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Removal and Recovery of Copper (II) Ions by Bacterial Biosorption

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Abstract

Studies were conducted to investigate the removal and recovery of copper (II) ions from aqueous solutions by *Micrococcus* sp., which was isolated from a local activated sludge process. The equilibrium of copper biosorption followed the Langmuir isotherm model very well with a maximum biosorption capacity (q_{max}) of 36.5 mg of Cu^{2+}/g of dry cell at pH 5.0 and 52.1 mg of Cu^{2+}/g of dry cell at pH 6.0. Cells harvested at exponential growth phase and stationary phase showed similar biosorption characteristics for copper. Copper uptake by cells was negligible at pH 2.0 and then increased rapidly with increasing pH until 6.0. In multimetal systems, *Micrococcus* sp. exhibited a preferential biosorption order: Cu ~ Pb > Ni ~ Zn. There is virtually no interference with copper uptake by *Micrococcus* sp. from solutions bearing high concentrations of Cl^-, SO_4^{2-}, and NO_3^- (0–500 mg/L). Sulfuric acid (0.05 *M*) was the most efficient desorption medium, recovering >90% of the initial copper sorbed. The copper capacity of *Micrococcus* sp. remained unchanged after five successive sorption and desorption cycles. Immobilization of *Micrococcus* sp. in 2% calcium alginate and 10% polyacrylamide gel beads increased copper uptake by 61%. Biomass of *Micrococcus* sp. may be applicable to the development of potentially cost-effective biosorbent for removing and recovering copper from effluents.

Index Entries: Biosorption; copper removal; bioremediation; immobilization; metal adsorption.

Introduction

The presence of copper ions in water poses serious environmental and human health hazards because of their toxicity, tendency to bioaccumulate,

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and abundance and persistence in the environment *(1)*. In Hong Kong and South China, copper pollution arises mainly from the effluents discharged from the electroplating and printed circuit-board factories. It has been reported that for a typical printed circuit-board factory, about 55 kg of copper was discharged per month into Hong Kong waters before enactment of the Water Control Ordinance. A high level of copper has also been found in the sediment of Victoria Harbor.

Metal-processing industries must pretreat or detoxify metal-rich effluents before discharging them into the aqueous environment. Current technologies for copper removal such as chemical precipitation, electrochemical treatment, and ion exchange provide only partially effective treatment and are costly to implement and use, especially when the metal concentration is low. The use of biological materials for the removal or recovery of heavy metals has gained importance in the last decade owing to their good performance and low cost. Microorganisms including bacteria *(2,3)*, microalgae *(4)*, and fungi *(5)* could accumulate large amounts of heavy metals with high selectivity even when they are dead or inactive. Accumulation of metals without active uptake is known as biosorption, which can be considered a collective term for a number of passive accumulation processes including ion exchange, coordination, complexation, adsorption, and microprecipitation *(4)*. These biomasses are capable of removing even trace levels of metal ions. Moreover, they can also be used to recover rare, precious, or strategic metals from waste solutions. The abundant bacterial biomass can be obtained inexpensively, because it is a waste byproduct of large-scale industrial processes such as fermentation and activated sludge wastewater treatment. Hence, biosorption coupled with desorption may provide an economic and effective alternative for the removal and recovery of heavy metals.

It is important to identify more microbial strains that can uptake metals with high efficiency and specificity as well as to design better bioprocess that effectively remove or recover heavy metals from aquatic systems. To optimize design and operation of biosorbent systems for removal and recovery of metals, a thorough understanding of biosorption behavior and desorption kinetic characteristics of microbial cells is needed. This motivated us to evaluate the feasibility and ability of microorganisms indigenous to biological treatment systems to remove metals in wastewater *(6)*.

