*Rhodotorula glutinis*에 의한 납 제거 메커니즘

조 대행, 김 의용
서울시립대학교 화학공학과

The mechanisms of Pb^{2+} removal from aqueous solution by *Rhodotorula glutinis*

Dae Haeng Cho, Eui Yong Kim
Dept. of Chemical Engineering, University of Seoul

Introduction

The potential for using microorganism in the treatment of metal-bearing wastewater has been studied intensively and many microorganisms including bacteria, fungi and algae have been found to remove metals from solutions. The biosorption of heavy metal ions by microorganisms may be placed into two categories: (i) metabolism-independent entrapment in the cellular structure and subsequent sorption on to the binding sites present in the cellular structure and (ii) metabolism-dependent transport across the cell membrane through the cell metabolic cycle. The metal-sorption mechanisms including complexation, ion exchange, coordination, adsorption, chelation, and microprecipitation are complex and dependent on the chemistry of the metal ions, surface properties of the microorganisms, and cell physiology [1].

It was recently reported that some soil yeasts including *Rhodotorula* sp. were resistant to heavy metal toxicity and shown to play a role in processes of mineral cycling. *Rhodotorula* sp. was frequently isolated from lagoon polluted by industrial and municipal sewage and mine drainage effluent. *Rhodotorula* sp. also has an aptitude for degradation of cyano-metals and bioleaching of mineral containing metals. These studies represent that *Rhodotorula* sp. has potential for bioremediation of soil and treatment of wastewater containing toxic metals. The interactions between *Rhodotorula* sp. and heavy metals have been focused on trace metal tolerance and accumulation of heavy metals by metabolically active biomass. Although the removal of cadmium and lead by viable and non-viable biomass of *R. rubra* was investigated, the metal uptake value was significantly lower than that of other biosorbents [2]. *R. glutinis* KCTC 7989, which produce EPS containing uronic acid, is expected to have potential for heavy metal biosorption.

In this study, we examined the biosorption of lead (Pb^{2+}) from aqueous solution using a powdered *Rhodotorula glutinis* biomass. The environmental factors for this metal biosorption were studied. In addition, we demonstrated that the biosorption mechanism of ion exchange by the biomass and precipitation by phosphate released from the biomass.

Materials and method

Microorganism : *Rhodotorula glutinis* KCTC (Korean Collection for Type Cultures) 7989 was isolated from soil and identified by morphological characteristics, biochemical properties and carbon assimilation test [3].

Medium and culture conditions : The growth medium consisted of 10 g/l of glucose, 3.0 g/l of yeast extract, 3.0 g/l of malt extract, and 5.0 g/l of peptone. The cells were grown in 500 ml Erlenmeyer flasks containing 100 ml of the growth medium. Cultures were incubated at 25℃ on an orbital shaker at 150 rpm.

Biomass preparation : At the end of the exponential growth phase, i.e. after 30 h incubation, the biomass was harvested by centrifugation at 10,000×g for 5 min. Once harvested, the biomass was washed twice with deionized distilled water. After washing, the biomass was dried at 70℃ for 24 h and powdered by mortar and pestle. The powdered *R. glutinis* biomass obtained was used in Pb^{2+} sorption studies.

Metal biosorption experiments : Stock Pb^{2+} solutions (1000 mg/l) were prepared using $Pb(NO_3)_2$. The desired concentrations of Pb^{2+} were prepared by dilution of stock solution with deionized distilled water. For all metal binding experiments, 0.1 g of biomass was added to 50 ml of Pb^{2+} solutions in 250 ml Erlenmeyer flasks shaken at 150 rpm in an orbital shaker at 25℃ for 3 h unless stated otherwise. To determine the effect of biomass concentration on Pb^{2+} biosorption, the amount of dissolved Pb^{2+} concentration was kept constant while the biomass concentration was varied from 0.1 g/l to 6 g/l. Before adding biomass, the pH of the metal solutions was adjusted to the desired values using 0.1N NaOH and 0.1N HNO_3. The effect of light metal ions was studied by using 0.1 g of biomass and 100 mg/l of Pb^{2+} solution containing the respective light metal ions such as Na^+, K^+, Ca^{2+} and Mg^{2+}. Blank samples without light metals were used as controls. After sorption, the biomass was then separated by centrifugation at 10,000×g for 5 min and the concentration of Pb^{2+} in the supernatant was determined by using a atomic absorption spectrophotometer (Varian AA-220FS, USA).

