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Biosorption of Lead, Mercury, and Cadmium Ions by *Aspergillus terreus* Immobilized in a Natural Matrix

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Abstract

Our aim was to investigate the biosorption of Pb^{2+}, Hg^{2+}, Cd^{2+} from aqueous solution by *Aspergillus terreus* (both free and immobilized on loofa sponge discs). Our results show that the adsorption capacity of fungal biomass on loofa sponge (FBLS) is superior to free fungal biomass (FFB). The adsorption selectivity by FBLS was in the order Pb^{2+}>Hg^{2+}>Cd^{2+}. The maximum metal ions adsorbed was 247.2, 37.7, 23.8 mg/g FBLS for Pb^{2+}, Hg^{2+} and Cd^{2+}, respectively. Metal uptake by FBLS was affected by the pH of the metal solution, but independent of temperature (10–50°C). The Langmuir model was more suitable than the Freundlich model to describe the biosorption process of FBLS. The regenerated FBLS was found to be effective for repeated use for five cycles without significant loss in adsorption capacity. This research demonstrates that FBLS possesses excellent capacity for Pb^{2+} biosorption from aqueous solution and industrial wastewaters.

Key words: biosorption, heavy metal, immobilization, industrial wastewater

Introduction

It is well recognized that the presence of heavy metals in the environment can be detrimental to a variety of living species, including man. Industrial wastewaters are considered the most important source of heavy metal pollution. The conventional technologies for treating these pollutants are precipitation, oxidation/reduction, ion exchange, filtration and electrolysis. However, some disadvantages, such as high cost, incomplete removal, high energy consumption, and/or generation of toxic wastes accompany these technologies. Therefore, a cost-effective treatment that efficiently removes heavy metals from industrial effluents is needed. Using microorganisms (*i.e.*, fungi, bacteria, algae and yeasts) as biosorbents to remove metal ions from wastewater offers a potential alternative to existing methods. Numerous studies have demonstrated that microorganisms have ability to remove heavy metals from wastewater with better performance and lower cost compared with conventional technologies (Congeevaram *et al*. 2007, Kapoor *et al.*, 1999; Liu *et al*., 2004; Moon *et al*., 2006).

Aspergillus, *Penicillum* and *Trichoderma* are the primary fungal groups that degrade cellulose, hemicellulose and lignin in agriculture wastes, soils, and the feces of cattle and sheep. Among the three fungal groups, *Aspergillus* spp. are the most frequently studied and applied in agriculture waste recycling and the biomass energy industry (Gawande and Kamat, 1999, 2000; Ghanem *et al.*, 2000). Various researchers have investigated the use of *Aspergillus* spp. as a biosorbent for metal ions removal. For example, studies have shown that *Aspergillus niger* can effectively remove uranium, lead, cadmium and copper ions (Kapoor *et al.*, 1999; Yakubu and Dudeney, 1986). Huang and Huang (1996) and Huang *et al.*, (1988) investigated the use of *Aspergillus oryzae* to remove cadmium and copper ions from aqueous solution. A strain of *Aspergillus terreus* has been shown to take up chromium, nickel and iron from metallurgical effluents (Dias *et al.*, 2002).

Small particle size with low density, poor mechanical strength and rigidity are some of the physical problems encountered when applying biomass as

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a biosorbent (Han *et al.*, 2005; McHale and McHale, 1994). Immobilization of the biomass within a suitable matrix can overcome these problems by offering ideal size, mechanical strength, rigidity and porous characteristics to the biological material (Trujillo *et al.*, 1995). Several immobilized biomass systems have been successfully used as adsorbing agents to remove heavy metals (Blanco *et al.*, 1999; Hu and Reeves, 1997; Pan *et al.*, 2005). Among the various immobilized biomass systems, a polymeric matrix is the most common method. However, these techniques have certain disadvantages: they are prohibitively expensive, they limit the rate of diffusion (Hu and Reeves, 1997), or they produce other waste products that require disposal (Wilde and Benemann, 1993).