The present study was conducted to characterize copper biosorption and desorption behavior of *Micrococcus* sp., which was isolated from a local activated sludge system. *Micrococcus* sp. was found to have high copper biosorption capacity *(6)*. The effects of initial copper concentrations, cell age, pH, competing cations, anions, desorption, and immobilization on the copper biosorption capacities by *Micrococcus* sp. have been studied systematically and extensively. The ultimate goals of this study were to develop novel and economical processes for removing and recovering copper (II) ions from aqueous wastes using bacterial-based biosorbents.

Materials and Methods

Isolation Procedures and Identification

Micrococcus sp. was isolated from fresh activated sludge collected from the return sludge channel at the Shatin Sewage Treatment Works in Hong Kong. The sludge was serially diluted in distilled deionized water. Aliquots (0.1 mL) were spread on nutrient agar (Difco) and cultivated in an incubator at 30°C for 3 d. Colonies were picked up and maintained on the same medium for the subsequent metal biosorption test. The isolates were identified using the MIDI Sherlock Microbial Identification System.

Preparation of Biosorbents

Colonies from freshly prepared agar plates were used to inoculate several sterilized 1-L conical flasks, each containing 400 mL of nutrient broth. The cultures were grown at pH 7.0 and 37°C on an orbital shaker at 250 rpm for 48 h. Cells were harvested and washed twice with distilled deionized water by centrifuging at 9000*g* force and 4°C for 20 min. The biomass was then resuspended in 200 mL of distilled deionized water for preparing a biomass stock solution. The concentration of biomass in stock was estimated by oven drying a designated portion of cells.

Biosorption Studies

Batch experiments for determining metal biosorption isotherms were carried out with the initial copper concentrations ranging from 1 to 150 mg/L and biomass concentrations ranging from 1 to 2 g of dry cell/L. The total reaction volume was 50 mL in 500-mL polyethylene bottles. The solution pH was adjusted to 5.0 by adding 0.1 *M* NaOH and 0.1 *M* HNO_3. Reaction bottles were agitated on an orbital shaker at 25°C for 12 h, which is more than ample time to reach metal biosorption equilibrium *(7)*. Bacteria were always the last component to be added to the reaction mixtures, and all experiments were performed in duplicate. At designated intervals, samples were taken and the biomass was separated by centrifuging. Copper concentrations in the supernatants were determined using a model 100 Perkin-Elmer atomic absorption spectrophotometer.

Effects on Competing Cations

To ascertain whether there was any competition between different metal ions for uptake by *Micrococcus* sp., biosorption of copper, zinc, nickel, and lead by *Micrococcus* sp. from single and multimetal ion solutions was studied. The initial concentration of each single metal ion for biosorption was 2 m*M*. Our previous study *(6)* has shown that a metal concentration of 2 m*M* would be high enough to saturate all the binding sites of *Micrococcus* sp. under the experimental conditions. For the binary metal systems, total metal concentration was 4 m*M*, whereas for the four-metal systems, the total metal concentration was 8 m*M*. The experiment was conducted according to the biosorption methodology described earlier at pH 5.0. All metal ion solutions were prepared by using nitrate salts to prevent possible salt precipitation.

Effects of Anions

Experiments examining the effect of various anions on copper removal by *Micrococcus* sp. were carried out by adding sodium salts of chloride, sulfate, or nitrate to obtain the additional anion concentrations of 0, 50, 100, and 500 mg/L, to solutions containing 100 mg/L of copper nitrate. Experiments were conducted according to the biosorption methodology described earlier.

Biosorption and Desorption Cycles

The bacterium *Micrococcus* sp. with a final concentration of 1.5 g of cell/L was suspended in solutions containing 50 mg of Cu^{2+}/L in centrifuge tubes. The final volumes were made up to 25 mL. The pH of the resulting mixtures was adjusted to 6.0 by adding HNO_3 and NaOH. The tubes were shaken at 250 rpm and 25°C for 6 h. Then, the copper-loaded biomass was centrifuged, rinsed with distilled deionized water, and resuspended in 10 mL 0.05 *M* separately for 45 min to recover the copper ions from cells. The regenerated biomass was again suspended in copper solutions for the next biosorption run. The biosorption and desorption steps just described were repeated five times. The copper concentrations in the supernatants were determined after the biosorption and desorption steps.

