

Biosorption of Heavy Metal Ions by Biomass of Marine Brown Algae in Cheju using Their Immobilization Techniques: Biosorption of Copper by *Undaria pinnatifida*

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The biosorption performances of copper were investigated by the immobilized biomass of nonliving marine brown algae *Undaria pinnatifida* by each of the Ca-alginate method(Ca-ALG), Ba-alginate method(Ba-ALG), polyethylene glycol method(PEG), and carrageenan method (CARR). The copper removal performance increased but the copper uptake decreased as the biomass amount was increased. However, the copper uptake by the immobilized biomass increased with increasing initial copper concentration. Among the immobilization methods, the copper uptake decreased in the following sequence: Ca-ALG > Ba-ALG > PEG > CARR. The pattern of copper uptake by the immobilized biomass fitted the Langmuir isotherm better than the Freundlich isotherm. Desorption of deposited copper with 0.05~0.5M HCl, resulted in no changes of the copper uptake capacity of the immobilized biomass by the immobilization methods except for PEG, through five subsequent biosorption/desorption cycles. There was no damage to the immobilized biomass which retained its macroscopic appearance in repeated copper uptake/elution cycles.

Key words: biosorption, copper, *Undaria pinnatifida*, immobilization method, Freundlich isotherm, Langmuir isotherm, desorption

1. Introduction

Recently many workers have shown clearly that different, dead algae have different affinities for metals, depending upon the adsorptive conditions (Gardes-Torresdey, 1988; Greene and Darnall, 1990; Kam, 1997; Lee, 1997). One major advantage for using dead algae to recover metal ions from aqueous solutions is that the algae can be used to bind and recover metal ions under conditions that normally would be toxic to living algae.

In order to apply a biosorbent to the waste-

water treatment and metal recovery usefully, it must be easy to employ the equipment used to contact the wastewater with the biosorbent. Furthermore, it must be easy to transfer or separate from the contactor device and must have good physical and chemical stability for extended wastewater treatment use and for regeneration/metal recovery. For these reasons, it is necessary to granulate or immobilize the biomass.

Various techniques have been used to immobilize or granulate algae, both viable and nonviable, and other microorganisms(Kennedy and Cabral, 1983; Robinson, 1986). Algae have

been granulated using either polyacrylamide or silica gel. The latter has an advantage because physical strength of the product is greater and cost of the final biosorption product is less than the former (Darnall, 1986). Among the immobilization techniques, the most widely used in the adsorption process of heavy metals is the one that immobilize the adsorbent by entrapment with calcium alginate, κ -carrageenan, and polyacrylamide etc. as immobilizing carriers. The granulation technology of choice must also possess a number of characteristics which result in a stable, active biosorption product at reasonable cost.

Only recently the industrial applications of immobilized algal cells for metal recovery operations have been considered feasible (Holan, 1993). As a result, there have been few reports in the specific use of nonviable, immobilized algae for industrial or environmental metal ion recovery. The purposes of this study are to investigate the biosorption of metal ions by nonviable, immobilized algae, to identify effective immobilizing carrier material which can be applied to the practical biosorption process, and to develop good algal immobilization methods.

In this study, the biomass of nonliving, dried *Undaria pinnatifida* which is known as predominant heavy metal biosorbent material among easily available and preferably abundant marine brown algae in the earlier studies (Kam, 1997; Lee, 1997), were immobilized by calcium alginate method (Ca-ALG), barium alginate method (Ba-ALG), polyethylene glycol method (PEG), and carrageenan method (CARR) among the immobilization methods (Asenjo and Merchuk, 1995) and the biosorption performances of copper were investigated in the batch-stirred reactor for each of the immobilized biomass by the immobilization method discussed above.

2. Materials and Methods

2.1. Biomass

The marine brown alga *U. pinnatifida* (Phaeophyta) harvested in the sea near Cheju Island were employed as biomass. This material was washed thoroughly with water, stored in refrigerator for one day, and removed water fully by the freeze dryer. Dried material was ground in the Waring blender and sieved. Finely powered biomass in the range of 70~100 mesh was used in this study.