Loofa sponge is a natural, environmentally friendly biomaterial. It is abundant, cheap, rigid, non-toxic, chemically inert and highly porous. The use of loofa sponge material for the immobilization of algae, fungal hyphae and yeast cells has been successfully demonstrated (Akhtar *et al.*, 2008; Iqbal and Edyvean, 2004; Iqbal and Zafar, 1994; Li *et al.*, 2008; Ogbonna *et al.*, 2001). However, the use of loofa sponge-immobilized *Aspergillus terreus* for metal biosorption has not been investigated. Our previous report showed that free *A. terreus* has high capacity for adsorbing metal ions from aqueous solutions (Ho *et al.*, 2006). The goal of the present study was to further investigate Pb^{2+}, Hg^{2+} and Cd^{2+} biosorption from solutions by loofa sponge-immobilized *A. terreus.* and to evaluate the applicability of the immobilized *A. terreus* for the removal of Pb^{2+} from real industrial wastewaters.

Experimental

Materials and Methods

Microorganism and culture medium. The fungus, *Aspergillus terreus* BCRC 32068, was obtained from the Bioresource Collection and Research Center, HsinChu, Taiwan. It was maintained in pure culture with potato dextrose agar (Difco) slants at 28°C. After incubating for 7 days, fungal spores were harvested with sterile solution (0.9% NaCl, 0.05% Tween 80), washed twice and enumerated. An aliquot amount of fungal spores (2×10^6) was transferred to a 250-ml Erlenmeyer flask containing 50 ml of growth medium (Hajjaj *et al.*, 2001). This growth medium consisted of 5 g l^{-1} KH_2PO_4, 5 g l^{-1} K_2HPO_4, 0.2 g l^{-1} $FeSO_4\times7H_2O$, 0.1 g l^{-1} $MnSO_4\times4H_2O$, 0.2 g l^{-1} $ZnSO_4\times7H_2O$, 5 mg l^{-1} $CuCl_2\times2H_2O$, 11 mg l^{-1} H_3BO_3, 5 mg l^{-1} $(NH_4)_6Mo_7O_{24}\times4H_2O$, 12.5 g l^{-1} $C_5H_8NNaO_4$, 45 g l^{-1} glucose, 0.1 g l^{-1} $MgSO_4\times7H_2O$, and 20 mg l^{-1} $CaCl_2\times2H_2O$. Once inoculated, the flasks were shaken in an incubator shaker (200 rpm) at 28°C for 24 h. These mycelium suspensions were then used for immobilization.

Pretreatment of the loofa sponge. Vegetable sponges composed of loofa, *Luffa aegyptica*, were purchased from the Madou local market (Tainan, Taiwan). They were used as immobilized media for *A. terreus*. The fibrous sponges were cut into discs of approximately 0.2 g, soaked in boiling water for 30 min, thoroughly washed under tap water, soaked for 24 hours and rinsed for 3–4 times with deionized water. Then, the sponge discs were sterilized by autoclaving at 121°C for 30 min. Finally, the sponge discs were oven dried at 70°C and stored in desiccators before further use.

Immobilization of *A. terreus* on loofa sponge discs. The immobilizing technique was performed according to the method described by Iqbal and Edyvean (2004), with some modifications.

An aliquot amount (approximately 2.5 ml) of mycelium suspension was taken from the fungal stock culture and inoculated into 250 ml flasks containing 50 ml of the growth medium and four pre-weighed loofa sponge discs. Culture flasks for free hyphal growth, with no loofa sponge discs in the medium, served as the control. The inoculated flasks were then incubated at 28°C and shaken at 200 rpm. After 15 days of incubation, both free (hereafter called free fungal biomass and abbreviated as FFB) and immobilized *A. terrues* (hereafter called fungal biomass on loofa sponge and abbreviated as FBLS) were harvested from the medium, rinsed twice with distilled water and oven dried at 70°C. The dry weight of the fungal biomass entrapped within the sponge discs was determined by weighing the sponge discs before and after fungal growth following 24 h of drying at 70°C.