Immobilization Studies

In the exponential phase of growth, *Micrococcus* sp. cells were centrifuged, washed, and resuspended in solution A containing 4% sodium alginate, 18.2% acrylamide, and 1.8% *N,N'*-methylene-*bis*-acrylamide. The volume ratio of cell suspension to solution A was 1:1. distilled deionized water was added instead of cell suspension for preparing the gel beads for controls. The resulting mixtures were dropped into solution B, which consisted of 4% calcium chloride, 0.1% *N,N,N',N'*-tetramethylene-diamine, and 0.1% ammonium persulfate. The drops gelled into 2.3 ± 0.3 mm diameter spheres on contact with solution B. The immobilized cell particles were kept in solution B at room temperature with constant and gentle stirring for at least 2 h to complete gel formation. The immobilized cell particles were prepared under aseptic conditions.

Results and Discussion

Biosorption Studies

Copper biosorption isotherms for *Micrococcus* sp. at pH 5.0 and 6.0 are presented in Fig. 1. The calculated model parameter values and the correlation coefficients for Langmuir and Freundlich isotherms are given in Table 1.

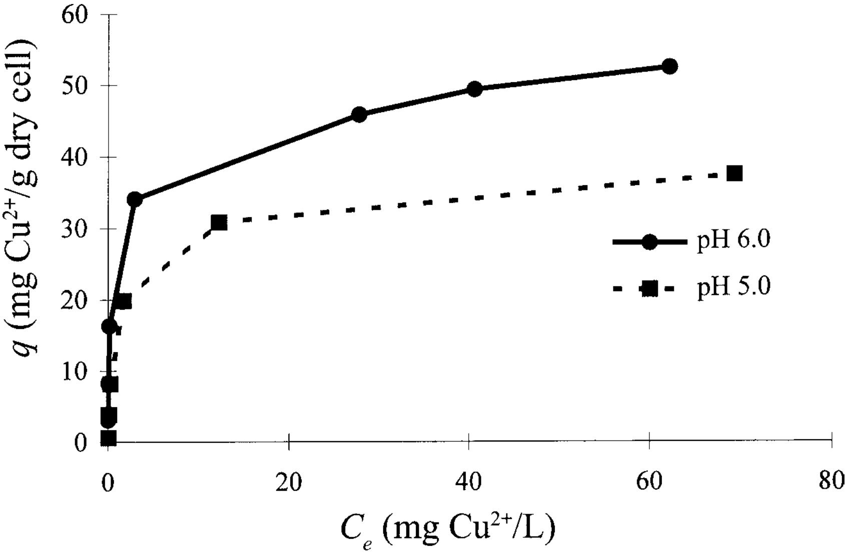

Fig. 1. Copper removal isotherm by *Micrococcus* sp. at pH 5.0 and 6.0.

Table 1
Calculated Parameters of Langmuir and Freundlich Isotherm Models
for Copper Biosorption by *Micrococcus* sp. at pH 5.0 and 6.0

	Langmuir equation			Freundlich equation		
	Q_{max} (mg/g)	b (L/mg)	r^2	K	n	r^2
pH 5.0	36.5	0.61	0.9993	8.24	0.49	0.8008
pH 6.0	52.1	0.66	0.9893	13.6	0.61	0.7827

The isotherms were found to follow the typical Langmuir adsorption pattern with r^2 of the linearized Langmuir isotherm equal to 0.999. The linearized Langmuir isotherm can be represented as follows:

$$C_e/q = 1/(q_{max} \cdot b) + C_e/q_{max}$$

in which q_{max} and b are the maximum biosorption capacity and affinity, respectively. The Freundlich isotherms did not fit the biosorption data very well, as shown by the r^2 of the linearized Freundlich plot (0.801). The results demonstrated that copper ions were well adsorbed by the mobilized *Micrococcus* sp. The maximum biosorption capacity (q_{max}), estimated by the Langmuir model, reached 36.5 mg of Cu^{2+}/g of dry cell at pH 5.0 and 52.1 mg of Cu^{2+}/g of dry cell at pH 6.0. The Freundlich coefficients K at pH 5.0 and 6.0 were 8.24 and 13.56, respectively. The parameters q_{max} and K reflect different characteristics: the Langmuir parameter, q_{max}, represents the saturation level of sorbed copper at high solution concentrations, whereas the Freundlich K represents the amount of copper sorbed when the solution concentration in the equilibrium is unity. Both the Freundlich

