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Biosorption of Heavy Metal Ions by Immobilized *Zoogloea* and Zooglan

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ABSTRACT

Immobilized *Zoogloea* and zooglan in calcium alginate-silica matrix was shown to have a high adsorption capacity for Cu and Cd ions. Our results showed that Cu-ion uptake in the presence of Ca and Mg ions can be enhanced using immobilized *Zoogloea* and zooglan. Heavy metal ion adsorption efficiency decreased in the following order: Cu>Cd>Zn>Cr. The adsorbed metal ions were desorbed completely using sulfuric acid. Immobilized *Zoogloea* and zooglan which was repetitively regenerated adsorbed heavy metal ions without a loss of adsorption capacity.

Index Entries: immobilized *Zoogloea* and zooglan; heavy metal adsorption; regeneration of biomass; packed-bed column.

INTRODUCTION

Microbially assisted removal and accumulation of metals from aqueous solutions developed very rapidly from a laboratory curiosity to a full-scale industrial process *(1–4)*. Using microorganisms as biosorbents for heavy metals offers a potential alternative to existing conventional methods for detoxification and for recovery of valuable metals from aqueous solutions containing heavy metals *(5)*.

Novel processes for the accumulation of metal ions from the dilute solutions have been proposed. Bacteria, yeast, and algae have been successfully used as adsorbing agents for heavy metals *(6,7)*.

Among a group of floc-forming bacteria indigenous to activated sludges or organically polluted water, *Zoogloea ramigera* 115, isolated by Friedman and Dugan *(8)*, produces an extracellular zoogloeal matrix in

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which the cells are embedded. The zoogloeal matrix of *Zoogloea ramigera* strain 115 was shown to be composed of *D*-glucose, *D*-galactose, and pyruvic acid in an approximate molar ratio of 11:3:1.5 *(9)*.

Zoogloea ramigera and its extracellular polymer (zooglan) have a number of potential applications, including use in the removal of organic matter from solution as a part of wastewater treatment processes *(10)*. The purified polymer from *Zoogloea ramigera* was reported to have properties of a polyelectrolyte, as it binds high concentrations of metal ions *(11)*.

Although *Zoogloea ramigera* as an adsorbing agent is effective in accumulating heavy metal ions *(12)*, there are only few reports on the use of immobilized *Zoogloea ramigera* and zooglan for enhancing the biosorption efficiency of heavy metals.

The objective of the present work was to investigate the biosorption efficiency of heavy metals by immobilized *Zoogloea* and zooglan. Besides competition of heavy metals for adsorption on immobilized *Zoogloea* and zooglan, removal and recovery of heavy metals from immobilized *Zoogloea* and zooglan were also investigated.

MATERIALS AND METHODS

Production and Immobilization of Biomass

Methods of culturing *Zoogloea ramigera* 115 and production of zooglan have been described previously *(10)*. The culture broth was withdrawn after 96 h and stored at 4°C. *Zoogloea ramigera* and zooglan were separated from culture supernatant by centrifugation at 10,000*g* for 20 min, and the supernatant fraction was discarded.

The precipitate obtained by centrifugation was composed of a mixture of 1.1 g of *Zoogloea ramigera*/L and 12.6 g of zooglan/L. The precipitate was stored at 4°C. The precipitate composed of *Zoogloea ramigera* and zooglan was directly dried for 2 h at 105°C without any separation between microorganism and its producing polymer because *Zoogloea* strongly embedded in zooglan and its separation can increase manufacturing cost of biomass. Therefore, in this work, the dried mixture of *Zoogloea* and zooglan was used and crashed to form biomass-alginate silica matrix which is made by using sodium alginate and sodium metasilicate.

In this immobilization process, 30 g of biomass was added to 0.85% NaCl solution containing 2% sodium alginate and 0.2% sodium metasilicate. To make immobilized biomass-silica bead, the mixed solution was slowly dropped to 1.47% $CaCl_2$ solution. The produced bead (2 mm diameter) was hardened using 1% polyethylene (PEI) for 2 h and washed using distilled water.

Metal Uptake Apparatus

In the batch procedure, 1 g of immobilized *Zoogloea* and zooglan was placed in Erlenmeyer flasks (500-mL) to directly contact with 100 mL of metal-bearing solutions ($CuCl_2$, $CdCl_2$, $ZnCl_2$, $MnCl_2$) of 10 g/L of initial metal concentration to determine heavy-metal removal capacity of immobilized *Zoogloea* and zooglan.