Analysis of the compound released from the biomass : The powdered biomass (0.1 g) was incubated as above in either deionized distilled water or 100 mg/l Pb^{2+} solution. The biomass was centrifuged and the concentration of Pb^{2+}, protein, carbohydrate and phosphate in the supernatant was determined. Protein content was determined by the Bradford method with bovine serum albumin as a standard. Total carbohydrate was quantified by the phenol-sulfuric acid method using glucose as a reference compound. The phosphate ion concentration was determined by ion chromatography (IC). IC analysis was performed on a Metrohm 761 compact IC fitted with Metrosup A Supp 4 column (250 x 4.0 mm). For elution, a mixture of 1.8 mM Na_2CO_3 and 1.7 mM $NaHCO_3$ was used at a flow rate of 1.0 ml/min.

Energy dispersive X-ray microanalysis (EDX) : Native and lead exposed cells of *R. glutinis* were dried and then covered with Pt for later scanning. EDX analyses were conducted using a Field-emission scanning electron microscope (Hitachi S-4700, Japan) with an X-ray analytic system (EDX). The acceleration voltage was constant at 15KV and the magnification was 450 times.

Results and discussion

The effect of biomass concentration in percentage sorption is depicted in Fig. 1. The percentage sorption increased steeply with the biomass concentration up to 2-g/l and thereafter remained more or less constant. This proved that Pb^{2+} sorption yield decreased when the biomass concentration increased. The 65-75% of dissolved Pb^{2+} was removed with 2-g/l biomass concentration.

The effect of the presence of light metal ions such as Na^+, K^+, Ca^{2+} and Mg^{2+} on Pb^{2+}

sorption capacity of *R. glutinis* is shown in Fig. 2. These ions were considered as quite likely to be present in industrial effluents. The effect of light metal ions was marginal even at a 10 mM concentration of these ions. These ions, at 50mM concentration, reduced the removal effiiency by around 8-11%. This result indicated that the light metal ions do not significantly interfere with the binding capacity and the *R. glutinis* biosorbent have much higher affinities for the lead ion than for the light metal ions studied.

R. glutinis was subjected to EDX analysis before and after Pb^{2+} sorption. The peaks at 3.3 KeV and 2.3 KeV were identified as that corresponding to K^+ and Pb^{2+}, respectively. The EDX spectra of the biomass before and after Pb^{2+} sorption showed that Pb^{2+} was observed on the after adsorption EDX spectrum while peak of K^+ disappeared (Fig. 3). It is well known that K^+ is an important constituent of cell membrane and cell walls. This indicates that Pb^{2+} has exchanged with K^+ on the cell wall of *R. glutinis*, thereby suggesting on ion exchange mechanism as one of mechanisms of metal biosorption for this strain.

When the powdered biomass was washed with deionized distilled water, biosorption efficiency by the washed biomass was significantly lowered than that of unwashed (data not shown). This result suggested that the release of some component associated with the biomass contributed significantly to biosorption of Pb^{2+} in solution. Therefore, we examined the effect of biomass washing on removal of Pb^{2+} from solution. The powdered biomass was added to either deionized distilled water or 100 mg/l Pb^{2+} solution. Protein, total carbohydrate and phosphate analyses performed on the supernatants revealed that significant amounts of these compounds were released from the biomass (Table 1).

Table 1. Protein, carbobydrate and phosphate release by R. glutinis biomass in deionized distilled water and 100 mg/l Pb^{2+} solution.

Compound	H_2O	Pb^{2+} (100 mg/l)
Protein (mg/l)	5.1±0.4	4.1±0.1
Carbohydrate (mg/l)	192.5±2.3	172.1±1.7
Phosphate (mg/l)	18.5±1.2	3.6±0.3

Protein was found in supernatant after incubation either in water or in 100 mg/l Pb^{2+} solution, but the variation of amounts was negligible. Large amounts of carbohydrates were detected in both cases. When the biomass was incubated in 100 mg/l Pb^{2+} solution, the amount of carbohydrate in the supernatant was decreased more or less than that of water. In particular, the biomass released a large amount of phosphate after incubation in water. However, a significant decrease in phosphate was observed after incubation in 100 mg/l Pb^{2+} solution. When Pb^{2+} was added to the supernatant after incubation in water, Pb^{2+} was found to precipitate in solution. It is well known that phosphates are precipitated in the form of metal phosphate in metal containing solution. These results indicated that the released phosphate from the biomass formed highly insoluble phosphate of Pb^{2+} in solution and as a result precipitation was responsible for the biosorption of *R. glutinis*. During in drying and grinding of the biomass, the yeast cell wall is damaged and as a result cell components could be released either from the cell wall or from the cell interior. In our study, it was found that the mechanism of Pb^{2+} removal by *R. glutinis* involved the biosorption by direct biosorptive interaction with the biomass through ion exchange and precipitation by phosphate released from the biomass.

