, the specific cadmium uptake of encapsulated whole cell exopolymer was nearly the

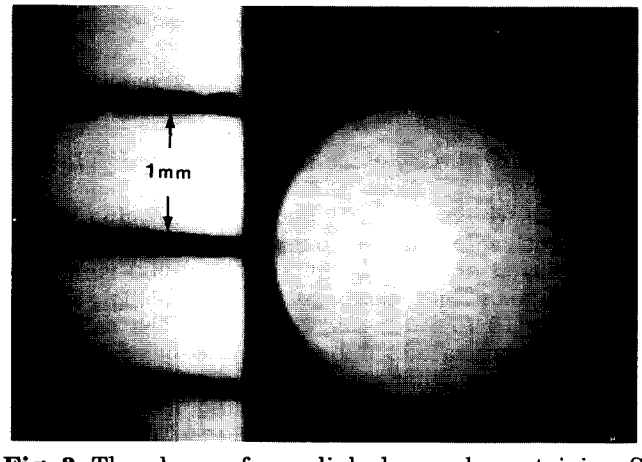

Fig. 3. The shape of crosslinked capsule containing *S. cerevisiae* cells.

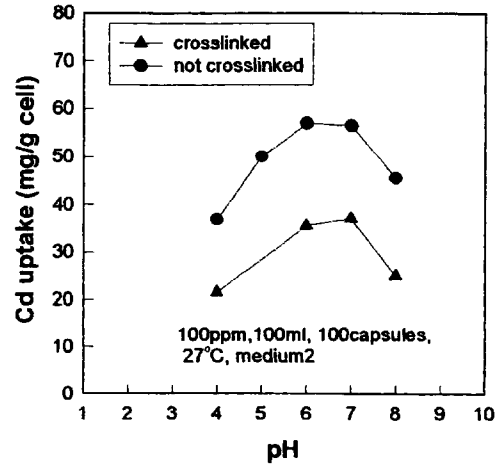

Fig. 5. Effect of pH on the adsorption capacity or crosslinked capsules containing *Z. ramigera.*

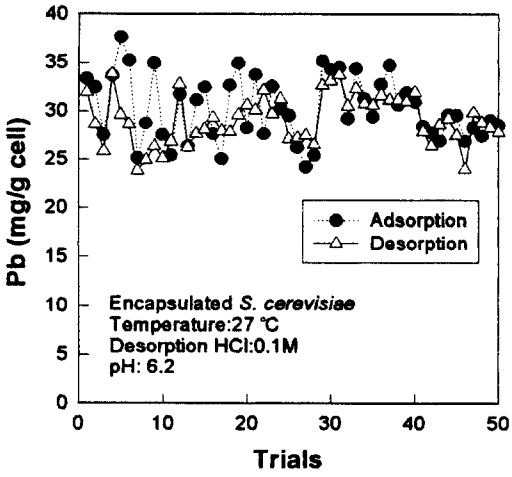

Fig. 6. Reusability of crosslinked capsules in adsorption-desorption process.

same with that of free cell exopolymer. When the capsule was crosslinked with triethylene tetramine and glutaric dialdehyde, the diameter of the capsule decreased from 2 mm to 1.8 mm and the specific cadmium uptake of biosorbent decreased about 33% of the original capacity. The adsorption isotherm was well fitted with Langmuir type.

Durability of Encapsulated Whole Cell Biosorbent

Capsules containing whole cell biosorbent were crosslinked and reused for the adsorption-desorption process. Cadmium adsorbed by the encapsulated whole cell exopolymer, which had been produced by *Z. ramigera* cells, was desorbed by the 0.1% (w/v) NTA (nitrilotriacetic acid trisodium salt and monohydrate) solution. Lead adsorbed on the encapsulated *S. cerevisiae* was desorbed by 0.1 M HCl solution. After 30 batches, encapsulated whole cell exopolymer was retained in the capsule and the specific cadmium uptake capacity increased gradually with repeated batches and reached to 125% of the initial batch. For the *S. cerevisiae*, the specific lead uptake capacity was constant and same with that of initial batch during 50 times adsorption-desorption trials as shown in Fig. 6. The capsule kept its initial shape until 30 batches but the capsule wrinkled after 50 cycles. The mechanical strength of the crosslinked capsule was found to be retained with naked eyes.

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