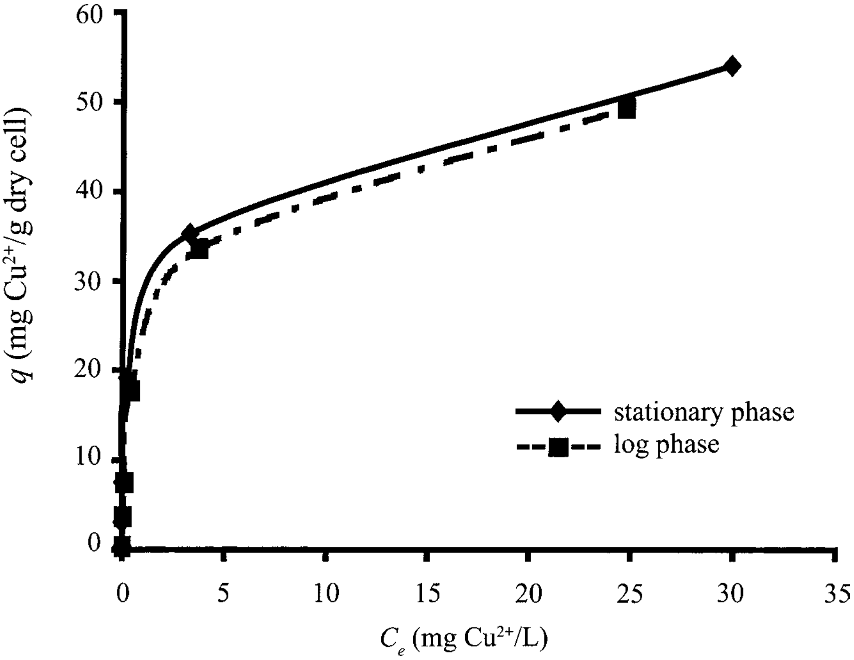

Fig. 2. Effect of culture age on copper biosorption.

parameter K and the Langmuir parameter b measure the effectiveness of copper biosorption at low copper concentration in solution. Higher values of b imply a higher biosorption level at low solution concentrations. The Freundlich parameter n measures the extent of impact on biosorption of a change in residual solution concentration from unity. High values of n imply a relatively large change in sorbed copper when the residual copper concentration deviates either above or below unity.

Effect of Culture Age on Copper Biosorption

Cells of two different culture ages were used to remove copper from metal-contaminated solutions (Fig. 2). Results showed that there was virtually no difference in copper uptake between cells harvested during log phase and those during stationary phase. Volesky and Holan *(4)* reported that metal ions bound mainly to the anionic functional groups present on the cell wall, such as carboxyl, hydroxyl, sulfate, phosphate, and amino groups. This may suggest that the surface composition and properties of the cell wall did not change much during the stationary phase.

Effect of pH

Because biosorption is a predominantly physio-chemical process taking place between positively charged metal ions and anionic groups of the cell surface *(8)*, metal removal is strongly influenced by the experimental conditions of solution pH, specific surface properties of adsorbent, the