2.2. Chemicals

Analytical grades of $\text{Cu}(\text{NO}_3)_2 \cdot 3\text{H}_2\text{O}$, sodium alginate, H_3BO_3 , NaOH (Yakuri Pure Chem. Co., Japan), κ -carrageenan, polyethylene glycol (PEG) 4000, $\text{K}_2\text{S}_2\text{O}_8$ (Junsei Chem. Co. Ltd., Japan), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, N,N,N',N' -tetramethylethylenediamine (Sigma Chem. Co., USA), Na_2CO_3 (Hayashi Pure Chem. Co., Japan), and HNO_3 (Hori Pharm. Co., Japan) were used and the solutions were prepared with deionized distilled water. The stock solution of copper ion were prepared as 1000mg/L and diluted as desired concentrations with water.

2.3. Immobilization Method.

2.3.1. Ca-ALG and Ba-ALG

1g of biomass was added to the 250mL Erlenmeyer flask containing 100mL of 1% sodium alginate solution, stirred thoroughly with magnetic stirrer, and the mixture was dropped to the solution containing 1% CaCl_2 or 1% BaCl_2 using a syringe with 1mm of inner diameter. The bead of spherical immobilized biomass formed was in the range of 2~3mm in diameter. It was left for one day to increase its stability, filtered with

Whatmann filter paper, and then washed with 2% KCl and then water.

2.3.2. PEG

1g of biomass and sodium alginate were added to the 250mL Erlenmeyer flask containing 100mL of 40% PEG solution, stirred thoroughly with magnetic stirrer. To this solution, 1mL of 3% $K_2S_2O_8$ and N,N,N',N' -tetramethylethylenediamine were added, stirred, and then the mixture was dropped to the solution containing 1% $CaCl_2$ using a syringe with 1mm of inner diameter, and then was performed with the same procedure as section 2.3.1. The bead of spherical immobilized biomass formed was in the range of 3~4mm in diameter.

2.3.3. CARR

1g of biomass was added to the 250mL Erlenmeyer flask containing 100mL of 4% κ -carrageenan solution in a constant water bath controlled at 60°C, stirred thoroughly with magnetic stirrer, and the mixture was dropped to the solution containing 1% $CaCl_2$ using a syringe with 1mm of inner diameter, and then was performed with the same procedure as section 2.3.1. The bead of spherical immobilized biomass formed was in the range of 3~4mm in diameter.

2.4. Methods

The batch reactor experiments were performed to investigate not only the effects of initial copper concentration, the biomass amount, pH, and acid concentration in desorption of immobilized biomass loaded with copper but also biosorption isotherm. The immobilized biomass with fixed amount and copper concentration with fixed concentration were placed in a 1-liter flask

container. The solution was stirred with a magnetic stirrer. During the experiment, 2mL of sample in the solution was taken at a given time interval and used for the measuring metal ion concentrations in the solution. Water bath was used to maintain the constant temperature(20°C) in the system.

In the biosorption isotherm experiments, a relationship between the equilibrium concentration and the amount of copper ion adsorbed per unit mass of biomass in a immobilized biomass was obtained by employing a series of tests in the range of initial copper concentration, 25~500mg/L.

In the desorption experiments, following the copper biosorption experiments, each of the immobilized biomass loaded with copper was separated by filtration, returned into 150mL Erlenmeyer flask containing 50mL of hydrochloric acid at different concentrations(0~0.5M), and stirred with magnetic stirrer to equilibrium(or longer up to 12hr). Following the filtration recovery of immobilized biomass, it was thoroughly washed with deionized distilled water until pH 7 of the effluent was reached. The regenerated immobilized biomass was used again in the next copper biosorption experiment.

All experimental vessels were pyrex glass and all glasswares were leached in a 14% nitric acid and washed thoroughly with deionized distilled water prior to use. The concentrations of copper in solution were determined by an atomic absorption spectrophotometer(GBC 904AA) at 324.7nm.

