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Removal of Lead by *Artherobacter sp.*

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The biosorption of heavy metals has received a lot of attraction for application of metal ions treatment. In this work, we studied with *Arthrobactor sp.*, screening from a wastewater containing heavy metals. The Pb uptake capacity of *Arthrobactor sp.* was nearly 146.9 mg Pb/g dry biomass(initial concentration, 500 mg/L), whereas the Pb uptake capacity of *Saccharomyces cerevisiae* and *Saccharomyces uvarum* were only around 39.40 and 35.65 mg Pb/g dry biomass, respectively. The Pb and Cr were removed from metal solution much more efficiently than were the other metals(Cd and Cu). The Pb uptake capacity of *Arthrobactor sp.* increased with increasing in pH(1.8, 3.0 and 4.0) and decreased with increasing of biomass concentration. At pH 4.0, the Pb uptake capacity reached 244 mg Pb/ g dry biomass in Pb initial concentration of 1000 mg/L. The Pb uptake capacity of *Arthrobactor sp.* treated by KOH and $CaCl_2$ were increased above values obtained with untreated *Arthrobactor sp.* However, the Pb uptake capacity of *Arthrobactor sp.* treated by NaOH was decreased. The removal efficiency of Pb was kept above 99% before the breakthrough points were reached.

Key words : biosorption, heavy metal, immobilization, uptake, lead

1. Introduction

Microorganisms will have many binding sites for heavy metals, so may uptake for metal-containing wastewater and selectively uptake for metal cations. Cell surfaces are anionic due to the presence of ionized groups such as carboxylate, hydroxyl and phosphate in the various biopolymers (Martin and Roberty, 1989). Uncharged groups such as peptide N atoms may also function as ligands to complete the coordination number requirements of the metal ion.

The removal of heavy metal from various industrial wastewater has been mostly solved by chemical and physical methods of treatment. However, these procedures have significant disadvantages, such as incomplete metal removal, high consumption of chemicals, energy requirements, generation of toxic sludge or other waste products and are generally very expensive when the contaminant concentrations are in the range of 10 - 100 mg/L(Sag, 1995). The treatment of low charge effluents, conciliating economic and technical constraint is impossible with traditional physico-chemical processes.

The biosorption processes are an alternative to traditional physico-chemical processes of heavy metal removal. The biosorption is usually used to describe the removal of metal from solution by microbial cells. The adsorption of metal ions onto microbial cells results in the rapid binding of cation to negatively charged sites on the cell wall(Ehrich, 1990).

Recently, the biosorption of heavy metals has received a lot of attraction for application of hazardous waste treatment(Ahn 1995, Ahn 1996). We are interested in the role of microorganisms in the removal of Pb. The objectives of this work were to determine the metal uptake characteristics of *Artherobacter sp.* after screening from metal-bearing activated sludge at wastewater plant. The biomass has been chemically pretreated in order to enhance the biosorption performance. It was investigated uptake capacity in packed-bed bioreactor with immobilized *Artherobacter sp.* by polyacrylamide.

2. Material and Methods

2.1 Preparation of Microorganism

The *Artherobacter sp.* strain used in this study was isolated from industrial wastewater plant. The growth medium used contained Bacto-beef extract 3 g, Bacto-pepton 5 g, NaCl 1g in 1000mL distilled water. Cells for uptake studies were obtained by culturing in liquid medium for 36 hr at 25 ℃.

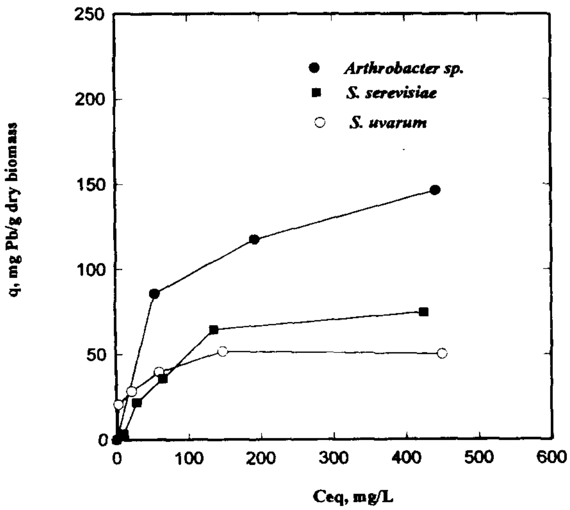

Fig. 1. Pb biosorption isotherms of *Arthrobactor sp.*, *S. cerevisiae* and *S. uvarum*.