Metal solutions. All reagents used were analytical-grade. The stock solutions of Pb^{2+}, Hg^{2+} and Cd^{2+} (6000 mg l^{-1} for Pb^{2+}, 2000 mg l^{-1} for Hg^{2+} and 2000 mg l^{-1} for Cd^{2+}) were prepared by dissolving exact quantities of $PbCl_2$, $HgCl_2$ and $CdCl_2\times2H_2O$ in doubly distilled water, respectively. The working solutions containing single Pb^{2+} or Hg^{2+} or Cd^{2+} were prepared just before use by diluting the stock solutions. The initial metal ion concentration ranged from 1000 to 6000 mg l^{-1}, in the case of Pb^{2+} and from 200 to 2000 mg l^{-1}, in the case of Hg^{2+} and Cd^{2+}. Except for a pH-shift experiment, the pH of the working solution was adjusted to pH 5.0 with 0.1 N HCl or 0.1 N NaOH.

Biosorption studies. Batch experiments were conducted to determine the optimum Pb^{2+}, Hg^{2+} and Cd^{2+} biosorption conditions. One hundred milligrams of FBLS or FFB were mixed with 100 ml of the metal solutions (6000 mg l^{-1} for Pb^{2+}, 800 mg l^{-1} for Hg^{2+} and 300 mg l^{-1} for Cd^{2+}) in 250 ml Erlenmeyer flasks. The effects of contact time, pH, temperature and rotation speed on the biosorption of Pb^{2+}, Hg^{2+} and Cd^{2+} were studied. The effect of contact time was evaluated

in the range of 10–180 min. The flasks were agitated on an orbital shaker (200 rpm) at 30°C. In the latter experiments the contact time of 90 min was applied. The effect of pH on the biosorption capacity was investigated at initial pH values range of 2.0–6.0. The designated pH of the solutions was achieved by adding HCl or NaOH at the beginning of the experiment without further adjustment afterwards. As above, the flasks were agitated on an orbital shaker (200 rpm) at 30°C. In experiments to test the effect of temperature, the flasks were agitated (200 rpm) at different temperatures ranging from 10 to 50°C. In experiments to find the effect of rotation speeds, the flasks were agitated at 30°C using different agitation speeds (0, 50, 100, 150, 200 and 250 rpm). In experiments to assess the effect of the initial metal ion concentration, 100 ml of single metal solutions (1000–6000 mg l^{-1} for Pb^{2+}, 200–2000 mg l^{-1} for Hg^{2+} and 200–2000 mg l^{-1} for Cd^{2+}) were contacted with 100 mg of FBLS for 90 min at 200 rpm and 30°C. At the end of biosorption, FFB was removed from metal solution by centrifugation at 1500 g for 5 min, whereas FBLS was separated from the solution by simple decantation. Residual metal concentrations in the supernatant were determined using an atomic absorption spectrophotometer (Perkin-Elmer AAnalyst 1003). The adsorption efficiency of metals by FBLS and FFB were then derived.

All glassware for the biosorption experiments was cleaned by immersion in 10% HNO_3 overnight and extensive flushing with deionized water.

Data analyses. The biosorption capacities for metal ions adsorbed per unit weight of FFB and FBLS were determined by Equation (1).

$$q = V\,(C_i - C_{eq}) / M \quad (1)$$

where q is the metal uptake capacity (mg metal ions/g dry weight of fungal biomass); V is the volume of metal solution (l); C_i is the initial concentration of metal ions in the solution (mg l^{-1}); C_{eq} is the residual concentration of metal ions in the solution (mg l^{-1}); M is the dry weight of fungal biomass (g). All experiments were replicated in triple runs.

The Langmuir and Fruendlich equilibrium models were used to describe our equilibrium batch sorption tests. Langmuir isotherm assumes monolayer adsorption, and is presented by the Eq (2).

$$q_e = (q_{max} K_L C_e) / (1 + K_L C_e) \quad (2)$$

where q_e and q_{max} are the equilibrium and maximum uptake capacities (mg/g biosorbent); C_e is the equilibrium concentration (mg l^{-1}); and K_L is the equilibrium constant (l/mg).

