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Removal, Recovery, and Process Development of Heavy Metal by Immobilized Biomass Methods

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Heavy metal adsorption by microbial cells is an alternative to conventional methods of heavy metal removal and recovery from metal-bearing wastewater. The waste *Saccharomyces cerevisiae* is an inexpensive, relatively available source of biomass for heavy metal biosorption. Biosorption was investigated by free and immobilized-*S. cerevisiae*. The order of biosorption capacity was Pb>Cu>Cd with batch system. The biosorption parameters had been determined for Pb with free cells according to the Freundlich and Langmuir model. It was found that the data fitted reasonably well to the Freundlich model. The selective uptake of immobilized-*S. cerevisiae* was observed when all the metal ions were dissolved in a mixed metals solution(Pb, Cu, Cr and Cd). The biosorption of mixed metals solution by immobilized-cell was studied in packed bed reactor. The Pb uptake was investigated in particular, as it represents one of the most widely distributed heavy metals in water. We also tested the desorption of Pb from immobilized-cell by using HCl, H_2SO_4 and EDTA.

Key words : biosorption, heavy metal, selective uptake, immobilized-cell, desorption

1. Introduction

The wide expansion in the use of heavy metals over the past several decades has inevitably resulted in an increased flux of metal pollutants in natural waters. The environmental pollutions by heavy metals result in a serious health problem because of their toxicities of those pollutants even at low concentrations. Birth defects, cancer and a number of chronic diseases have been all linked to heavy metals. Heavy metals can exert adverse effects on biological wastewater treatment processes. Although biological treatment systems can tolerate and actually accomplish considerable removal of heavy metals, excessive concentrations can impair the viability of the microorganisms resulting in inadequate performance or even complete failure.

Petroleum refining, mineral smelting, chemical manufacturing and electroplating are some of the leading sources of heavy metal pollutants and significant concentrations of heavy metals may be also occur in landfill leachates, combined sewer overflows, and cooling tower blowdowns.

Conventional methods for removing heavy metals from a heavy metal bearing-wastewater include as follows: Chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, membrane technology and evaporation recovery. But, this methods may be ineffective or extremely expensive, especially when the metals are in solutions containing in the range of 1 to 100 mg/L(Volesky, 1990).

Microbial cells may be used to remediate wastewater contaminated with heavy metals(Pradhan, 1992). Alternative metal removal methods are being considered which are based on metal-uptake properties by certain biological materials.

The biosorption is usually used to describe the removal of metal cations, and related elements or compounds from solution by microbial cells(Fourest, 1992). Exposure of microbial cells to heavy metals results in the rapid binding of cations to negatively charged sites on the cell wall because cell surfaces are anionic due to the presence of ionized groups such as carboxylate, hydroxyl and phosphate in the various cell wall polymers. Microorganisms will have various distributions of charge and geometry for these binding groups, and so may well selectively bind certain heavy metals(Pighi et al., 1989). *Saccharomyces cerevisiae* is an inexpensive, easily available source of biomass for bioremediation of wastewater (Brady et al., 1994; Ahn et al., 1996; Ahn et al, 1995)

The industrial application of biosorption has mainly been directed towards immobilized-cell systems. The most widely used materials for immobilized-cell systems are polyacrylamide, κ-carrageenan, calcium alginate and agar(Tanaka etal, 1993; Tyagi 1990). And a biosorption processes for the removal and/or of heavy metals are batch-stirred reactor, continuous-flow stirred-reactor, fixed packed-bed reactor and fluidized-bed reactor.

The objective of this study is to investigate a biosorption process, which uses a waste biomass such as *S. cerevisiae* utilized in fermentation industries to produce ethanol. The biosorption parameters had been determined for Pb with free cells according to the Freundlich and Langmuir model.

We also were investigated for their ability to absorb Pb, Cr and Cu by using packed-bed with the immobilized-cells in sodium alginate. Pb uptake was investigated in particular, as it represents one of the most widely distributed heavy metals in water.

