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Biosorption of lead by *Phanerochaete chrysosporium* in the form of pellets

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Abstract: The growth of *Phanerochaete chrysosporium* (ATCC 24725) in pellets was influenced by culture time, medium pH, C/N, surfactant concentration, spore number in inoculum, and shaking rate. The removal of Pb^{2+} from aqueous solution by this kind of mycelial pellets was studied. The results indicated that many factors affected biosorption. These factors included pH, Pb^{2+} concentration, co-ion, adsorption time, and chemical pretreatments of biomass. Under optimum biosorption conditions(pH 4.5, 27℃, 16h), the highest lead uptake of 108 mg/g, was observed with mycelial pellets of 1.5 – 1.7 mm in diameter which were treated with 0.1 mol/L NaOH solution before adsorption. Pretreatment of biomass with NaOH further increased its biosorption capacity.

Keywords: *Phanerochaete chrysosporium*; mycelial pellets; lead ion; biosorption

Introduction

Biosorption of heavy metals is receiving increasing attention as a potential method for removing or recovering heavy metals from aqueous solution. This method is becoming an alternative to existing techniques for its scientific novelty and application potential. Among its advantages are the rapid uptake, the high selectivity and the great uptake capacity (Khummongkol, 1982). Previous studies on the mechanism of removal of metal ions by microorganisms have shown that the cell wall was the primary site of metal ion accumulation(Tobin, 1984). It has also been well established that the cations were adsorbed by reacting with groups on cell wall(Crist, 1981). However, the fundamental aspects of metal ion uptake are not well understood and conclusions about the mechanisms are divergent.

The culture and application of mycelial pellets have been researched(Sharma, 1985; Linko, 1988). In industrial fermentation processes, mycelial pellets have been used for the production of a number of compounds including citric acid, mushroom mycelia, enzymes, and antibiotics. Fungal pellets have also been used for the immobilization of biomass and enzymes(Prosser, 1991). Dispersed mycelial growth is perferred for many processes, but increases the viscosity of the culture medium can wrap around impellers. The biomass is generally separated from the broth by filtration or centrifugation(Friis, 1986; Norberg, 1984). During the course, the loss of biomass is inevitable. This problem may be resolved by growing the microorganism in the form of pellet, which also makes the biosorption process easy to operate.

The primary objective of our investigation was to examine the conditions of pellet formation. The adsorption capacity of Pb^{2+} from the dilute aqueous solution by this kind of mycelial pellets was also studied.

1 Materials and methods

1.1 Culture of mycelial pellet

The microorganism used in this study was *Phanerochaete chrysosporium* (ATCC 24725) which was cultured in nitrogen-limited medium(Kirk, 1978). It contains Basal III medium(100 ml/L), glucose(10 g/L), 0.1 mol/L dimethylsuccinate(100 ml/L), thiamin(0.001 g/L), $NH_4H_2PO_4$(0.25 g/L), trace elements solution(60 ml/L), 1% Tween 80 solution(50 ml/L), and spores suspension(abs = 0.5, 100 ml/L). Spores from malt extract agar slant were suspended in sterile NaCl solution(0.85%). This suspension was inoculated to nitrogen-limited medium in flasks and the flasks were shaken at 39℃ for 3

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days. The harvested smooth, white mycelial pellets by filtration were used in the adsorption experiments.

1.2 Batch biosorption experiments

The lead solution of desired concentration was prepared by dissolving lead powder in nitric acid solution(10%, V/V). The solution pH was adjusted to desired value with 0.5 mol/L NH_4OH and 0.2 mol/L HNO_3. Batch adsorption experiments were performed in 250 ml Erlen-meyer flasks (0.2g dry biomass per 100 ml lead solution) on shaker(150 r/min) for 16h. The concentration of lead in solution before and after adsorption were determined by atomic absorption spectrophotometry (AAS). The metal uptake was calculated as follows:

$$q_e = (C_i - C_e)/m. \quad (1)$$

Removal efficiency was expressed as:

$$R = (1 - C_e/C_i) \times 100\%. \quad (2)$$

1.3 Reagents and apparatus

Analytical grade NaOH, NH_4OH, HNO_3, HCl and lead, cadmium, copper and zinc were used in the experiments. A Perkin-Elmer atomic absorption spectrometer(model 3030) was used for the determination of Pb^{2+} concentration. Scanning electron microscope(HITACHI, model S-520) was employed to observe cells.