The pH and temperature of metal containing solution was 5.0 and 25°C, respectively. After 2 h, which is sufficient to reach sorption equilibrium of Cu, Cd, Zn, and Mn because there is no variations of sorption efficiency, immobilized *Zoogloea* and zooglan was removed by centrifugation, and the supernatant fraction was analyzed for the remaining metal ions. No adjustment of pH was necessary to achieve flocculation because immobilized *Zoogloea* and zooglan in silica was easily separated from the solution.

In this experiment, commercial ion-exchange resin that is styrene-type strong-acidic cation-exchange resin (SK1B, Samyang, Korea) was also used to compare the heavy metal ion-uptake efficiency of immobilized *Zoogloea* and zooglan to commercial ion-exchange resin.

The characteristics of SK1B was as follows; empirical formula: $(C_{26}H_{24}S_2O_6Na_2)n$, apparent density: 825 g/L, water containing rate: 43–50%, exchange ability: above 1.9 meq/mL, effective size: 0.4–0.6 mm, durable temperature: below 120°C, effective pH range: 0–14.

Metal ions were analyzed using Atomic Absorption Spectrophotometer (Perkin Elmer, Norwalk, CT, Model: 2280) and followed the procedures described in the standard method *(13)*. In using AAS, wavelength, slit and flame were adjusted to the adequate value to the concerned metal.

The schematic diagram of packed bed column is shown in Fig. 1. The height and inner diameter of packed-bed column (total volume: 0.61 L) were 40 cm and 4.4 cm, respectively. In the column procedure, 100 g of immobilized *Zoogloea* and zooglan (60% of empty-bed total volume) was poured into a column and the solution containing metal ions was then passed through the column (flow rate: 12 mL/min), and the effluent was analyzed for metal ions. The temperature of the metal solutions was 25°C and the retention time in the column was 30 min.

Competition between heavy metal ions for adsorption on immobilized *Zoogloea* and zooglan, and the removal efficiency of heavy metal ions by regenerated immobilized *Zoogloea* and zooglan were investigated in these column experiments. 0.48% of nitrotriacetic acid (NTA) and 1 *N* sulfuric acid were compared to determine the regeneration ability of immobilized *Zoogloea* and zooglan.

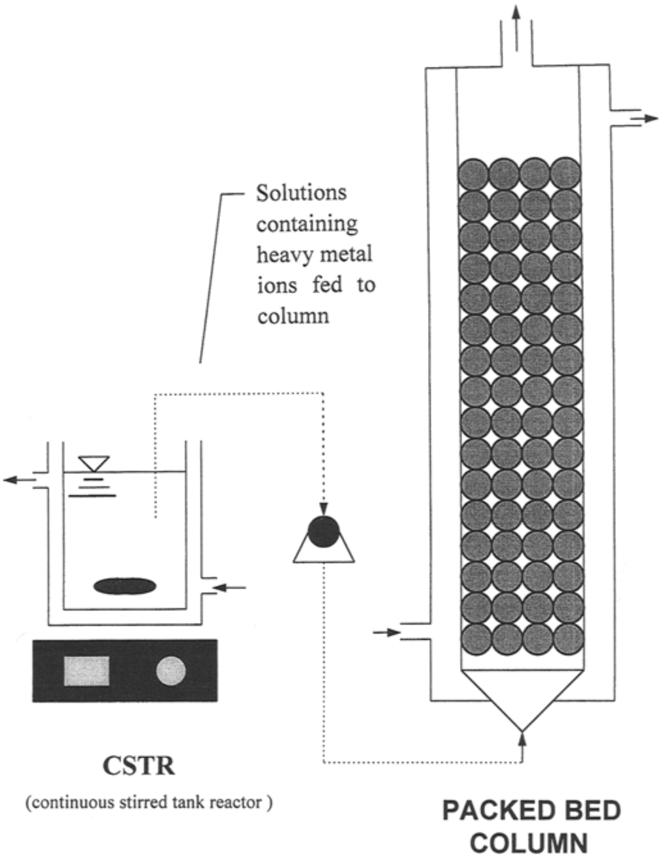

Fig. 1. Schematic diagram of packed-bed column used in column test.

RESULTS AND DISCUSSION

Figure 2 shows the heavy-metal-ion uptake efficiency of immobilized *Zoogloea* and zooglan and commercial ion-exchange resin in batchwise tests. Immobilized *Zoogloea* and zooglan in silica showed higher adsorption capacity for Cd and Cu ions than for Zn and Mn ions. Compared with commercial ion-exchange resin, higher Cd-ion uptake capacity was observed in immobilized *Zoogloea* and zooglan.