The Freundlich isotherm model is presented by Eq (3).

$$q_e = K_F C_e^{1/n} \quad (3)$$

where K_F and n are Freundlich constants that characterize the system.

Desorption studies. For batch desorption studies, five different desorbing agents (0.1N HNO_3, 0.1N HCl, 0.1N CH_3COOH, 0.1N H_2SO_4 and deionized H_2O) were used. One hundred milligrams of FBLS were contacted with 100 ml of 6000 mg l^{-1} of Pb^{2+} solution for biosorption and 100 ml desorption solution for desorption in 250 ml Erlenmeyer flasks. The flasks were agitated on an orbital shaker (200 rpm) at 30°C. The contact time of biosorption and desorption was maintained 90 min for achieving biosorption or desorption equilibrium. The amount of Pb^{2+} adsorbed on FBLS was obtained by difference between the initial Pb^{2+} concentration and the final one after biosorption equilibrium was reached. After desorption, the FBLS was removed and the final Pb^{2+} concentration in the aqueous phase was determined by atomic absorption spectrophotometer. The desorption ratio was calculated from the amount of Pb^{2+} adsorbed on FBLS and the final Pb^{2+} concentration in the desorbing agent.

In order to determine reusability of the biosorbent, the FBLS was reused in five biosorption-desorption cycles. After each cycle of biosorption-desorption, FBLS was recovered by decantation, washed repeatedly with distilled water and reconditioned for biosorption in the succeeding cycle.

Results and Discussion

Immobilization of *A. terreus* on loofa sponge discs. The biomasses of *A. terreus* hypha grown on the loofa sponge discs in 30-day incubation were monitored. Serving as a growth substratum, the porous sponges did have a positive influence on the growth of *A. terreus*. Typical exponential growth curves were observed after the first 5 days, and, probably due to the complete coverage of the sponges by fungal hypha, reached a stationary phase on the seventh day (data not shown).

In order to examine whether the biosorption of FBLS depends on the growth phases of *A. terreus*, the lead uptake by FBLS in different incubation periods (3, 7, 15, 18, 23 and 30 days) were compared. The results show that the Pb^{2+} adsorption, with fixed concentration of 6000 mg l^{-1} of Pb^{2+}, increased as the incubation time of FBLS increased (Fig. 1). The lead uptake capacity increased remarkably when the immobilized *A. terreus* cells were in stationary phase (7^{th} day; Fig. 1). The highest lead uptake was observed on day 15. However, the lead uptake decreased significantly for incubation time longer than 18 days. Based on these observations, we chose 15 days as the optimal incubation period for preparing FBLS in subsequent experiments.

Chemical and physical stability of FBLS. The chemical and physical stability of biosorbents are important in wastewater treatment (Gadd and White, 1993). FBLS was immersed in a pH neutral solution

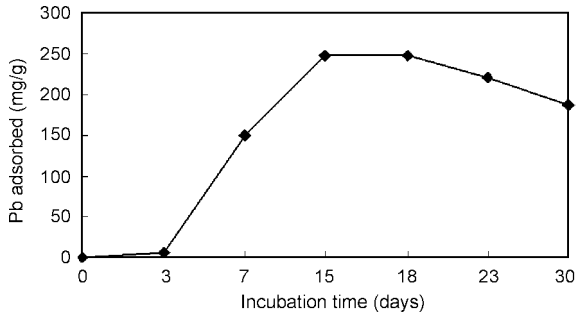


Fig. 1. Effect of growth phase on lead uptake by FBLS.
Biosorption conditions: concentration of Pb^{2+}: 6000 mg l^{-1}, pH 5, temperature: 30°C, FBLS: 100 mg, rotation speed: 200 rpm, contact time: 90 min.

for 20 days on a rotary shaker at different rotation speeds (50–300 rpm) at 30°C. No significant mechanical effect on FBLS was observed (Fig. 2). While immersing the FBLS in buffer solutions of pH ranging from 2.0 to 12.0 for 20 days (at 30°C and 200 rpm), no noticeable corrosive effect on FBLS was observed (Fig. 2).