2 Results and discussion

2.1 Pellet formation

2.1.1 Effect of medium pH

Mycelial pellets mass and diameter were influenced by medium pH. Lower pH and higher pH resulted in less biomass, and more biomass was obtained during pH 4.5-6.5(1.7-1.9 g/L). At pH 2.0 there were not pellets in medium. Pellet size became smaller as pH value increased. It indicated that pH value of the culture medium played an important role in the process of pellet formation, especially in the coagulation of spores. Metz and Kossen(Huang, 1990) suggested that surface properties of spores were affected by pH, which were responsible for the coagulation of spores. On the other hand, pH value of medium could influence the growth of spores and hyphae.

2.1.2 Effect of concentration of surfactant

The experiment results showed that the biomass increased with the addition of Tween 80 in medium, and the pellet size increased in the concentration ranging from 0.05% to 0.2%, then decreased in that more than 0.2%. But the changes of size were little. In the meantime, it was observed that the hyphae pellets were uneven and soft without Tween 80 in medium, but they became even, smooth and harder due to the addition of Tween 80. Tween 80 increased spores and hyphae coagulation, which could be attributed to its chemical structure. Being a nonionic surfactant, its hydrophilic group contains polyoxyethylene which has higher negative charge density and lower intrinsic viscosity than Tween 20 and Tween 60. So it avails the formation of even and smooth pellets.

2.1.3 Effect of C/N ratio

The effect of medium composition on pellet growth was also studied. At the same nitrogen concentration, the biomass weight increased, pellet diameter decreased and mechanical properties improved as carbon concentration increased. At the same carbon concentration, gradual increases in biomass weight and pellet size occurred with the increasing of nitrogen concentration, but the mechanical performance of pellets became bad. By comparison, high C and low N concentrations in medium resulted in better mechanical properties of pellets and more biomass.

2.1.4 Effect of spore number in inoculum

The inoculum concentration is generally recognized as an important factor in the process of pellet

formation. The result that the different spore numbers in the inoculum gave rise to bigger differences of pellets was obtained in experiments. Higher spore concentration in the inoculum resulted in harder, smooth and smaller pellets, and softer pellets with larger size were produced when spore number in inoculum was lower. An inverse relationship between pellet size and spore number in inoculum was found to exist. And the bigger the pellet, the lower the weight ratio of dry cells to wet cells. It implied that the bigger pellet contained more water in it which resulted in softer pellet. The smooth, hard pellets could be obtained when spore number in inoculum reached 10^6 ml^{-1}.

2.1.5 Effect of shaking rate

The pellet growth was, to a large extent, dependent upon agitation and shear. The influence of shaking rate on pellet growth was evident. As showing in experiments, the pronounced changes in morphology of pellets took place at different shaking rate. The pellet was bigger and softer with decreasing shaking rate. *P. chrysosporium* is aerobic. Increase of shaking rate can provide more oxygen, which leads to the increases of hyphae growth and biomass. On the other hand, the shaker rate can affect the rheologic properties of the medium, availability of nutrients and gases. Big pellet has hollow center, which was a probable result of cell autolysis due to lack of nutrients and oxygen.

Overall, pellet formation is not only influenced by biological factors, the environment in which the organism is grown plays an equally important role.

2.2 Batch biosorption experiments

2.2.1 Initial pH

Hyphae pellets used in adsorption experiments of Pb^{2+} were in the diameter range of 1.5 – 1.7 mm. The culture conditions as follows: medium pH was 4.5, Tween 80 concentration was 0.05%, C/N ratio was 137, spore number in inoculum was 10^6 ml^{-1} and at a constant speed of 150 r/min on a shaker at 39℃ for 3 days.

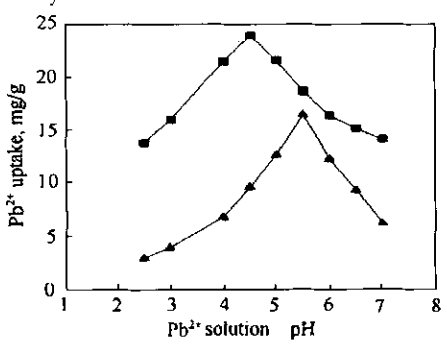

Fig. 1 Effect of Pb^{2+} solution pH on uptake
▲ no pretreatment; ■ treated by NaOH
(c_i = 50 mg/L; m = 2 g/L; 27℃, 16h)