2. Material and Methods Microorganism

S. cerevisiae was obtained from the Il-San Co. The growth medium contained glucose(100 g/L), yeast extract(8.5 g/L), ammonium chloride(1.32 g/L), magnesium sulfate(0.11 g/L) and calcium chloride (0.06g/L). Cells were harvested by centrifugation (3min, 3000 r.p.m.) and washed twice with de-ionized water(Millipore Milli-Q) and were repeatly centrifuged at 5000 r.p.m. for 10 min. The concentrated cells of *S. cerevisiae* were kept in the refrigerator at 4 ℃.

Immobilization

The method of immobilization was as follows. *S. cerevisiae*(10 g Dry Wt.) was suspended in 250 mL flask and sodium alginate(10 g) was dissolved in 250 mL deionized water. Sodium alginate solution and cell suspension were throughly mixed together. The mixture dropped in the 0.1 M CaCl. The beads of immobilized-cell left to harden in the $CaCl_2$ solution about one day.

Metal biosorption experiments

Metal solutions were prepared by dissolving the $Pb(NO)_3$, $Cd(NO_3)_2 \cdot 4H_2O$, and $Cu(NO_3)_2 \cdot 3H_2O$ in de-ionized water.

The batch metal-sorption experiments were carried out in 500 mL Erlenmeyer flasks containing 200 mL heavy metal solution at 30 ℃. Samples of 5mL were removed and centrifuged several times. The precipitation used to experiment in desorption. The metal concentration in the supernatant was measured by atomic absorption spectrometry(Shimazu 1200).

S. cerevisiae was immobilized with sodium alginate and metal solution was passed through a fixed packed-bed. The influent metal concentrations, the flow rate and the column volume were 50mg/L, 9.26 mL/min and 168mL, respectively.

The heavy metal uptake(q) was calculated from the initial concentration(C_i) and the final concentration(C_f) of the heavy metal in solution according to the following equation.

$$q = \frac{V(Ci - Cf)}{M}$$

Where V is the volume of solution in the contact batch flask(mL) and M is the initial *S. cerevisiae* dry weight(g).

3. Results and Discussion

The *S. cerevisiae* biomass was able to adsorb accumulating Pb, Cu and Cd from metal solutions. The batch biosorption experiment was observed relative to time, and the amounts of heavy metals taken up by *S. cerevisiae* were determined (Fig. 1). The uptake of Pb was 60 g Pb/g Dry Wt. and most effectively adsorbed by free *S. cerevisiae* biomass. At equilibrium state, the order of the biosorption capacity was Pb>Cu>Cd. A reason for different biosorption might be the ion radius or electrochemical properties of the metal ions(Tobin et al, 1984).

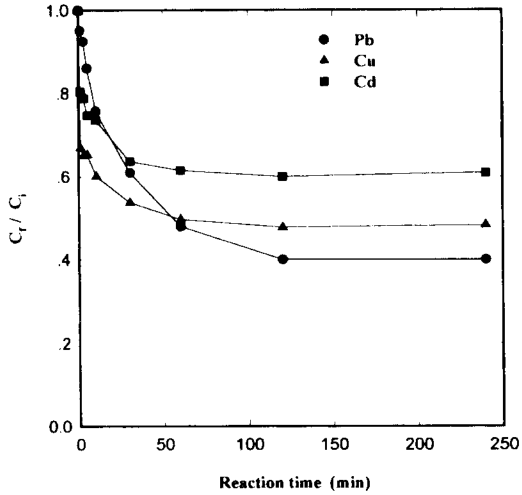

Fig. 1. Effect of reaction time on heavy metal adsorption by *S. cerevisiae*. C_i, initial concentration of heavy metal cation(mg/L); C_f,final concentration of heavy metal cation (mg/L); Biomass concentration, 1 g/L; Initial metal concentration, 100 mg/L.

About 80% of the soluble metals in solution was removed during the 30 min and the total uptake was reached within 60 min. The fast biosorption rate was probably almost entirely dependent on the biosorption of heavy metal cations to the cell wall. An initial rapid accumulation step is thought to involve cation binding at the surface(Brady and Duncan 1994) The data of biosorption equilibrium were used in the Freundlich and Langmuir model.

The Freundlich isotherm, which is commonly used to model heavy metal adsorption. This model uses two parameters, K and n to model adsorption

$$q = K \cdot C_{eq}^{1/n}$$

where q is the amount of metal absorbed per gram of biomass, Ceq is the equilibrium solution concentration, K is constant related to capacity, 1/n is constant related to affinity.