Effect of contact time on biosorption. The kinetic profiles of metal ions biosorption by FBLS and FFB are shown in Fig. 3. The lead uptake by these biosorbents was found to be extremely rapid, reaching an equilibrium in 20 and 10 min for FBLS and FFB, respectively. After reaching equilibrium, the amount of lead adsorbed did not change significantly over time. The maximum lead uptake capacity of FBLS is superior to that of FFB. This may be due to an increase in accessible binding sites on FBLS. On the other hand, the equilibrium biosorptions of cadmium and mercury ions onto both tested biosorbents were evidently low. The difference in sorption dynamics and capacity for different metals by the biosorbents has been explained in terms of the difference in the ionic size of metals, ionic radii, electrode potential, affinity to the functional groups on the biosorbents, as well as the mode of interaction between the metal ions and the biosorbent (Chandra Sekhar *et al.*, 2004).

Effect of pH on metal ion biosorption. It is well known that the biosorption of heavy metal ions onto fungal biomass is affected by pH (Iqbal and Edyvean, 2005). In fact, metals not only show different pH optima for their sorption, but may also vary from one kind of biosorbent to another. In order to evaluate the effect of pH on the amount of metal uptake by FBLS and FFB, the batch studies of different pH, ranging from 2.0 to 6.0, were conducted. As can be seen from Fig. 4, the pH has a profound influence on metal sorption by both FBLS and FFB. The metal uptake by FBLS and FFB were significantly low at pH 2.0 and then suddenly increased in pH from 2.0 to 3.0. For the lead adsorpted on FBLS, the biosorption plateau was attained at pH ranging from 3.0 to 5.0. This may be due to the large quantities of proton competing with the metal cations for the adsorption sites at pH 2.0 (Huang *et al.*, 1988). As the pH of the solution increases, the number of protons dissociated from the functional groups on the fungal cell wall increases and thus more negative-charge groups for the complexation of metal cations are provided. A similar result is reported (Yan and Viraraghavan, 2003) for the removal of lead using *Penicillium digitatum* and *Rhizopus nigricans*. Removal of lead, copper and zinc by *Phanerochaete chrysosporium* (Iqbal and Edyvean, 2004) also shows the same trend. It is notable that the metal uptake by FBLS has a milder pH-dependent effect than that of FFB (Fig. 4). Because most metal contaminated industrial wastewater is acidic, the pH-resistant property of FBLS may be advantageous for industrial applications.

Effect of temperature on metal ion biosorption. We conducted batch studies at different temperatures, ranging from 10 to 50°C. The results for FBLS and FFB are shown in Figure 5. There is no obvious difference for the amount of metal uptake by FBLS among different temperatures. However, higher metal uptakes at elevated temperatures were observed for FFB. This might be due to the increases in the binding sites by disaggregating the fungal pellets at higher

