BIOMEDICAL AND ENVIRONMENTAL SCIENCES **18**, 141-145 (2005)

Biosorption of Cadmium by Fungal Biomass of *Aspergillus niger*[1]

QI YANG[#,△], JIAN-LONG WANG[2,#], AND ZHI XING[◊]

[#]*Laboratory of Environmental Technology, INET, Tsinghua University, Beijing 100084, China;* [△]*School of Water Resource and Environment, China University of Geoscience, Beijing 100083, China;* [◊]*Analysis Center of Tsinghua University, Beijing 100084, China*

Objective To investigate the removal of cadmium from aqueous solution by waste fungal biomass of *Aspergillus niger*, originated from citric acid fermentation industry. **Methods** Batch adsorption test was used to study the biosorption equilibrium and isotherm. The Cd^{2+} concentration was measured with atomic adsorption spectrophotometer (AAS) HITACHI 180-80. **Results** The biosorption achieved equilibrium within 30 min. The adsorption isotherm could be described by Freundlich adsorption model, and the constants K_F and 1/n were determined to be 2.07 and 0.18, respectively, and the correlation efficiency was 0.97. The optimal pH for Cd adsorption was 6.0. The cadmium-laden biomass could be effectively regenerated using 0.1 N HCl. **Conclusion** The waste biomass of *Aspergillus niger*, a by-product of fermentation industry, is a potential biosorbent for the removal of cadmium from aqueous solution.

Key words: Cadmium; Heavy metal pollution; *Aspergillus niger*; Biomass; Biosorption

INTRODUCTION

Heavy metal contamination exists in aqueous-waste streams of many industries, such as metal plating facilities, mining operations, and tanneries. The soils surrounding many military bases are also contaminated and pose a risk of metal contamination on ground and surface water. Some metals associated with these activities are cadmium (Cd), chromium (Cr), lead (Pb), and mercury (Hg). Heavy metals are not biodegradable and tend to accumulate in living organisms, causing various diseases and disorders[1].

Treatment processes for metal-contaminated waste streams include chemical precipitation, membrane filtration, ion exchange, carbon adsorption and the like. Cost effective alternative technologies or adsorbents for treatment of metal-contaminated waste streams are needed.

Biosorption and related phenomena are of importance because the removal of potentially toxic and/or valuable metals and radionuclides from aqueous effluents can result in detoxification and therefore safe environmental discharge[2-4]. Furthermore, appropriate treatment of loaded biomass can enable recovery of valuable elements for recycling or further containment[5].

Living and dead cells of fungi are able to remove heavy metal ions from aqueous solutions. Uptake of heavy metal ions by fungal microorganism may offer an alternative method for their removal from wastewater. For such application, fungal biomass would have to be easily available in substantial quantities. Fungi are used in a variety of industrial fermentation processes, which could serve as an economic and constant supply source of biomass for the removal of metal ions. Fungi can also be easily cultivated in substantial amounts using unsophisticated fermentation techniques and inexpensive growth media. Therefore, a fungal biomass could serve as an economical means for removal/recovery of metal ions from aqueous solution.

Fungi belonging to the genera *Rhizopus* and *Penicillium* have already been studied as a potential biomass for the removal of heavy metals from aqueous solutions[6-8]. But little is known about the removal of heavy metals such as lead, cadmium, copper and nickel from aqueous solutions using *Aspergillus niger*, which is applied in a variety of industrial fermentation processes, such as citric acid production. It was demonstrated by Yakuba and

[1]The project was supported by the National Natural Science Foundation of China (Grant No. 50278045) and the Basic Research Fund of Tsinghua University (Grant No. JC2002054).

[2]Correspondence should be addressed to Dr. Jian-Long WANG, Laboratory of Environmental Technology, INET, Tsinghua University, Beijing 100084, China, Tel: 86-10-62784843. Fax: 86-10-62771150. E-mail: wangjl@tsinghua.edu.cn

Biographical note of the first author: QI YANG, male, born in 1966, Ph. D. degree, majoring in the field of water pollution control.