A second common model is Langmuir isotherm, which describes a monolayer ad-

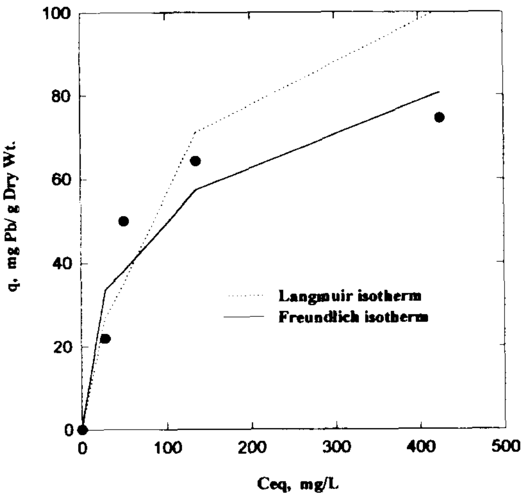

Fig. 2. Comparision of different models for fitting of experimental Pb biosorption by *S. cerecisiae*.

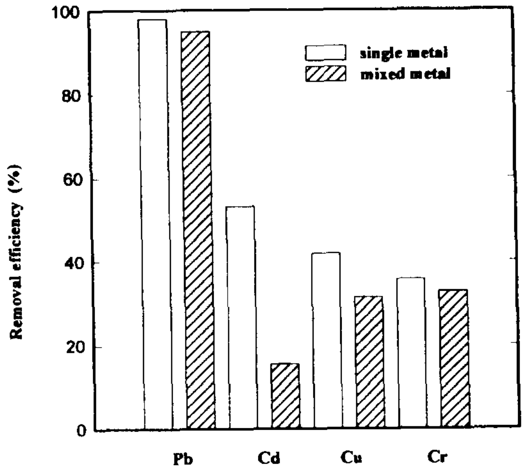

Fig. 3. Removal efficiency of heavy metals by *S. cerevisiae* from single-metals and mixed metals. Initial concentration, 1000 mg/L; Heavy metal solution, 50 mL; added 5 mL bead.

sorption on the cell surface. The commonly quoted form is

$$\frac{1}{q}=\frac{1}{(Qb)}\frac{1}{Ceq}+\frac{1}{Q}$$

where b is a constant related to the energy or net enthalpy of adsorption, Q is the maximum uptake upon complete saturation on the surface, q and C_{eq} are as defined previously.

The biosorption equilibrium values expressed in the Freundlich and Langmuir model are shown in Fig. 2. The constants K, 1/n, Q and b of the two different models were evaluated according to the least squre fitting method, experimental C_f and q values. As seen in Fig. 2, the Langmuir model did not fit the experimental data well. It was also found that the data fitted reasonably well to the Freundlich model.

It appears that the removal efficiency by immobilized *S. cerevisiae* from single-metals and mixed metals(Fig. 3, Initial heavy metal concentration was 1000 mg/L). The selective uptake was observed when all the metal ions were dissolved in a mixed solution. Selective binding of metals might be an effect of competition, metal-ligand interaction include the polarizing power and hard-soft character of the cation, together with special factors such as the chelate and synergic effects. Pb was bound by immobilized-cell in large amounts from solutions with one metal as well as from mixed solutions. The biosorption of Cr, Cu and Cd were lower when Pb present in the mixture.

It is very difficult for free cell to apply industrialization because of cell separation problems. Whenever the immobilized-cell used, it may be industrial application. The advantages of immobilized-cell during metal biosorption over cell suspensions include improved biomass retention, improved levels of biomass reuse, higher biomass concentrations, and higher flow rates. Mixed metal solutions of three metals(Pb, 'Cu and Cr) species were passed through column set up to investigate the extent of selective uptake of the cations due to differences for sorption bind-