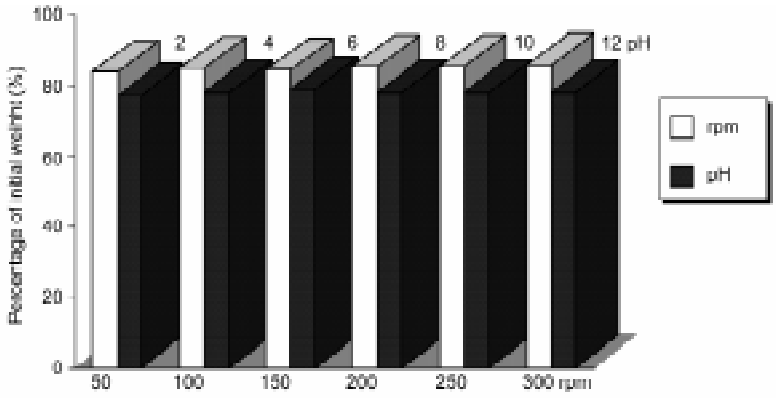


Fig. 2. Physical and chemical stability of FBLS

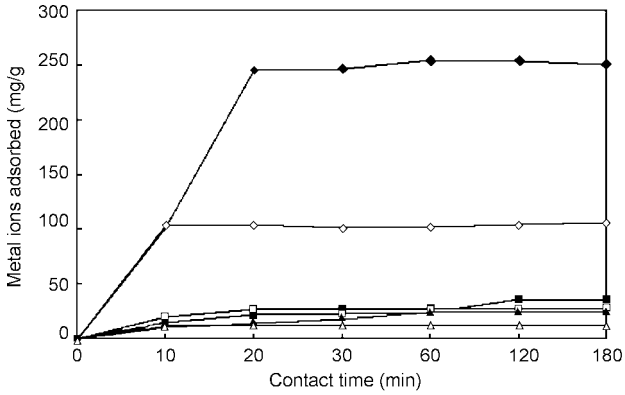


Fig. 3. Biosorption kinetics of metal ions by FBLS and FFB; 100 ml single metal solutions (Pb^{2+} = 6000 mg l^{-1}, Hg^{2+} = 800 mg l^{-1}, Cd^{2+} = 300 mg l^{-1}) were contacted with 100 mg of FBLS or FFB. Biosorption conditions: pH 5, temperature: 30°C, rotation speed: 200 rpm. (FBLS: Pb(◆), Hg(■), Cd(▲); FFB: Pb(◇), Hg(□), Cd(Δ).

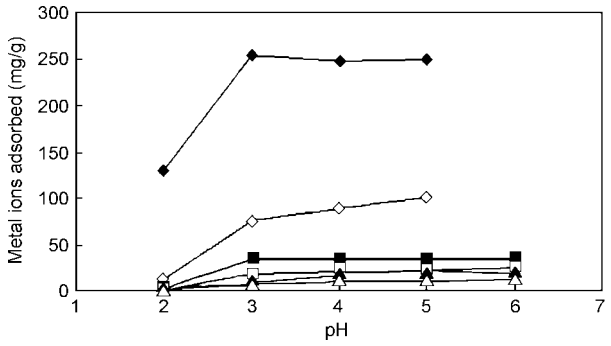


Fig. 4. Effect of pH on metal ions biosorption by FBLS and FFB; 100 ml single metal solutions (Pb^{2+}=6000 mg l^{-1}, Hg^{2+}=800 mg l^{-1}, Cd^{2+}=300 mg l^{-1}) were contacted with 100 mg of FBLS or FFB. Biosorption conditions: temperature: 30°C, rotation speed: 200 rpm, contact time: 90 min. (FBLS: Pb(◆), Hg(■), Cd(▲); FFB: Pb(◇), Hg(□), Cd(Δ).

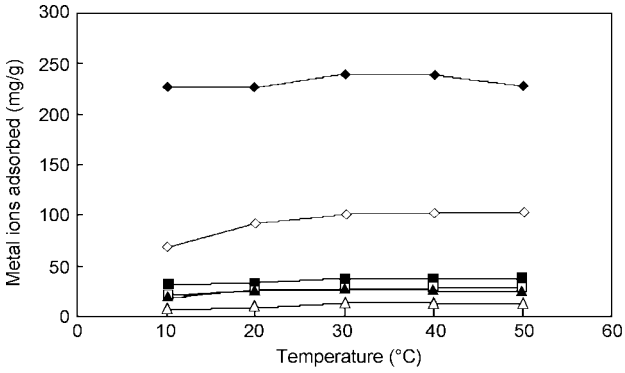


Fig. 5. The effect of temperature on metal ions biosorption by FBLS and FFB. 100 ml single metal solutions (Pb^{2+} = 6000 mg l^{-1}, Hg^{2+} = 800 mg l^{-1}, Cd^{2+} = 300 mg l^{-1}) were contacted with 100 mg of FBLS or FFB. Biosorption conditions: pH 5, rotation speed: 200 rpm, contact time: 90 min. (FBLS: Pb(◆), Hg(■), Cd(▲); FFB: Pb(◇), Hg(□), Cd(Δ).