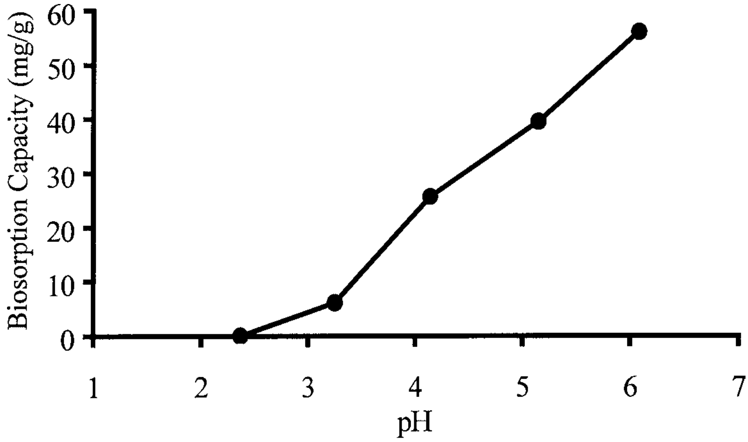

Fig. 3. Effect of pH on 100 mg/L copper biosorption by *Micrococcus* sp.

concentration of adsorbate, and the presence of coions in solutions. It has been consistently reported that pH is the dominant solution parameter controlling biosorption and that cation biosorption increases as solution pH increases *(6,9,10)*.

Copper biosorption by *Micrococcus* sp. was strongly affected by solution pH, as indicated in Fig. 3. Copper uptake was negligible at pH 2.0 and then increased rapidly with increasing pH. It is very likely that hydrogen ions compete with copper ions for the sorption sites of cells *(11)*. At lower pH, biosorption of hydrogen ions was preferred over that of copper ions, but at higher pH, more copper ions were taken up. The pH biosorption studies were not conducted at pH values above 6.0, because insoluble copper hydroxide precipitates from the solution at higher pH values, making true biosorption studies impossible. The pH profile suggested that copper binding to cell walls and external surfaces was most likely one of the dominant removal processes.

Effects on Competing Cations

As with hydrogen ion on pH effect, the cocations existing in the solution have the same effect. Table 2 gives the percentage decrease in the metal removal by *Micrococcus* sp. in the presence of one or three other metals. The presence of additional metal ions does not significantly impair the biosorption of copper. Copper biosorption was more sensitive to the presence of lead than zinc and nickel. On the other hand, removal of zinc and nickel by *Micrococcus* sp. was affected conspicuously by the presence of copper. When all four metals were present, the inhibition influence on copper biosorption by *Micrococcus* sp. did not accumulate proportionally. On the contrary, the removal capacities for copper and lead were increased when compared with their two metal systems. *Micrococcus* sp. bound the heavy metals in the following order: Cu ~ Pb > Ni ~ Zn. These results showed high similarity to the study carried out by Shuttleworth and Unz

Table 2
Percentage Decreases in Metal Removal by *Micrococcus* sp.
in Presence of Two-, Three-, or Four-Metal Systems

	Cu + Zn	Cu + Pb	Cu + Ni	Cu + Zn + Pb + Ni
Cu	20.0	39.1	22.8	29.9
Zn	88.2	—	—	88.9
Pb	—	30.9	—	18.8
Ni	—	—	80.3	90.0

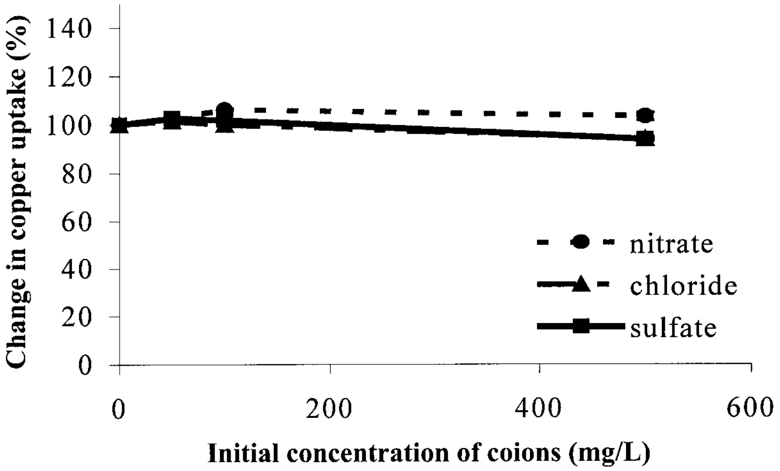

Fig. 4. Effects of three anions (chloride, nitrate, and sulfate) on the biosorption of 100 mg/L of copper by *Micrococcus* sp.