0895-3988/2005
CN 11-2816/Q

Dudeney[9] that *Aspergillus niger* is able to effectively remove uranium ions. Huang and his coworkers[10] demonstrated that *Aspergillus oryzae* could remove cadmium and copper ions from aqueous solutions.

The objective of this study was to investigate the removal of cadmium from aqueous solution by waste biomass of *Aspergillus niger*, a citric acid-producing microorganism used for citric acid production in our laboratory[11-13].

MATERIALS AND METHODS

Microorganism

Aspergillus niger W1-2, a citric acid-producer, was isolated in our laboratory and maintained on malt extract slants, stored at 4℃ and renewed every other month[14].

A loopful conidia of *Aspergillus niger* was inoculated into a malt extract agar slant and incubated at 30℃. Six days after incubation, 5.0 mL of sterile physiological saline was added, and the water-inoculum combination was shaken violently. The spore suspension was then collected and used as inoculum.

Fermentation Medium

The fermentation medium for citric acid production contained 120 g/L sucrose, 2.0 g/L NH_4Cl, 1.0 g/L KH_2PO_4, 0.25 g/L $MgSO_4 \cdot 7H_2O$, and 0.3 mg/L $FeSO_4$, 0.4 mg/L $ZnSO_4$, 0.15 mg/L $MnSO_4$, 0.4 mg/L $CuSO_4$.

Preparation of Bioabsorbent

Fermentation of citric acid was carried out in a 2.0 L fermentor. After fermentation, biomass was harvested by filtering the cultured medium. Once harvested, the biomass was washed with distilled water and then suspended in designated heavy metal solutions for adsorption experiments.

Adsorption Experiments

Bio-adsorption experiments were performed in an orbital shaker at 150 rev min^{-1} and 25℃ using 250 mL shake flasks containing 50 mL Cd^{2+} solution. The initial pH value of the solution was adjusted with HCl or sodium hydroxide using a pH meter. Biomass of *Aspergillus niger* was added to each flask. After the flasks were shaken for 4 h, the reaction mixture was filtered through filter paper and the filtrate was used for analysis.

The amount of cadmium adsorbed by the biomass was calculated using the following equation:

$$q = (C_0 - C_e)\ V/W \quad (1)$$

where:

q = amount of cadmium adsorbed by biomass (mg/g);

C_0 = initial concentration of cadmium (mg/mL);

C_e = concentration of cadmium at equilibrium (mg/mL);

V = initial volume of cadmium solution (mL);

W = weight of the biomass (g).

All experiments were conducted in duplicate and mean values were used in the analysis of data.

Desorption Experiments

The experimental conditions for desorption of cadmium on biomass were similar to those of the batch adsorption tests except for a higher concentration (100 mg/L) and higher doses (5 g/L) of biomass. After being subjected to adsorption process for 4 h, the reaction mixture was filtered and the biomass was washed repeatedly with distilled water to remove any un-adsorbed cadmium. The desorption studies were performed in the shake flask on an orbital shaker, 0.1 mmol/L HCl was used as desorbent.

Measurement of Cadmium

The heavy metal adsorbate used in this study was cadmium chloride, and its concentrations in solutions were measured with Polarized Zeeman atomic adsorption spectrophotometer (AAS) HITACHI 180-80. Before determination, the heavy metal solutions were appropriately diluted with de-ionized water to ensure that the sample concentration was linearly dependent on the absorbance detected.

RESULTS

Time Course of Cadmium Adsorption

In order to determine the time required for achieving adsorption equilibrium, the adsorption equilibrium experiments were performed. The results (Fig. 1) showed that the adsorption equilibrium was reached within 10 minutes under experimental conditions. No change of cadmium ion concentration was observed during prolonged shaking. Therefore, a shaking time of 4 h which is long enough for achieving adsorption equilibrium in the whole system, was used in this study.