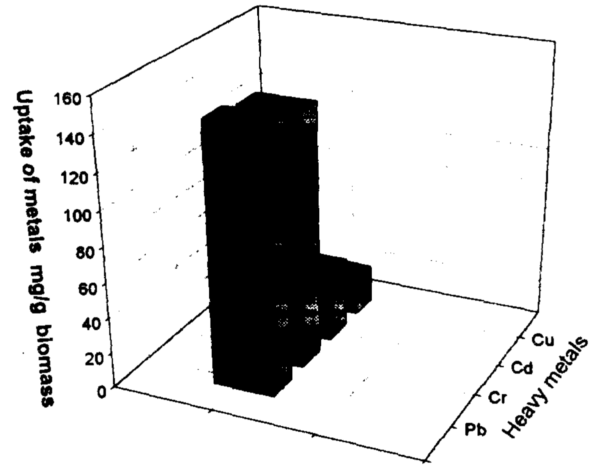

Fig. 2. Uptake of Pb, Cr, Cd and Cu by *Arthrobactor sp.*. Initial concentration, 500 mg/L.

Cells were harvested by centrifugation (3min, 3000 r.p.m.) and washed twice with deionized water (Millipore Milli-Q) and were repeatedly centrifuged at 5000 r.p.m. for 10 min. The concentrated cells of *Artherobacter sp.* were kept in the refrigerator at 4 ℃.

2.2 Immobilization

The method of immobilization was that of Brady et al.(1994) and was as follows: 10 g wet mass of *Artherobacter sp.* was suspended in 20 mL of 0.15 M NaCl at 8 ℃ : acrylamide monomer(7.5 g) plus N, N'-methylene-bisacrylamide(0.4 g) was dissolved in 24 mL deionized water and cooled to 8 ℃ : the monomer solution and cell suspension were throughly mixed together with a rod. Added to this mixture was 1 mL of 2.5% N,N,N',N', tetramethylethylenediamine(TEMED), and 5 mL of 1% ammonium persulfate. The temperature of the solution/suspension was maintained below 50 ℃ during the exothermic polymerization process so as to not damage the biomass. Allow polymerization to proceed for 1 hour.

2.3 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 with known initial metal concentration at 30 ℃. 5mL samples were removed and centrifuged several times. The precipitate was used to experiment in desorption. The metal concentration in the supernatant was measured by atomic absorption spectrometry (Shimazu 1200).

Pre-treatments of *Arthrobactor sp.* were performed by suspending biomass in 0.1M NaOH, KOH and $CaCl_2$ for 4 hour, than washing with deionized.

The packed bed is very simple and consists of a tubular column, with the immobilized *Arthrobactor sp.* using polyacrylamide. It was operated in the upflow, i.e., the heavy metal solution is introduced at the bottom of the column.

3. Results and Discussion

Among a variety of different biomass types for biosorbent potential, the uptake results obtained from batch experiments(Fig. 1) were presented. In this work, Pb uptake capacity of *Arthrobactor sp.* was higher than that of *Saccharomyces cerevisiae* and *Saccharomyces uvarum*. There were no significant differences in Pb uptake among yeast (*Saccharomyces cerevisiae* and *Saccharomyces uvarum*). As indicated in Fig. 1, the Pb uptake capacity of *Arthrobactor sp.* was nearly 146.9 mg Pb/g dry biomass, whereas the Pb uptake capacity of *Saccharomyces cerevisiae* and *Saccharomyces uvarum* were only around 39.40 and 35.65 mg Pb/g dry biomass, respectively.

Fig. 2 shows that the uptake of Pb, Cr, Cd, and Cu were carried out from heavy metal solution. As shown in Fig. 2, the uptake capacity of Pb and Cr to the *Arthrobactor sp.* were found to be similar.

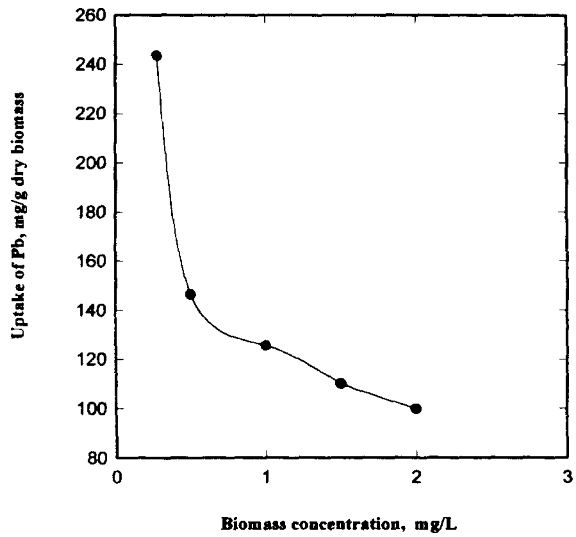

Fig. 3. Uptake of Pb by increasing biomass of *Arthrobactor sp.* Initial concentration, 500 mg/L.