3. RESULTS AND DISCUSSION

3.1. Effect of initial copper concentration

In order to investigate the effect of initial copper concentration, each of the immobilized

biomass containing 0.6g of biomass for Ca-ALG, Ba-ALG, and PEG or the immobilized biomass containing 1.5g of biomass for CARR was added to the solution containing 25, 50, 100, 200, 300, and 500mg/L. As shown in Fig. 1, represented as the solution concentration with time, all the immobilized biomass attained the final equilibrium copper uptake after 2hr. This result shows much more time is required, compared to the biomass which is not immobilized(Kam, 1997; Lee, 1997). This feature illustrates the contact time between copper ion and biomass increases with the increase in biomass size in order to attain to the equilibrium state.

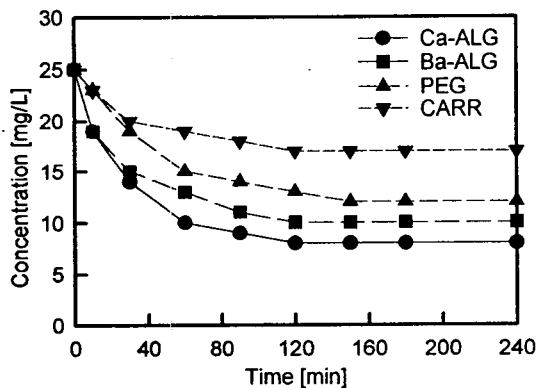


Fig. 1. Concentration profiles for copper biosorption by the immobilized biomass of *U. pinnatifida*(initial copper concentration: 25 mg/L, biomass amount: 0.6g for Ca-ALG, Ba-ALG and PEG, 1.5g for CARR).

Figure 2 show how the variation of the initial copper concentration affects the biosorption performance. As shown in figure, the experimental result shows the better copper uptake as initial copper concentration is increased. This feature causes the increase of driving force of mass transfer with increasing the initial copper concentration. Comparing the copper uptake among each of the immobilization methods, the copper

uptake was 86mg/g, 60mg/g, 48mg/g, and 18mg/g at final equilibrium state, for Ca-ALG, Ba-ALG, PEG, and CARR, respectively, when 500mgCu/L of initial concentration was used. Therefore it is considered that Ca-ALG is the most efficient among the immobilization methods used in this study for removing copper to the practical water and wastewater treatment.

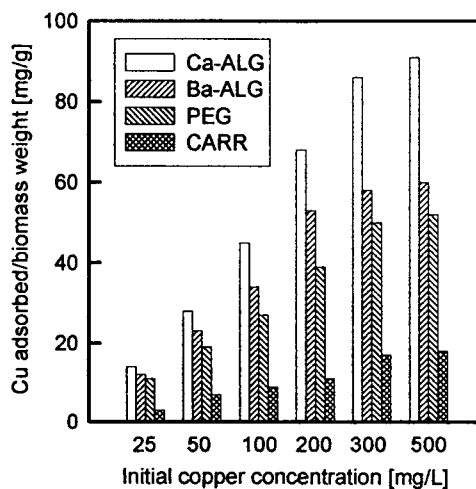


Fig. 2. Effect of copper uptake on initial copper concentration for biosorption by the immobilized biomass of *U. pinnatifida*.

3.2. Effect of biomass amount

The effect of biomass amount on the metal removal for each of the immobilization methods used was examined. Each of the immobilized biomass containing 0.15, 0.3, and 0.6g of biomass for Ca-ALG, Ba-ALG, and PEG, 0.375, 0.75, and 1.5g of biomass for CARR was added to 500mL solution containing 25mg Cu/L at 20°C.