The uptake of Pb^{2+} was strongly affected by initial pH of solution as shown in Fig. 1. The Pb^{2+} uptake by increased as initial pH increased, and the maximal adsorption occurred around pH 5.5 (16.45 mg/g), and decreased after pH 5.5. At pH 2.0 the adsorption capacity was very low, because large quantities of protons competed with Pb^{2+} for the adsorption sites, which resulted in lower uptake. As initial pH of solution increased, the number of H^+ dissociated from the functional groups on cell wall increased and provided more negative groups for complexation of Pb^{2+}. But at higher initial pH, the uptake decreased. Pb^{2+} which were adsorbed on the cells gave rise to some changes of cell wall structure, and resulted in the release of Pb^{2+} with some soluble materials releasing from the cells into solution (Huang, 1990). Above pH 7.0, precipitation of lead hydroxide complexes was probable to form and disturb the biosorption process. So in the following experiments, the initial pH of solution was controlled bellow pH 7.0.

2.2.2 Chemical pretreatment

Alkali treatment of biomass was carried out by soaking the hyphae pellets in NaOH solution for 40 min, followed by filtration of the suspension and rinsing the biomass with distilled water until the filtrate pH close to be neutral. The results of adsorption by pellets treated with different concentration NaOH were given in Table 1. It was seen that the optimum concentration is 0.1 mol/L. The effect of pH on uptake by

alkali treated biomass is also shown in Fig. 1. It followed a similar trend to that of Pb^{2+} uptake by untreated biomass. Apparently, alkali treatment improved the adsorption capacity. The maximum uptake for the treated biomass reached 24 mg/g(at pH 4.5) compared to 16 mg/g for the untreated biomass(at pH 5.5).

Luef and Prey *et al*.(Luef, 1991) reported that the removal of some amorphous polysaccharides from the cell wall due to the alkali treatment which changed the structures of cell wall composition, such as glucosamine and chitin, and generates more accessible space within the glucan-chitin skeleton, and allowing more Pb^{2+} to be adsorbed on cell surface. On the other hand, some materials on cell surface which interfere in biosorption process may be dissolved by alkali treatment, thus more active sites were exposed to Pb^{2+} for adsorption. Finally, the alkali treatment resulted in the increase of ionization of negative functional groups, which was also one of reasons of increased adsorption capacity. In the following experiments, the biomass were treated by 0.1 mol/L NaOH before adsorption.

Table 1 Effects of chemical treatments on biosorption

Treatment methods	Concentration, N	q_e, mg/g	R, %
No treatment		16.06	64.25
Soaked in NaOH solution for 40 min	0.05	16.44	65.75
	0.10	23.66	94.65
	0.20	21.38	85.50
	0.50	19.53	78.13
	1.00	15.16	60.63

Note: biosorption conditions: c_i = 50 mg/g; m = 2 g/L; pH = 5.5; T = 27℃; t = 16h

2.2.3 Time

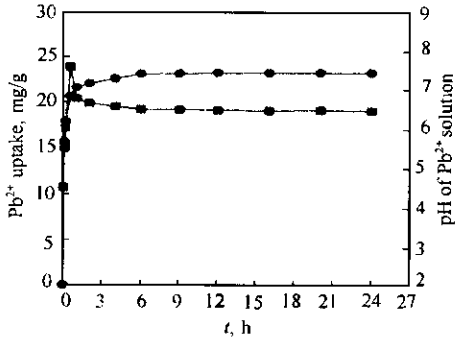

Fig.2 Effect of time on Pb^{2+} uptake and solution pH
● q_e; ■ pH
(c_i = 50 mg/L; m = 2 g/L; pH = 4.5; 27℃; 24h)

Most of metal uptake happened during a short period immediately after the adsorption process began. As illustrated in Fig. 2, hyphae pellets had already adsorbed 76% (17.94 mg/g) of the total amount of Pb^{2+} (23.35 mg/g) adsorbed within the initial 10 min. The lead uptake rate gradually decreased afterwards and the uptake increased by only 4.14 mg/g which was 17.74% of the total from 10 min to 2h. Sorption equilibrium was almost established after 6h. This implied that the surface binding occurred first and took place rather fast. Further Pb^{2+} uptake may be controlled by the diffusion process through the cell wall or regulated by the intracellular metabolic processes. Since the biomass was treated by NaOH before adsorption and the cell died, the metal uptake could not have been metabolically mediated. Residual enzymatic activity, however, could not be entirely excluded. So the intracellular diffusion may be the rate limiting step. Nevertheless, by contrast to the surface binding, the intracellular uptake is insignificant. Tsezos and Volesky(Tsezos, 1982a; 1982b) have also suggested that surface binding is the key to the rapid establishment of an uptake equilibrium.