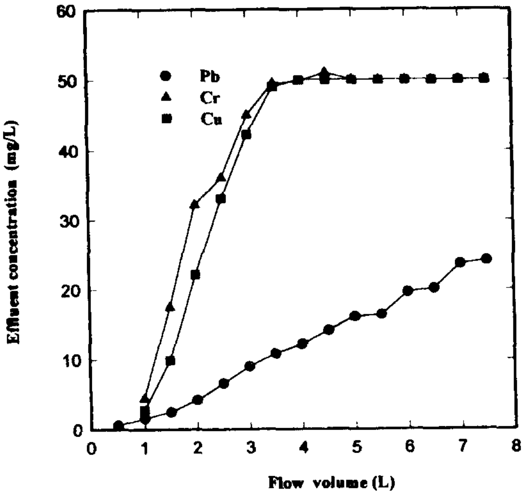

Fig. 4. Biosorption of Pb, Cr and Cu by columns packed with immobilized *S. cerevisiae*. Initial concentration,

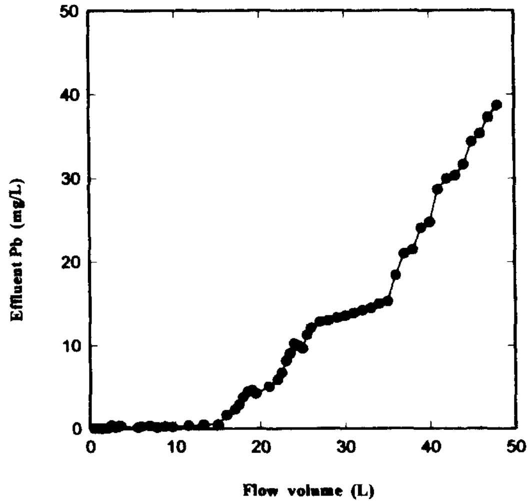

Fig. 5. Biosorption of Pb by column packed with alginate gel immobilized *S. cerevisiae*. Metal concentrations, 50 mg/L; Flow rate, 9.26 mL/min; Column volume, 168 mL; Temperature, 30℃.

ing sites on the immobilized-cell. Fig. 4 has also shown to adsorb a mixed Pb, Cr and Cu. The effluent concentration of Cu and Cr begin to increase sharply, as soon as the mixed metal solutions feed in

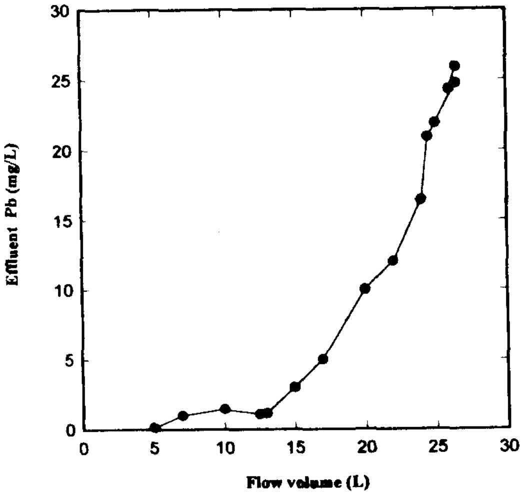

Fig. 6. Re-biosorption of Pb by regenerated *S. cerevisiae*.

the column. When the flow volume reached to 3.2 L, no more the uptake was appeared for Cu and Cr. For Pb, removal of more than 80% was achieved over the total volume of 3 L effluent passed through the column.

As Fig. 5 indicates, the removal of Pb is achieved with immobilized-cells. The removal efficiency of Pb was kept above 99% before the breakthrough points reached. But, On passing a breakthrough points, the concentration of effluent Pb begin to increase sharply. The flow volume was a 40 L when the removal efficiency of Pb was approached up to 50%.

Desorption is needed for the recovery of metals and for the regeneration of immobilized-cell. We tested the desorption of Pb from immobilized-cell by HCl, H_2SO_4 and EDTA.

The acid was used in concentrations between 0.0001 and 1 M. The desorption of Pb was efficient with 0.1 M and the immobilized-cells were stressed by up to 0.1 M acids. The 0.1 M HCl and 0.1 M H_2SO_4 were obtained similar desorption results. But, the EDTA solution was not efficient in the desorption of Pb because the immobilized-cells melted. In Fig. 6, re-uptake of Pb was possible after desorption of binding-metal with the 0.1 M HCl.

4. Conclusions

The waste *S. cerevisiae* was found to be capable of adsorbing heavy metal cations from metal solutions and utilizing in metal removal or recovery from wastewater.

In the batch biosorption, the uptake of Pb was 60 mg Pb/g Dry Wt. and most effectively adsorbed by free *S. cerevisiae* biomass. The orderof the biosorption capacity was Pb>Cu>Cd at equilibrium state.