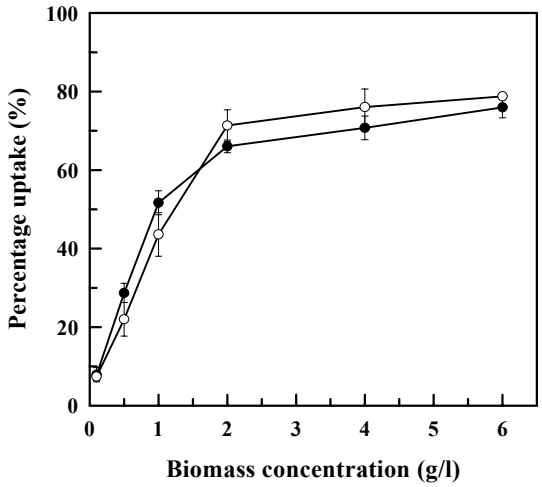

Fig. 1. Effect of biomass concentration on Pb^{2+} biosorption by *R. glutinis*.

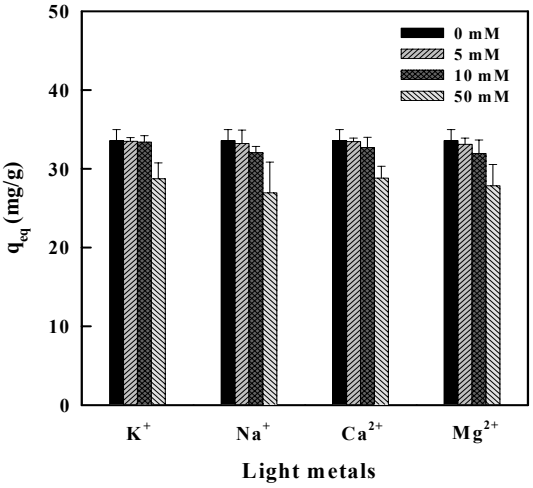

Fig. 2. Effect of Light metal ions on Pb^{2+} biosorption by *R. glutinis*.

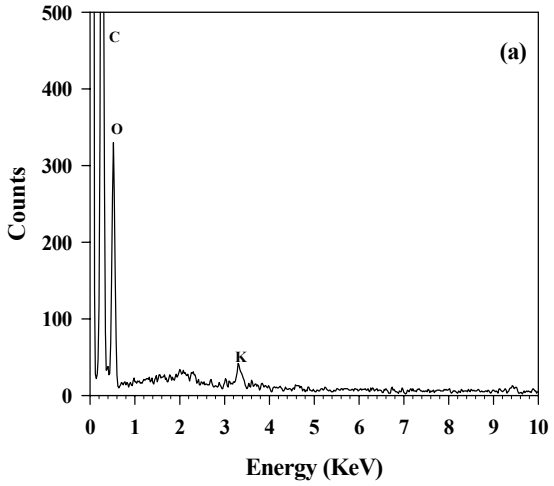

Fig. 3. EDX spectrum. (a) before Pb^{2+} sorption

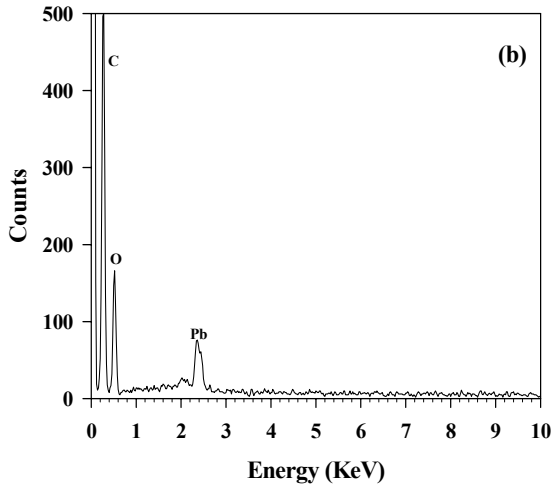

Fig. 3. EDX spectrum. (b) after Pb^{2+} sorption

References

1. Hu MZC, Norman JM, Faison BD, Reeves ME. Biotechnol. Bioeng. 51:237-247 (1996)
2. Salinas E, Orellano M, Rezza I, Martinez L, Marchesvky E, Tosetti M. Bioresour. Tech. 72:107-112 (2000)
3. Kim EY, Park PK, Chae HJ. Kor. J. Biotechnol. Bioeng. 13: 58-64 (1998)