(10). In their investigation, the affinity of the filamentous bacterium *Thiothrix* strain A1 for metals was Cu > Zn ~ Ni.

The concentration of each metal ion used in this experiment was 2 m*M*. This value was known from our previous study *(6)* to be high enough for saturating all the binding sites of *Micrococcus* sp. under the experimental conditions. If the metal ion-binding sites on biosorbents have not been attained for saturation, no or an insignificant effect on the competition of metals may be observed. Hence, misleading results would be obtained.

Effects of Anions

Anions such as chloride, sulfate, and nitrate are often found in industrial wastewater together with heavy metal ions. The presence of these ligands is usually assumed to reduce the sorption of metals to bacteria because only free metal ions are bioavailable. Figure 4 illustrates the effect of anions (Cl^-, SO_4^{2-}, and NO_3^-) on the biosorption of copper by *Micrococcus* sp. There was virtually no interference with copper uptake by *Micrococcus* sp. from the three anions (Cl^-, SO_4^{2-}, and NO_3^-), although a wide range of

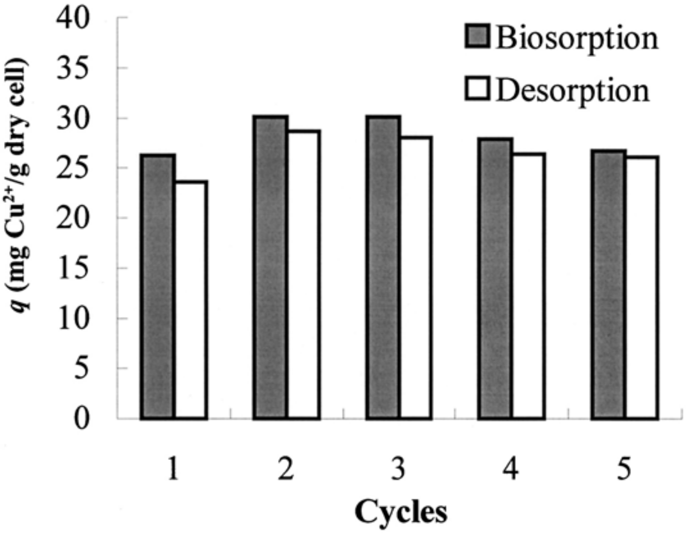

Fig. 5. Five regeneration cycles by 0.05 *M* sulfuric acid.

anion concentrations (0–500 mg/L) was tested. The result obtained was promising for applying *Micrococcus* sp. to detoxification of heavy metals bearing industrial wastewaters.

Biosorption and Desorption Cycles

Repeated biosorption and desorption operations were performed to examine the reusability and metal recovery efficiency of the biomass. Five consecutive regeneration cycles by *Micrococcus* sp. using 0.05 *M* sulfuric acid as a desorption medium are illustrated in Fig. 5. The copper biosorption capacity of the biomass had no significant difference from cycle 1 to cycle 5. For all runs, more than 90% of sorbed copper could be recovered and concentrated in a small volume by the desorption eluent. The results indicated that *Micrococcus* sp. possessed high reuse potential for removal and recovery of copper (II) ions from wastewater. A slight enhancement in copper uptake was observed in the second operation in both experiments. It is likely that the cell particles became much finer after the first treatment of desorption medium. Thus, the increase in surface area of the biomass may be a cause of the increase in removal capacity. Chang et al. *(9)* and Wong et al. *(14)* reported similar observations. They attributed the behavior to HCl-induced structural changes, which increased surface binding of the metals. Because suspended cells were used in our study, about 35% loss in cell concentration from the working solution was observed after five regeneration cycles. This loss of biomass is most likely owing to repeated centrifugation and rinse operations. Using immobilized cell systems can minimize the problems associated with the loss of biomass owing to solid-liquid phase separations.