The Pb and Cr were removed from metal solution much more efficiently than were the other metals (Cd and Cu) at 500 mg/L of initial concentration for heavy metal ions. The *Arthrobactor sp.* showed a great deal of uptake capacity for Pb and Cr with 146.9 mg Pb/g dry biomass and 143.20 mg Cr/g dry biomass, respectively. The uptake capacity of metal ions by *Arthrobactor sp.* were related to the affinity and availability of binding sites.

Fig. 3 represents the uptake of Pb at various biomass concentration. It shows that the uptake of Pb decreases with the increasing biomass concentration. Therefore, it is not useful to increase the biomass concentration beyond 0.5 g/L. Reduction in biomass concentration in the suspension at a given metal concentration enhances the metal/biosorbent ratio, and thus increase metal uptake per gram of biosorbent. These results reported that the reduction is attributable to metal concentration shortage in solution(Eric et al, 1995) and the electrostatic interaction between cells is a significant factor in the biomass dependence of metal adsorption(Rome and Gadd, 1987).

pH is likely to be a major factor in the quantity of metal uptake owing to cation competition effects with hydrogen ions. The effect of pH on Pb uptake is represented in Fig. 4. It was shown that Pb uptake capacity of *Arthrobactor sp.* increased with the increase in pH(1.8, 3.0 and 4.0). At pH 4.0, the Pb uptake capacity reached 244 mg Pb/g dry biomass in initial concentration, 1000 mg/L.

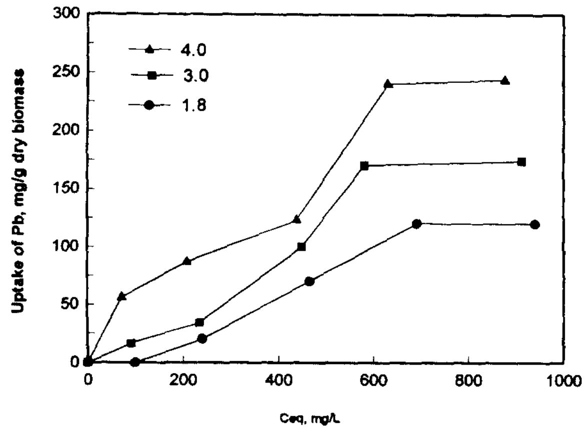

Fig. 4. Pb uptake isotherm of *Arthrobactor sp.* at various pH. pH:1.8, 3.0 and 4.0.

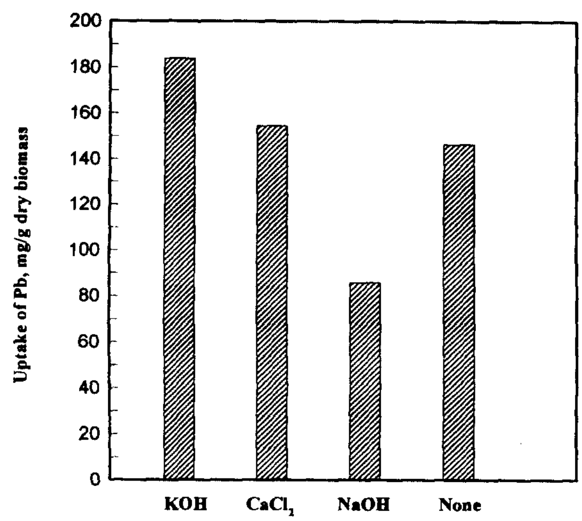

Fig. 5. Effect of KOH, $CaCl_2$ and NaOH treatment upon Pb adsorption by *Arthrobactor sp.*. Initial concentration, 500 mg/L.