Figure 3 show the comparison of copper removal efficiency with the biomass amount for each of the immobilization methods. The rate of copper removal increased as the biomass amount added was increased. This result means that the

increase of biomass amount contributes to the increase of surface available for biosorption. This figure also shows that different copper removal efficiency was obtained according to the immobilization method used, i.e., for each of 0.15, 0.3, and 0.6g of biomass, the removal efficiencies was 30, 48, and 68% in the case of Ca-ALG; 27, 44, and 52% in the case of Ba-ALG; 23, 38, and 52% in the case of PEG, at the equilibrium state: in the case of CARR, for each of 0.375, 0.75, and 1.5g of biomass, 13, 20, and 32%. This finding demonstrates that different immobilization method exhibit different metal binding characteristics at a given pH and is also similar to the result of Greene et al.(1987) and Gardea-Torresdey(1988). Among the immobilization methods, the copper removal efficiency was in the following sequence: Ca-ALG > Ba-ALG > PEG > CARR.

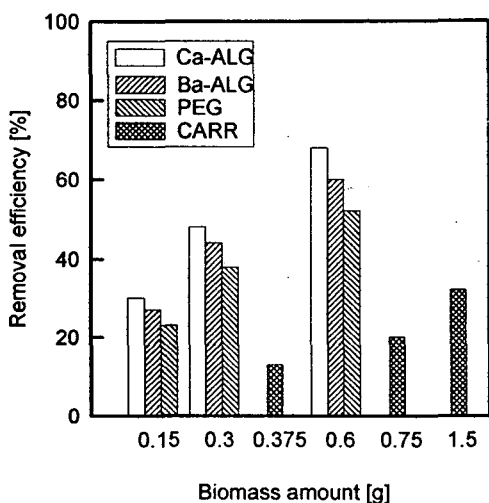


Fig. 3. Comparison of copper removal efficiency with the biomass amount of *U. pinnatifida* for each of the immobilized methods(initial copper concentration: 25mg/L).

Figure 4 illustrates the copper uptake, i.e., the amount of copper adsorbed per unit weight of dry biomass, at constant loading on the variation of biomass amount. This figure shows that the

equilibrium copper uptake decreases as the amount of biomass added is increased. This result is similar to that by Horikoshi et al.(1981) that uranium adsorbed per unit weight of micro-organism decreases as its amount added is increased, using *Acinomyces levoris* and *Streptomyces viridochromogens*. This finding gives us that biosorbent efficiency for copper removal will decrease as much more biomass is used. It will be possible to determine the suitable biomass amount added from the result in this experiment and that in the effect of initial copper concentration.

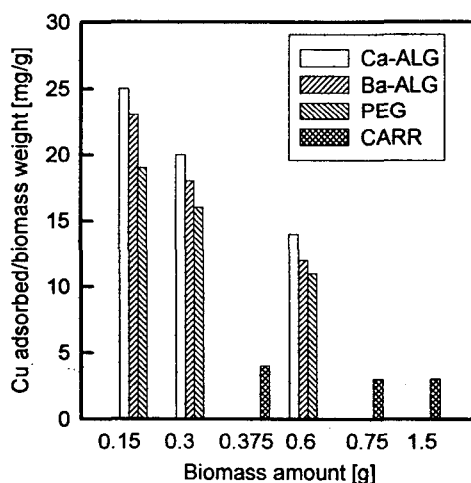


Fig. 4. Effect of biomass amount on the copper uptake for biosorption by the immobilized biomass of *U. pinnatifida*(initial copper concentration: 25mg/L).

3.3. Effect of pH

It is known that the pH of the solution is more sensitive than temperature and heavy metal biosorption increases as pH approaches to neutral (Norberg and Persson, 1984) or the optimum pH is present according to the biosorbents used (Haug, 1974; Strandberg, 1981).

Figure 5 shows the effect of pH on the copper uptake by the immobilized biomass in a solution containing 25mgCu/L. A constant pH for batch equilibrium biosorption experiment was maintained at pH 2, 3, 4, 5, and 6, respectively. Each pH was controlled with $\text{Na}_2\text{CO}_3(10^{-4}\text{N})$ and HNO_3 or NaOH . Although the starting pH of copper solution with no pH adjustments were 5.1, it increased to 5.3 for Ca-ALG, Ba-ALG, and PEG, and to 5.8 for CARR as soon as the immobilized biomass was added. But the pH in the immobilized biomass biosorption systems decreased to 5.0~5.1 for Ca-ALG, Ba-ALG, and PEG, and to 5.6~5.7 for CARR at the equilibrium state. The final pH values were substantially lower than the initial pH values, a result suggesting that metal-cation binding occurred at least in part by an ion-exchange mechanism in which some of the counter ions associated with the metal-binding sites were protons.