The constants of the two models were evaluated according to the least squre fitting method. Freundlich model reasonably fitted the experimental data than Langmuir model.

In the immobilized-cell systems, mixed metal solutions(Pb, Cu and Cr) were passed through a column to investigate the extent of selective uptake of the cations. The selective uptake was observed when all the metal ions were dissolved in a mixed solution. Pb was uptaken by immobilized-cell in large amounts from solutions with one metal as well as from mixed solutions.

When one metal solution species(Pb) was passed through a packed-bed, the effluent concentration of Pb was kept above 99% before the breakthrough points were reached. But, on passing a breakthrough points, the concentration of effluent Pb begin to increase sharply. Reuptake of Pb was possible after desorption of binding-metal with the 0.1 M HCl.

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References

Ahn K. H. and K. H. Suh, 1996, Pb biosorption by *Saccharomyces cerevisiae*. Korean J. Biotechnol. Bioeng. 11(2), 173 ~ 180.

Ahn K. H. and K. H. Suh, 1995, Biosorption of heavy metal by *Saccharomyces uvarum*, J. of the Korean Environmental Sciences Society, 4(5), 527 ~ 534.

Brady D., A. D. Stoll, L. Starke, and J. R. Duncan, 1994, Chemical and enzymatic extraction of heavy metal binding polymers from isolated cell walls of *Saccharomyces cerevisiae*, Biotechnol. and Bioeng., 44, 297 ~ 302.

Chong K. H. and B. Volesky, 1995, Description of two-metal biosorption equilibria by langmuir-type models, Biotechnol. and Bioeng., 47, 451 ~ 460.

Fourest E. and J. C. Roux, 1992, Heavy metal biosorption by fungal mycelial by-products: Mechanisms and influence of pH, Appl. Microbiol. Biotechnol., 37, 399 ~ 403.

Pighi L., T. Pumpel and F. Schinner, 1989, Selective accumulation of silver by fungi, Biotechnol. Lett., 11(4), 275 ~ 280.

Pradhan A. A. and A. D. Levine, 1992, Experimental evaluation of microbial metal uptake by individual components of a microbial biosorption system, Wat. Sci. Tech., 26, 2145 ~ 2148.

Tanaka A., T. Tosa and T Kobayashi, 1993, Industrial application of immobilized biocatalyst, Marcel Dekker, New York. 377pp

Tobin J. M., D. G. Cooper and R. J. Neufeld, 1994, Uptake of metal ons by *Rithizopus arrhizus* biomass, Appl. and Envir. Micro., 47(4), 821 ~ 824.

Tyagi R. D. and K. Vembu, 1990, Wastewater treatment by immobilized cells, CRC Boco Raton, 3pp.

Volesky B., 1990, Biosorption of heavy metals, CRC, Boca Raton. 3pp

미생물 고정화법에 의한 중금속 제거, 회수 및 공정개발

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(1996년 8월 2일 접수)

미생물에 의한 흡착법으로 중금속을 효과적으로 제거, 회수할 수 있다. 알콜 발효 후 부산물로 생성되는 폐 Saccharomyces cerevisiae는 비교적 가격이 저렴하고, 중금속 생체흡착에 유용한 자원으로 이용될 수 있다. 생체흡착 실험에 사용된 미생물은 부유 및 alginate에 고정화된 S. cerevisiae로 수행하였다. 회분식 실험에서 생체흡착량은 Pb > Cu > Cd의 순으로 이루어졌다. Pb 이온의 흡착 평형은 Freundlich와 Langmuir 모델로 설명하였고, Freundlich 모델이 실험자료와 잘 부합되었다. 고정화된 S. cerevisiae를 이용한 혼합용액(Pb, Cu, Cr 및 Cd) 흡착 실험에서 각 중금속들은 선택적 흡착 특성을 나타내었다. 고정화 미생물을 고정층 반응기에 충진하여 혼합 중금속 용액의 생체흡착 실험을 수행한 결과 Pb 이온이 가장 많이 흡착을 하였다. 고정화된 미생물에 흡착된 Pb의 탈착실험에서 0.1M의 HCl 및 H_2SO_4가 효과적이었다.