Adsorption Isotherm Analysis

The studies of cadmium adsorption on the fungal biomass were carried out, by varying the initial cadmium concentration from 10 mg/L to 250 mg/L. The results are illustrated in Fig. 2.

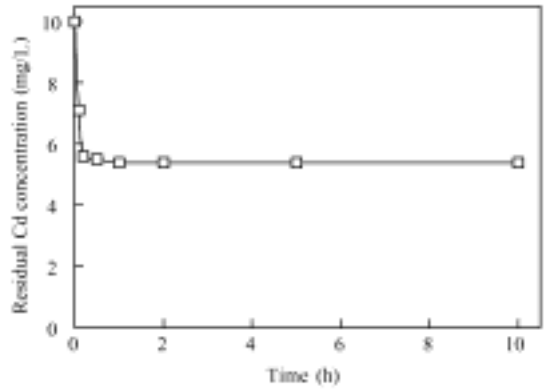

FIG. 1. Time course of cadmium adsorption.

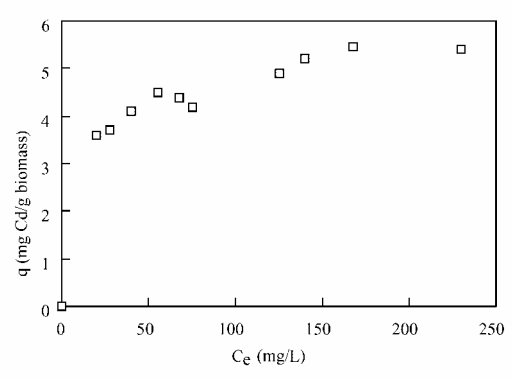

FIG. 2. Adsorption isotherm of cadmium.

The Freundlich model was used in this work to fit the adsorption data. Attempts to use the Langmuir equation to fit the adsorption isotherm failed to provide a satisfactory correlation (data not shown).

The Freundlich equation is used for heterogenous surface energies in which the energy term in the Langmuir equation varies as a function of the surface coverage strictly as a result of variation in the heat of adsorption. The Freundlich equation has the general form:

$$q_e = K_F c_e^{1/n} \tag{1}$$

This equation can also be modified as:

$$q_e = \frac{x}{M} = \frac{c_0 - c_e}{M} = K_F c_e^{1/n} \tag{2}$$

i.e.

$$M = \frac{c_0 - c_e}{x/M} \tag{3}$$

Where:

x is the amount of cadmium sorbed on biomass;

c_0 is the initial cadmium concentration;

c_e is the equilibrium concentration of adsorbate;

M is the amount of bio-absorbent.

The absorbent dosage, M, is required to reduce the initial concentration, c_0, so the desired final concentration, c_e, is calculated from equation (3). The value x/M at c_0 can be read from the plot of the Freundlich adsorption isotherm. This is helpful for large-scale applications of batch systems. A logarithmic plot linearizes the equation, enabling the exponent *n* and the constant K_F to be determined:

$$\ln q_e = \ln K_F + \frac{1}{n} \ln c_e \tag{4}$$

Where:

q_e is the amount of sorbate per unit mass of absorbent;

K_F is sorption capacity, indicator of adsorption intensity;

c_e is the equilibrium concentration.

The representative Freundlich isotherm for cadmium adsorption onto the fungal biomass is shown in Fig. 3.

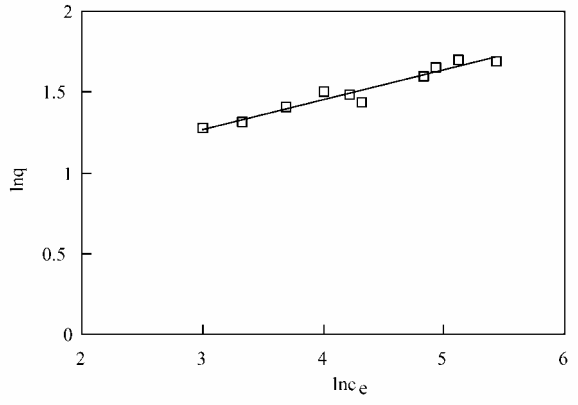

FIG. 3. Freundlich isotherm of cadmium adsorption.