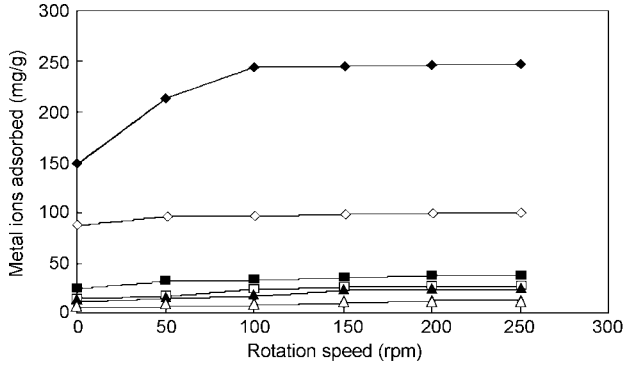


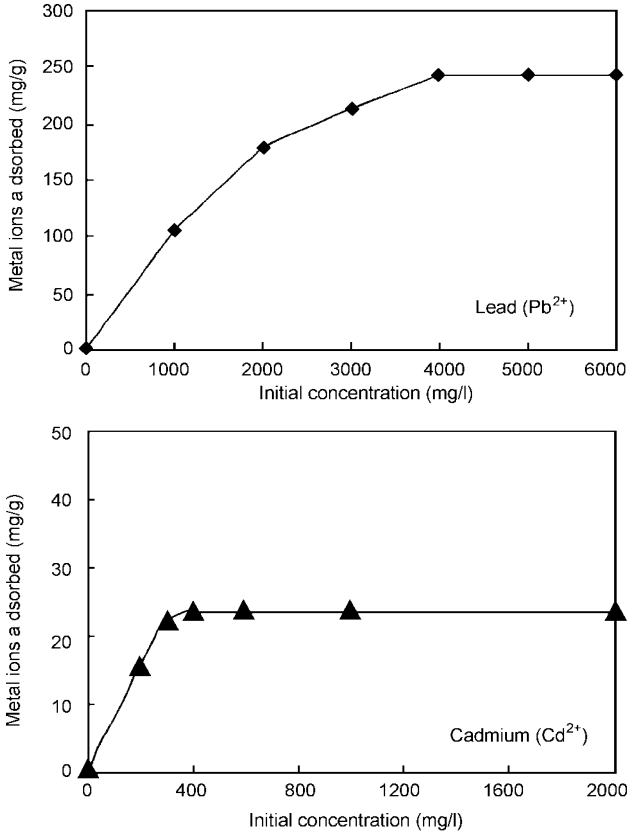
Fig. 6. The effect of rotation speed on metal ions biosorption by FBLS and FFB. 100 ml single metal solutions (Pb^{2+} = 6000 mg l^{-1}, Hg^{2+} = 800 mg l^{-1}, Cd^{2+} = 300 mg l^{-1}) were contacted with 100 mg of FBLS or FFB. Biosorption conditions: pH 5, temperature: 30°C, contact time: 90 min. (FBLS: Pb(◆), Hg(■), Cd(▲); FFB: Pb(◇), Hg(□), Cd(Δ).

temperatures (Iqbal and Edyvean, 2004). The thermal stability of FBLS makes it more suitable than FFB for industrial applications.

Effect of rotation speed on metal ion biosorption. We conducted batch studies of different rotation speeds, ranging from 0 to 250 rpm. The results are shown in Figure 6. Metal uptake by both FBLS and FFB increased with increasing rotation speed, especially in the case of lead adsorption by FBLS. Furthermore, FBLS with a stereo matrix shows a stronger affect by rotation speed, in comparison with FFB without the physical structure. The enhanced sorption ability at higher rotation speeds is probably due to an increase in the mobility of the sorbing species (Bulut and Baysal, 2006).