Generally, in contrast to the intracellular uptake of metal cations by microorganisms, which involves an energy-dependent system sensitive to pH and temperature, metal adsorption to negatively charged groups (carboxyl group, phosphoryl groups, amino groups) on the bacterial surface is rapid, reversible, and occurs whether or not a carbon or energy source is present in the medium *(14)*. Therefore, this result of high Cd-ion uptake by immobilized *Zoogloea* and zooglan may be caused by the pyruvic acid of extracellular zooglan in which *Zoogloea* is embedded. Immobilized

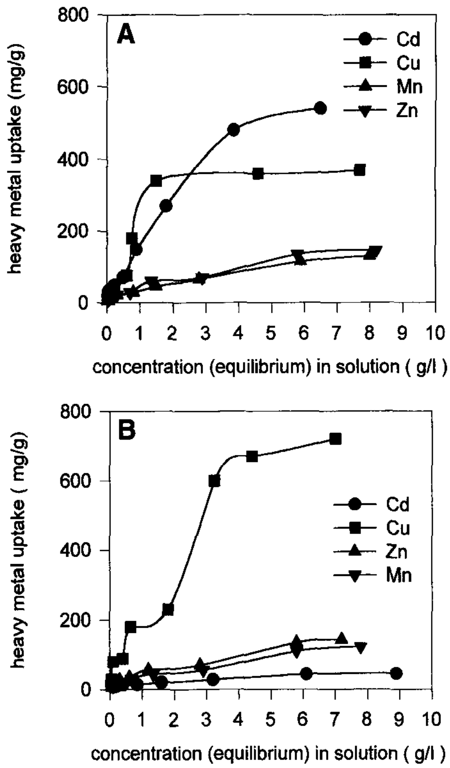

Fig. 2. Heavy-metal-ion uptake efficiency of immobilized *Zoogloea* and zooglan **(A)** and commercial ion-exchange resin **(B)**.

Zoogloea and zooglan was extremely hard and resisted fragmentation. Nevertheless, it may be sufficiently porous that all potential metal-ion binding sites are capable of being occupied since calcium alginate as a supporting material in this system have good porosity *(15,16)*.

For considering underground water, we compared Cu-ion adsorption capacity of immobilized *Zoogloea* and zooglan to commercial ion-exchange resin in the presence of 100 mg of Ca ion/L (as $CaCl_2$) and 100 mg of Mg ion/L (as $MgCl_2$). Despite of the presence of Ca and Mg ions, Cu-ion uptake capacity of immobilized *Zoogloea* and zooglan was not reduced, as shown in Fig. 3.

However, Cu-ion uptake capacity of ion-exchange resin was reduced in the presence of Ca and Mg ions because reactive functional group of commercial-ion exchange resin is composed of Na type and the ionization tendency of Ca and Mg ion is higher than heavy metal ion. These results show that heavy-metal removal of solutions that contain both heavy metal and Ca and Mg ions can be enhanced using immobilized *Zoogloea* and zooglan. Therefore, immobilized *Zoogloea* and zooglan can be applicable to the removal of heavy metals in ground water.

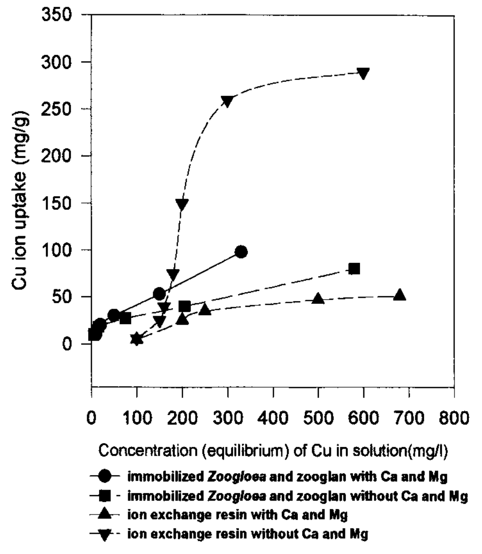

Fig. 3. Comparison of Cu uptake of immobilized *Zoogloea* and zooglan to commercial ion-exchange resin with/without Ca and Mg.