The values of constants K_F and 1/n were calculated by the least square method, which were 2.07 and 0.18, respectively. The correlation coefficient was 0.97. The high value of correlation coefficient indicated that the data conformed well to the Freundlich model.

Effect of pH

Ambient pH was likely to be a major factor in the quantity of metal ion bio-adsorption owing to cation competition effects with hydrogen ions. The result of effect of pH on cadmium adsorption is presented in Fig. 4.

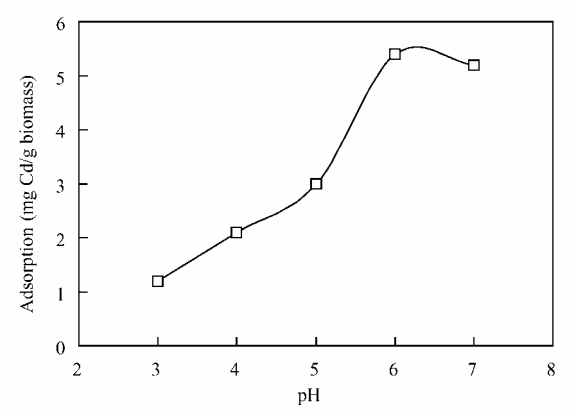

FIG. 4. Effect of pH on cadmium adsorption.

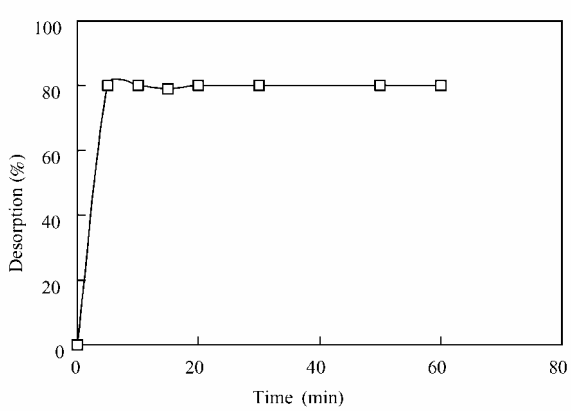

FIG. 6. Desorption of cadmium from Cd-laden biomass.

Desorption Kinetics of Cadmium-loaded Biomass

The bio-absorbed cadmium was eluted from fungal biomass using 0.1 mmol/L HCl, to investigate the removal of bio-absorbed acdmium ion from cadmium-loaded biomass, i. e., to investigate the possibility of regenerating the bio-absorbent.

To determine the effects of pH on desorption of Cd, solutions containing Cd-laden biomass were treated with 0.1 mmol/L HCl to adjust pH to designated values. The amount of cadmium released from the acid-treated biomass with different pH was then measured. The results are depicted in Fig. 5.

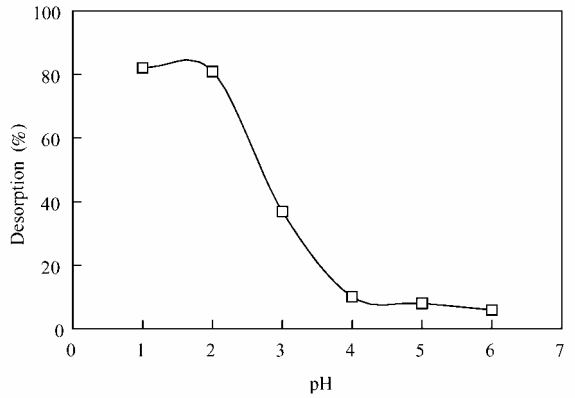

FIG. 5. Effect of pH on Cd desorption from Cd-laden biomass.