As shown in Fig. 5, a low pH of 2 resulted in a markedly lower copper uptake and a higher uptake was obtained in the range of pH 4.0-6.0. The reason for this is to be expected due to a higher hydrogen ion concentration, which in turn prevents the immobilized biomass-copper binding. The experimental result demonstrates that pH of the solution is a very important factor in copper uptake by the immobilized biomass. Crist *et al.* (1981) demonstrated that mechanism of metal-ion binding to nonliving algal cells may depend on the species of metal ion, the algal organism, and the solution conditions. They presented evidence that metallic cation binding to *Vaucheria* sp. occurred at least in part by an ion-exchange mechanism and suggested that electrostatic attraction plays an important role in metal uptake process. It is considered that the pH dependence of the copper-cation binding generally occurs when the active metal-binding sites can also bind protons. Thus, copper ions and protons

compete for the same binding sites.

The solution of copper salt for biosorption studies should not exceed pH 7 because insoluble copper hydroxide starts precipitation from the solutions at higher pH values making the true biosorption studies impossible.

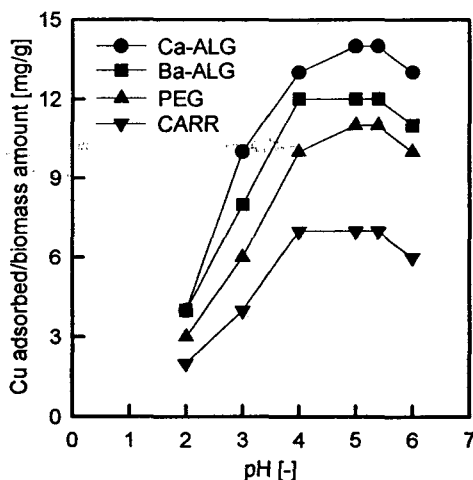


Fig. 5. Effect of pH on the copper uptake by the immobilized biomass of *U. pinnatifida* (initial copper concentration: 25mg/L, biomass amount: 0.6g for Ca-ALG, Ba-ALG and PEG, 1.5g for CARR).

3.4. Biosorption isotherm

Adsorption from aqueous solution at equilibrium is usually correlated by one of the two following adsorption isotherm, the Langmuir isotherm or the Freundlich isotherm.

Figure 6 shows copper biosorption isotherms for the immobilized biomass by each of the Ca-ALG, Ba-ALG, PEG, and CARR, in the range of initial copper concentration, 25~500 mg/L. The data demonstrated that immobilized biomass by Ca-ALG had relatively high biosorption capacity for copper. However, the copper biosorption capacity by CARR were very low,

compared to those by the other immobilization methods. This is assumed due to the difference in the structure of the immobilizing carrier (Asenjo and Merchuk, 1995).

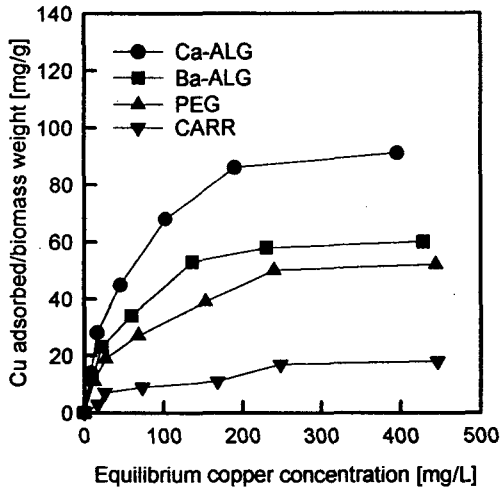


Fig. 6. Copper biosorption isotherms for the immobilized biomass of *U. pinnatifida*.