However, as shown in Fig 1, the Pb uptake was 146.9 mg Pb/g dry biomass in initial concentration, 500 mg/L. It is known that the biosorption of Pb is depended on pH and the initial concentration of Pb. Eric and Jean-Claude (1995) observed the optimal pH of around 7.5 for Zn and Ni, and approximately 5.0 for Pb. Several authors previously described an optimal pH around 4.9(Tobin *et al.*, 1984; Tsezo and Volesky, 1981). They said the high Pb concentration(1000 mg/L), if pH over 5.0, the precipitation of Pb as Pb hydroxides occurred in metal solution.

The clean *Arthrobactor sp.* has been chemically pretreated in order to regenerate anionic sites without significant modification of the cell surface structure and to reinforce the biosorption per-

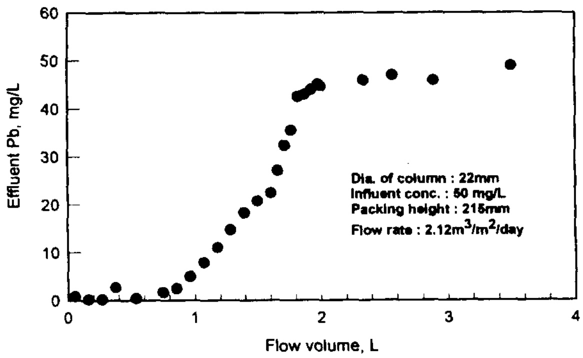

Fig. 6. Breakthrough curve of Pb in packed-bed column with immobilized *Arthrobactor sp.*.

formance before sorption experiments. Fig. 5 shows the Pb uptake capacity of *Arthrobactor sp.* treated by KOH and $CaCl_2$ were increased above values obtained with untreated *Arthrobactor sp.* However, the Pb uptake capacity of *Arthrobactor sp.* treated by NaOH was decreased. It shows the independence of Pb biosorption on hydroxides complex. Eric *et al*(1995) reported that the biomass treated with NaOH resulted in uptake reduction for specific biomass.

The advantage of the immobilized *Arthrobactor sp.* is to maximize heavy metal ions in the biosorbent, which if desired, the heavy metal may be recovered. The packed-bed is used to evaluate the performance of the immobilized *Arthrobactor sp.* in continuous reactor operation and to use factor to scale-up the biosorption process. And, it is very simple in nature and be able to apply in metal bearing wastewater easily. Breakthrough curve for immobilized *Arthrobactor sp.* is represented in Fig. 6. The biosorbent bed was able to maintain up to 15 hour of operation at constant flow rate of 2.12 m^3/m^2/day. The removal efficiency of Pb was kept above 99% before the breakthrough points were reached.

4. Conclusions

A wastewater containing heavy metal was treated in batch reactor, using free cell and packed-bed, using immobilized *Artherobacter sp.* in polyacrylamide. The Pb uptake capacity of *Arthrobactor sp.* was nearly 146.9 mg Pb/g dry biomass (initial concentration, 500 mg/L). The Pb uptake capacity of *Arthrobactor sp.* increased with the increase in pH(1.8, 3.0 and 4.0) and decreased with the increasing biomass concentration. The Pb uptake capacity of *Arthrobactor sp.* treated by KOH and $CaCl_2$ were increased above values obtained with untreated *Arthrobactor sp.* The packed-bed was able to maintain up to 15 hour of operation at constant flow rate of 2.12 m^3/m^2/day.

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*Artherobacter sp.*에 의한 납 제거

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(1997년 5월 20일 접수)

중금속 폐수를 처리하는 활성슬러지로 부터 *Arthrobactor sp.*를 부터 분리하여 생체흡착 실험에 사용하였다. *Arthrobactor sp.*의 납 흡착량은 초기농도 500 mg/L에서 146.9 mg Pb/g dry biomass로 *Saccharomyces cerevisiae*와 *Saccharomyces uvarum*보다 약 4배 더 많은 흡착량을 보여주었다. pH가 1.8, 3.0 및 4.0으로 높아질수록 납의 흡착량은 증가하였고, biomass의 양이 많아질수록 단위 미생물당 납 흡착량은 감소하였다. Biomass에 0.1M KOH, $CaCl_2$ 및 NaOH로 4시간 동안 전 처리하여 흡착실험을 수행한 결과 KOH로 전 처리된 biomass의 납 흡착량은 1.26배 증가하였으나 NaOH로 처리한 것은 납의 흡착량이 감소하였다. Polyacrylamide에 고정화된 biomass를 반응기에 충진한 연속실험에서, 유속 2.12 $m^3/m^2/day$ 에서 15시간 동안 납 제거효율이 99%이상 되었다.