2.2.4 Temperature

The effect of temperature on lead uptake is shown in Fig.3. There were only small changes between 28℃ and 35℃. However, an significant decrease in lead uptake was observed at 15℃. This indicated that the process of adsorption was a process of needing heat. In the range of 15 – 35℃, the increase of temperature is favourable.

2.2.5 Initial Pb^{2+} concentration

Fig.4 shows the initial rate of Pb^{2+} uptake by *P*. chrysosporium at different initial concentrations. The initial removal rate of Pb^{2+} (mg/(g·min)) were roughly calculated using the data within 5 min. The

initial rate of Pb^{2+} uptake increased with the initial metal ion concentration. However the lower the initial concentration, the higher the removal efficiency. So biosorption is potentially an effective method for the treatment of wastewater which contains metal ions of low concentration.

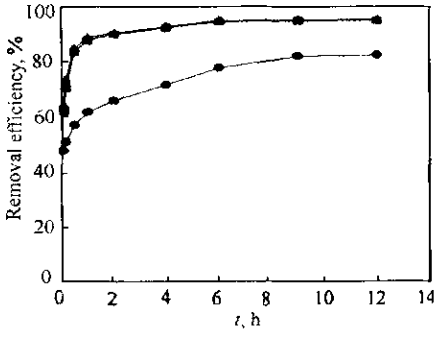

Fig.3 Effect of temperature on biosorption of Pb^{2+}
▲ 35℃; ■ 28℃; ● 15℃
(c_i = 50 mg/L; m = 2 mg/L; pH = 4.5; 12h)

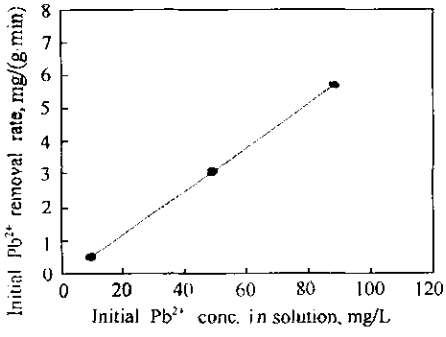

Fig.4 Effect of initial Pb^{2+} conc. on initial removal rate of Pb^{2+}
● c_i = 90 mg/L; ■ c_i = 50 mg/L; ▲ c_i = 10 mg/L
(m = 2 g/L; pH = 4.5; 28℃; 9h)

2.2.6 Biosorption isotherm

The adsorption capability of lead could be evaluated by plotting the sorption isotherms. *P. chrysosporium* exhibited the highest lead uptake capacity of approximately 108 mg/g at pH 4.5 and 27℃ as given in Fig.5. Langmuir model is:

$$q_e/q_{max} = bC_e(1 + bC_e), \tag{3}$$

and Eq.(3) can be changed into Eq.(4):

$$C_e/q_e = 1/bq_{max} + C_e/q_{max}. \tag{4}$$

Fig.6 showed that $C_e/q_e - C_e$ was nearly linear. It can be seen from Fig.5 and Fig.6 that the sorption isotherm coincided with Langmuir sorption model well. It suggested that surface sorption was the primary mechanism of biosorption. According to Langmuir sorption model, the saturation uptake could be calculated which was 144 mg/g and b was 1.095 L/mg.

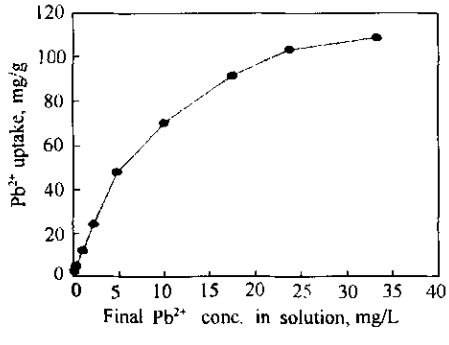

Fig.5 Biosorption isotherm of lead ion
(m = 2 g/L; pH = 4.5; 27℃; 16h)

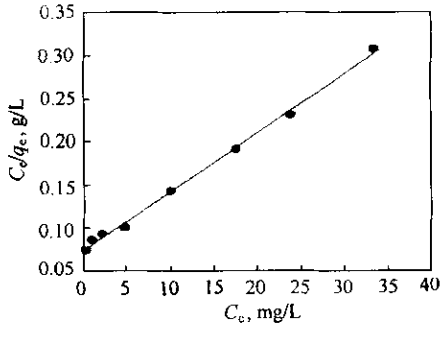

Fig.6 Langmuir plot
(m = 2 g/L; pH = 4.5; 27℃; 16h)

2.2.7 Co-ion

The effect of presence of co-ions in the solution on lead uptake capacity was examined by adding Cu^{2+}, Zn^{2+} and Cd^{2+} respectively. These co-ions were considered quite likely to be present in solutions originating from a real industrial source.