Basically, metal release was invisible when pH was over 4.0, whereas as pH decreased the desorption efficiency increased rapidly, until it reached a plateau when pH was below 2.0.

The time course of cadmium desorption from Cd-loaded biomass is demonstrated in Fig. 6.

The cadmium adsorbed by biomass could be eluted very quickly, the desorption reached equilibrium within 10 minutes. Around 80% of Cd could be recovered.

DISCUSSION

Interactions between metal cations and electron-rich functional groups on the biomass may be strongly sensitive to the pH value of environment. The way in which pH changes the adsorption of metal ions to biomass varies with the types of adsorbents (biomass) and adsorbates (metal ions). The optimal pH for adsorption of Pb by mycelial by-products of *Rhizopus arrhizus* is 5.0[15], and the optimal pH for adsorption of the same metal ion is around 4.5 for biomass of *Penicilluium chrysogenum*[16]. An optimal pH of 6.5 for the adsorption of Cu is found using *Saccharomyces cerevisiae* as the bioabsorbent[17]. For the bio-adsorption of Cd by fungal biomass, the maximal capacity occurs at pH 8.0[18]. The Hg^{2+} uptake by *Pseudomonas aeruginosa* PU21 reaches maximum at pH 7-8[19].

Alteration in pH over a wide range does not measurably change the average intracellular pH[20], suggesting that the binding sites must be located peripherally, under the influence of extracellular pH rather than sites exposed to the constancy of intracellular pH. This view does not take into account that a hydrogen ion gradient across the cell membrane must be present to allow for cation uptake[2].

It is apparent that when the initial pH<5.0, the adsorption capacity decreases dramatically, which may be due to the fact that the hydrogen ion as a cation, can compete with binding sites of the biomass surface for cadmium ions.

As shown in Fig. 4, pH of the adsorption medium is a key parameter that shows a substantial effect on bio-adsorption capacity of removing the cadmium ion from aqueous solution.

The recovery of heavy metals from metal-laden biomass is carried out by utilizing various desorption agents, including HCl[21], H_2SO_4, Na_2CO_3[22-24],

EDTA and β-mercaptoethanol. It appears that HCl has the best desorption efficiency among the chemical reagents tested, and thus was selected as the desorption agent in this study. The experimental results indicate that HCl is able to effectively elute the biosorbed cadmium from fungal biomass. The optimal procedure for cadmium recovery in this case is to adjust pH to approximately 2.0 with 0.1 N HCl. The recovery rate of Cd could reach about 80%.

CONCLUSIONS

The results of the present study show that it is possible to use waste fugal biomass for the removal of cadmium ion from aqueous solution. The adsorption equilibrium can achieve within 30 minutes. The adsorption process can be described by Freundlich isotherm model. The adsorbed cadmium can be eluted using 0.1 mmol/L HCl. The desorption can be completed within 10 minutes, with about 80% of cadmium recovered. To employ waste biomass to treat heavy metal polluted wastewater is an environmental-friendly technology.