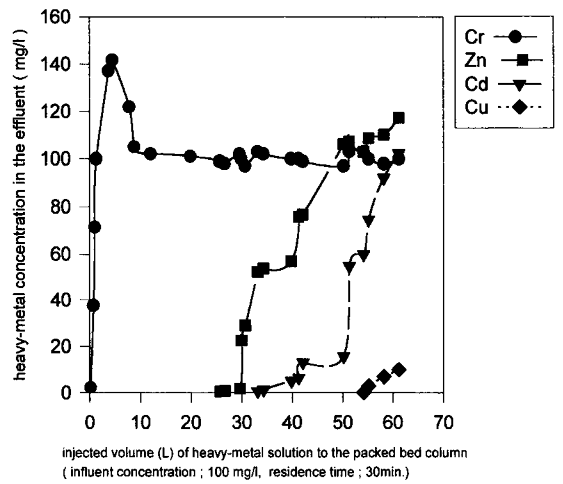

Fig. 4. Comparison of heavy-metal ions for adsorption on the immobilized *Zoogloea* and zooglan.

To investigate the competition of heavy-metal ions for adsorption on the immobilized *Zoogloea* and zooglan, the solutions containing Cr, Zn, Cd, and Cu ions were supplied to the column that was packed with immobilized *Zoogloea* and zooglan.

As shown in Fig. 4, heavy-metal-ion adsorption efficiency decreased in the following order: Cu>Cd>Zn>Cr. Compared to the batch results of ion-uptake capacity of the immobilized *Zoogloea* and zooglan, this result is in contrast to the batch result, that is, Cd-ion uptake capacity was higher

Table 1
Comparison of Regeneration Ability of Immobilized *Zoogloea* and Zooglan Using 0.48% of Nitrotriacetic Acid and 1 *N* Sulfuric Acid

Heavy metal ion	NTA (0.48%)	H_2SO_4 (1N)
Cd	60%	100%
Cu	72%	100%
Zn	70%	100%
Mn	20%	100%

than that of Cu ion in the batch test. According to Mittleman and Geesey *(17)*, of the various metals of toxicological concern in aquatic environments, copper exhibits the greatest tendency to associate with organic matter than cadmium. Our result also shows that copper has a higher affinity to the exopolymer when Cu and Cd ions are simultaneously supplied to the immobilized *Zoogloea* and zooglan. Meanwhile, the result of outlet concentration of Cr and Zn ions is temporarily higher than the inlet concentration may be adsorbed site by Cr and Zn is substituted by the following competitive other heavy metal, and the result of that, effluent have accumulating Cr and Zn ion.

Table 1 shows the regeneration ability of immobilized *Zoogloea* and zooglan using 0.48% of nitrotriacetic acid (NTA) and 1 *N* sulfuric acid in batchwise test. In this regeneration procedure, 20 g of immobilized *Zoogloea* and zooglan bead that is already used in heavy-metal adsorption was added to the 100 mL solution containing 0.48% of NTA and 1 *N* sulfuric acid for 2 h, respectively. Compared with 0.48% of NTA, the metal adsorbed on immobilized *Zoogloea* and zooglan was desorbed completely using sulfuric acid. No deformation of the shape of immobilized *Zoogloea* and zooglan was observed after the regeneration with 1 *N* sulfuric acid.

Figure 5 shows the heavy-metal ion removal efficiency of immobilized *Zoogloea* and zooglan which was repetitively regenerated using 1 *N* sulfuric acid. Immobilized *Zoogloea* and zooglan after repetitive regeneration adsorbed heavy-metal ions without a loss of adsorption capacity. This result showed that the continuous use of immobilized *Zoogloea* and zooglan is possible.

We conclude from this study that immobilized *Zoogloea* and zooglan on silica is an excellent biosorbent for heavy-metal ions, and better than ion-exchange resin in the presence of Ca and Mg ions. In addition, the immobilized *Zoogloea* and zooglan can be repetitively regenerated for continuous use. In future study, chemical modification of functional

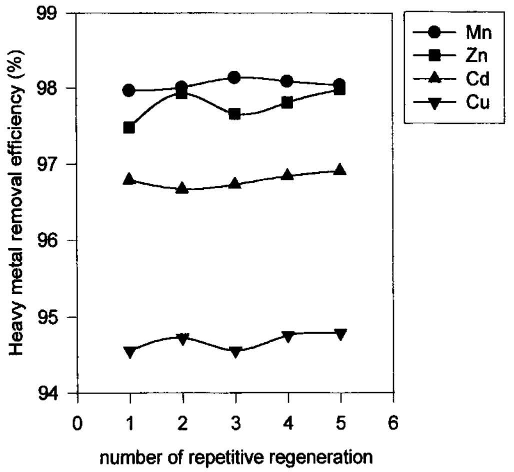

Fig. 5. Heavy-metal-ion removal efficiency of immobilized *Zoogloea* and zooglan after repetitive regeneration using 1 *N* sulfuric acid.

groups of zooglan to acquire specific selectivity to the heavy metal ions will be conducted and operational parameters of packed bed reactor will be investigated.

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