Figure 7 represent the Freundlich isotherm plot for copper. The values of K , n , and correlation coefficient (r^2) for the immobilized biomass sorption systems are summarized in Table 1. The values of n obtained were in the range of 1.785~2.353. The values of K giving a measure of adsorbent capacity decreased in the order of Ca-ALG, Ba-ALG, PEG, CARR among the immobilization methods.

Table 1. Langmuir and Freundlich isotherm parameters for copper uptake by the immobilized biomass.

Immobilization methods	Langmuir			Freundlich		
	q_{max}	b	r^2	K	n	r^2
Ca-ALG	105.1	0.011	0.991	6.62	2.111	0.928
Ba-ALG	68.61	0.021	0.995	5.39	2.317	0.937
PEG	50.67	0.023	0.991	4.33	2.353	0.948
CARR	25.18	0.003	0.992	1.48	1.785	0.955

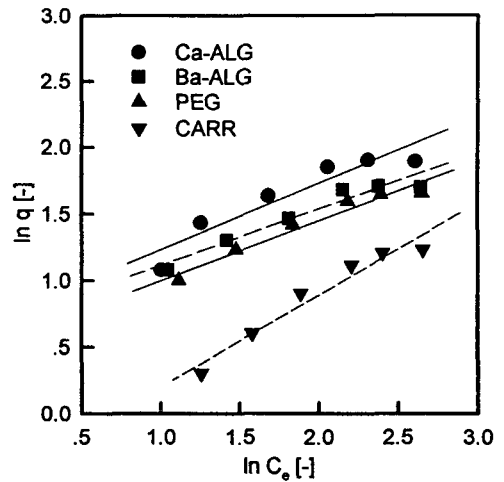


Fig. 7. Freundlich isotherm plot of copper for the immobilized biomass of *U. pinnatifida*.

The Langmuir isotherms for copper are plotted in Fig. 8. Obtained values of q_{max} , b , and r^2 are given in Table 1. As shown in Table 1, calculated biosorption maximum of copper for the immobilized biomass *U. pinnatifida* by Ca-ALG was found to be 105mg Cu/g of biomass.

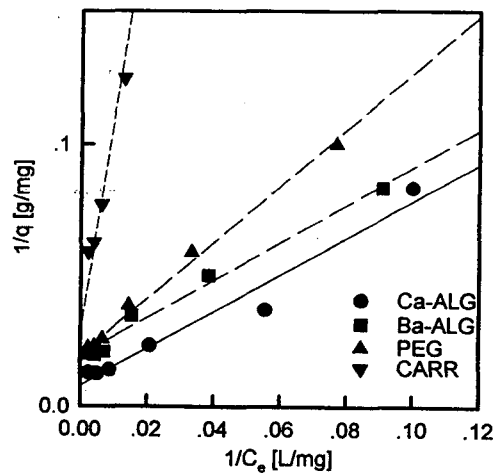


Fig. 8. Langmuir isotherm plot of copper for the immobilized biomass of *U. pinnatifida*.

Figure 9 illustrates the comparison of calculated values by the Freundlich and Langmuir correlation with the experimental data of copper biosorption. From the results in Fig. 9 and r^2 in Table 1, it was found that the pattern of copper biosorption by the immobilized biomass fitted the Langmuir isotherm better than the Freundlich isotherm.

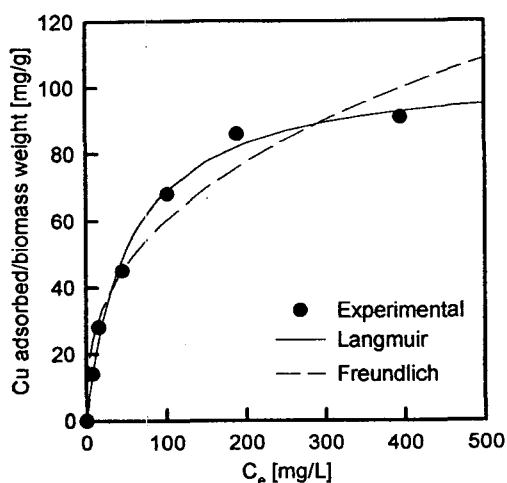


Fig. 9. Comparison of calculated values by the Langmuir and Freundlich model with experimental data of copper biosorption isotherms for the immobilized biomass of *U. pinnatifida* by Ca-ALG.