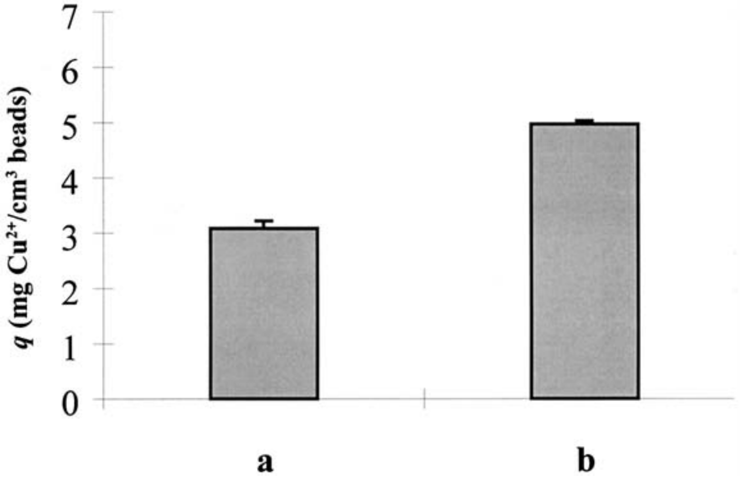

Fig. 6. Copper removal capacity by 2% calcium alginate and 10% polyacrylamide: (a) 10% polyacrylamide and 2% calcium alginate (without cells); (b) 10% polyacrylamide and 2% calcium alginate mixed with 42.3 g dry cell/L.

Immobilization Studies

The high surface-to-volume ratio of the microbial cells allows better contact between the biosorbent surface and soluble ions and molecules *(1,12)*. However, because of the difficulty of separating cells from the solution phase, cell immobilization procedures may be required for the development of large-scale recovery processes. The most common strategies used to attain this goal include the colonization of cells on a solid substrate (i.e., biofilm), or their immobilization into a polymeric or porous matrix *(2,13,14)*.

Micrococcus sp. was immobilized in 2% calcium alginate and 10% polyacrylamide gel beads to remove copper (II) ions from wastewater. As shown in Fig. 6, the copper removal capacity of the biomass increased by 61% when it was immobilized into gel beads. Polyacrylamide-entrapped cells have good mechanical properties and are inert to microbial degradation *(15)*. Calcium alginate is also a well-known biopolymer. It is biocompatible, chemically resistant, inexpensive, and easy to regenerate. In addition, it has a loose structure for overcoming diffusion limitations and provides a good model system for adsorption. Hence, the combined calcium alginate–polyacrylamide-based immobilization matrix offers promising potential for whole-cell immobilization in order to detoxify the metals-bearing solutions.

Conclusion

Copper biosorption by *Micrococcus* sp. was strongly dependent on the pH and copper concentration of the solution. Cells harvested at exponential growth phase and stationary phase showed similar biosorption characteristics for copper. The biosorption of copper decreased with decreasing pH from 6.0 to 2.0, which suggested that metal cations and protons compete for the same binding sites on the cell wall as pH decreased. In multimetal systems, *Micrococcus* sp. exhibited a preferential biosorption order: Cu ~ Pb > Ni ~ Zn. There is virtually no interference with copper uptake by *Micrococcus* sp. from solutions bearing a high concentration of Cl^-, SO_4^{2-}, and NO_3^- (0–500 mg/L). Sulfuric acid (0.05 *M*) was an efficient desorption medium. The copper capacity of *Micrococcus* sp. remained after five successive sorption and desorption cycles. Immobilization of *Micrococcus* sp. in 2% calcium alginate and 10% polyacrylamide gel beads increased copper uptake by 61%. These results show that biomass of *Micrococcus* sp. may be applied to the development of potentially cost-effective biosorbents for the removal and recovery of copper from effluents.

Acknowledgments

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