REFERENCES

1. Gadd, G. M. (1990). Heavy metal accumulation by bacteria and other microorganisms. *Experientia* **46**, 834-840.
2. Wang, J. L. (2002). Microbial immobilization techniques and water pollution control. Beijing, Science Press.
3. Wang, J. L. (2002). Biosorption of Copper (II) by Chemically Modified Biomass of *Saccharomyces cerevisiae*. *Process Biochemistry* **37**(8), 847-850.
4. Wang, J. L., Zhan X. M., Ding D. C., and Zhou, D. (2001). Biosorption of lead (II) from aqueous solution by fungal biomass of *Aspergillus niger*. *Journal of Biotechnology* **87**, 273-277.
5. Tsezos, M. and Deutschmann, A. A. (1990). An investigation of engineering parameters for the use of immobilized biomass particles in biosorption. *J. Chem. Tech. Biotechnol.* **48**, 29-39.
6. Tobin, J. M., Cooper, D. G, and Neufeld, R. J. (1984). Uptake of metal ion *by Rhizopus arrihus* biomass. *Appl. Environ. Microbiol.* **47**, 821-824.
7. Siegel, S. M., Galum, M., and Siegel, B. Z. (1990). Filamentous fungi as metal biosorbents: a review. *Wat. Air and Soil Pollut.* **53**, 335-344.
8. Volesky, B. and Holan, Z. R. (1995). Biosorption of heavy metals. *Biotechnol. Prog*. **11**, 235-250.
9. Yakubu, N. A. and Dudeney, A. W. L. (1986). Biosorption of uranium with *Aspergillus niger*. In Eccles, HH, Hunt, S. (Eds.) Immobilization of ions by biosorption. Ellis Horwood, Chichester, UK. pp.183-200.
10. Huang, C. and Huang, C. P. (1996). Application *of Aspergillus oryzae* and *Rhizopus oryzae* for Cu (II) removal. *Wat. Res.* **30**, 1985-1990.
11. Wang, J. L. and Liu, P. (1996). Comparison of citric acid production by *Aspergillus niger* immobilized in gels and cryogels of polyacrylamide. *J. Ind. Microbiol.* **16**, 351-353.
12. Wang, J. L. and Liu, P. (1998). Phytate as a simulator of citric acid production by *Aspergillus niger*. *Process Biochem.* **33**, 313-316.
13. Wang, J. L. (1998). Improvement of citric acid production by *Aspergillus niger* with addition of phytate to beet molasses. *Bioresource Technol*. **65**, 243-245.
14. Zhou, D. and Wang, J. L. (1992). Study on breeding of citric acid-producer and its fermentation. *Food Ferment Ind*. **5**, 45-49. (in Chinese)
15. Fourest, E. and Roux, J. C. (1992). Heavy metal biosorption by fungal mycelial by-products: mechanisms and influence of pH. *Appl. Microbiol. Biotechnol.* **37**, 399-403.
16. Niu, H., Xu, X. S., Wang, J. H., and Volesky, B. (1993). Removal of lead from aqueous solutions by *Penicilluium* biomass. *Biotechnol. Bioeng*. **42**, 785-787.
17. Brady, D. and Ducan, J. R. (1994). Bioaccumulation of metal cations by *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* **41**, 149-154.
18. Kiff, R. J. and Little, D. R. (1986). Biosorption of heavy metals by immobilized fungal biomass. In Eccles, HH, Hunt, S. (Eds.) Immobilization of ions by biosorption. Ellis Horwood, Chichester, UK. pp. 71-80.
19. Chang, J. S., Law, R., and Chang, C. C. (1997). Biosorption of lead, copper and cadmium by biomass of *Pseudomonas aeruginosa* PU21. *Wat. Res.* **31**, 1651-1658.
20. Pothstein, A. and Hayes, A. D. (1956). The relationship of the cell surface to metabolism. XIII. The cation-binding properties of the yeast cell surface. *Arch. Biochem. Biophys.* **63**, 87-99.
21. Wong, P. K., Lam, K. C., and So, C. M. (1993). Removal and recovery of Cu (II) from industrial effluent by immobilized cells of *Pseudomonas putida* II-11. *Appl. Microbiol. Biotechnol*. **39**, 127-131.
22. Nakajima, A. and Sakaguchi, T. (1993). Accumulation of uranium by basidiomycetes. *Appl. Microbiol. Biotechnol.* **38**, 574-578.
23. Pons, M. P. and Fuste, M. (1993). Uranium uptake by immobilized cells of *Pseudomonas* strain EPS 5028. *Appl. Microbiol. Biotechnol.* **39**, 661-665.
24. Kapoor, A., Viraraghavan, T., and Cullimore, D. R. (1999). Removal of heavy metals using the fungal *Aspergillus niger*. *Bioresource Technol.* **70**, 95-104.

(*Received October* 28, 2003 *Accepted December* 21, 2004)