3.5. Desorption of copper-laden immobilized biomass

Exploratory desorption tests for copper-laden immobilized biomass were conducted under batch conditions. The copper-laden immobilized biomass of *U. pinnatifida* with initial copper concentration of 500mgCu/L, was contacted with hydrochloric acid eluent at different concentrations(0~0.5M). Table 2 summarizes the copper release efficiency. The increase of copper release for acid wash in the range of 0.05~0.5M HCl, irrespective of the

immobilization method used, was only very marginal. But the size of the immobilized biomass by PEG, decreased to that by Ca-ALG or Ba-ALG, irrespective of hydrochloric acid concentration. The lower concentration of 0.05M for copper was used for elution in subsequent tests, for the immobilized biomass by the immobilization methods except for PEG. The biosorption-desorption experiment was consecutively performed during five cycles. Table 3 summarizes the biosorption efficiency by regenerated immobilized biomass. The copper uptake capacity of the immobilized biomass in the fifth cycle was comparable to that in the first cycle.

Table 2. Desorption of copper from the immobilized biomass of *U. pinnatifida*.

HCl (M)	Desorption efficiency (%)			
	Ca-ALG	Ba-ALG	PEG	CARR
0	6	7	6	28
0.005	72	70	63	72
0.01	79	80	76	82
0.025	85	86	86	88
0.05	98	98	93	97
0.1	97	98	98	95
0.2	98	98	98	98
0.5	99	97	100	98

Table 3. The biosorption efficiency of copper by regenerated immobilized biomass of *U. pinnatifida*.

Cycle	Biosorption efficiency* (%)		
	Ca-ALG	Ba-ALG	CARR
1 [†]	100	100	100
2	98	97	95
3	96	96	95
4	95	96	94
5	94	95	94

*Biosorption efficiency was calculated, comparing the copper uptake in each cycle with that in the first cycle.

[†]First cycle represents the first biosorption experiment

4. CONCLUSIONS

Using the immobilized biomass of nonliving marine brown algae *U. pinnatifida* harvested in the sea near Cheju Island by each of the Ca-ALG, Ba-ALG, PEG, and CARR, batch reactor experiments were performed in order to investigate the effect of initial copper concentration, biomass amount, and pH for their biosorption performances of copper and the results were compared among the immobilization method used.

The experimental result showed that each of the immobilized biomass-Cu²⁺ system attained the final equilibrium plateau after 2hr. The copper removal performance increased but the copper uptake decreased as the biomass amount was increased. However, the copper uptake by the immobilized biomass increased with increasing initial copper concentration. Among the immobilization methods, the copper uptake decreased in the following sequence: Ca-ALG > Ba-ALG > PEG > CARR.

Equilibrium parameters based on the Langmuir and the Freundlich isotherm were determined. The values of Freundlich constant *n* obtained in this study were in the range of 1.785~2.353. The experimental data of the copper biosorption by the immobilized biomass were fitted to the Langmuir isotherm better than the Freundlich isotherm.

The immobilized biomass by the immobilization methods except for PEG could be desorbed easily with 0.05~0.5M HCl, with practically negligible subsequent changes of each biosorbent capacity and macroscopic appearance in five subsequent biosorption/desorption cycles.

Nomenclature

- b Langmuir constant(related to energy of adsorption)
C concentration of copper in the solution

	[mg/L]
C _e	equilibrium concentration of copper in the solution [mg/L]
C _o	initial concentration of copper in the solution [mg/L]
K	Freundlich constant(adsorbent capacity)
n	Freundlich constant(adsorption intensity) [-]
q	copper uptake(amount of copper adsorbed per unit weight of adsorbent) [mg/g]
Q _{max}	maximum copper uptake

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