The presence of these co-ions had a negative effect on the lead uptake capacity by the biomass regardless of the initial co-ion concentration. As seen from Fig.7 the extent to which the Pb^{2+} uptake was

affected varied from metal to metal. Zn^{2+} had a stronger effect than Cd^{2+} and Cu^{2+} did. The uptake of Pb^{2+} exhibited a significant decrease as the initial concentrations of co-ions increased. This phenomenon indicated that the metals competed for the same active sites. It was documented that the extent of binding between metal ion and biological ligand depended on their polarizing power-that is, on the ratio of charge to radius of the cation (Brady, 1995). Because of the different features of metal ions, the degrees of the negative effects of co-ion on the sorption capacity of Pb^{2+} are quite different. In the meantime, this phenomenan also confirmed that the primary mechanism of the biosorption was a kind of surface binding due to reaction between metal ions and biological ligands.

2.2.8 pH variation

For the biomass without alkali treatment, the pH of solution had a slight fall from 4.5 to 3.95 during the biosorption process. But for the alkali treated biomass, the solution experienced a much bigger change in pH due to the residual OH^- inside the pellets which may diffuse from the inside of pellets into the bulk solution. It could be explained as a kind of ion exchange. When Pb^{2+} were adsorbed onto the cell surface, H^+ released into solution from cell surface leading to the slight decrease of pH. This kind of ion exchange is not the prevailing mechanism for Pb^{2+} uptake because the number of released H^+ from cell was very little.

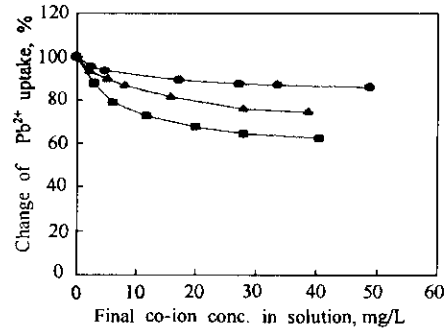

Fig.7 Effect of co-ion on Pb^{2+} uptake
●Cd^{2+}; ▲Cu^{2+}; ■Zn^{2+}
(m = 2 g/L; pH = 4.5; 27℃; 9h)

3 Conclusions

P. chrysosporium can grow in the form of pellet under certain culture conditions. The factors which affect pellet size, biomass and mechanical properties include medium composition, pH, spore number in inoculum and shaking rate. Biosorption of Pb^{2+} by *P. chrysosporium* hyphae pellets was strongly affected by pH, initial Pb^{2+} concentration, the presence of co-ions and chemical pretreatment. The alkali treatment before adsorption enhanced adsorption capacity, which could be attributed to the changes of the structures of cell wall composition. The maximum uptake capacity of Pb^{2+} 108 mg/g was at pH 4.5. Most of metal adsorption happened within the initial 10 min. The initial rate of Pb^{2+} uptake increased with the initial Pb^{2+} concentration, however, the removal efficiency at lower initial Pb^{2+} concentration was higher. In the same concentration range, Zn^{2+} strongly interfered in the Pb^{2+} uptake while Cd^{2+} and Cu^{2+} had slight effect on Pb^{2+} uptake. The mechanism of Pb^{2+} biosorption was illustrated preliminarily by series of experiments, including the effect of alkali treatment, the influence of co-ion, the change of pH during biosorption process. The surface binding is the primary mechanism along with less ion exchange. The further studies on biosorption mechanism will be made in the future work.

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Notation:

m: Biomass concentration, g dry biomass/L; C_i: Initial metal ion concentration, mg/L;
C_e: Equilibrium metal ion concentration, mg/L; q_e: Equilibrium uptake, mg metal ion/g dry biomass;
q_{max}: Ideal saturation uptake, mg metal ion/g dry biomass; R: Removal efficiency, %;
DBW: Dry biomass weight, g biomass/L medium; D/W: Weight ratio of dry biomass to wet biomass.

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