Effect of initial metal ion concentration. Heavy metal ion biosorption capacities of FBLS are presented as a function of the initial concentration of Pb^{2+}, Hg^{2+} and Cd^{2+} within the aqueous solution in Fig. 7. These experiments were performed for 90 min using single metal solutions (Pb^{2+}: 1000–6000 mg l^{-1}, Hg^{2+}: 200–2000 mg l^{-1}, Cd^{2+}: 200–2000 mg l^{-1}) at pH 5.0. Figure 7 shows that the metal ion biosorption capacity of FBLS increased with the increase in initial concentration of metal ions. This trend was also observed for the removal of lead and copper by *Phanerochaete chrysosporium* (Iqbal and Edyvean, 2004). It is worth mentioning that the isotherms for all three metals were steep at lower concentrations, indicating the suitability of FBLS for treatment of dilute solutions.

Adsorption isotherms. In order to determine the biosorption isotherm model of FBLS, we examined the most widely used isotherm models: Langmuir and Freundlich. The Langmuir isotherm assumes a monolayer adsorption while the Freundlich isotherm is an empirical equation based on sorption at a heterogeneous surface. Our results suggest that the Langmuir model is better than the Freundlich model



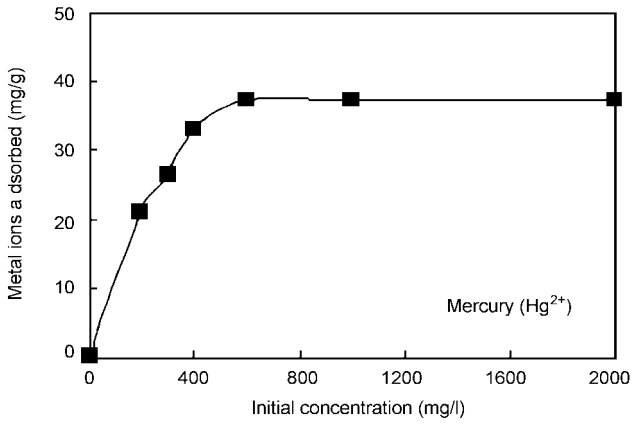


Fig. 7. The effect of initial metal concentration on metal ions biosorption by FBLS; 100 ml single metal solutions (1000–6000 mg l^{-1} for Pb^{2+}, 200–2000 mg l^{-1} for Hg^{2+} and 200–2000 mg l^{-1} for Cd^{2+}, pH 5.0) were contacted with 100 mg of FBLS for 90 min at 30°C, 200 rpm. (Pb(◆), Hg(■), Cd(▲).

in describing the experimental data. Model parameters for Pb^{2+}, Hg^{2+} and Cd^{2+} were further calculated (Table I). The excellent correlation coefficients ($r^2 > 0.99$) for all the three metal ions tested clearly indicate that the Langmuir isotherm model is suitable for describing the biosorption isotherms of FBLS in the studied concentration ranges. Higher q_{max} values for Pb^{2+}, as compared to Hg^{2+} and Cd^{2+} confirm the stronger bonding affinity of FBLS to Pb^{2+} than that of Hg^{2+} and Cd^{2+}.

Table I
Parameters of Langmuir and Freundlich biosorption model of FBLS

Metal ions	Langmuir model			Freundlich model		
	q_{max}(mg/g)	K_L(l/mg)	R^2	K_F	n	R^2
Pb^{2+}	253.16	0.01	0.999	57.68	0.18	0.724
Hg^{2+}	41.26	0.02	0.997	7.75	0.26	0.806
Cd^{2+}	25.72	0.07	0.999	6.69	0.24	0.853

Biosorption studies. We conducted biosorption tests for 90 min using single-metal solutions (Pb^{2+} = 6000 mg l^{-1}, Hg^{2+} = 800 mg l^{-1}, Cd^{2+} = 300 mg l^{-1}) at pH 5.0. Table II demonstrates that the metal uptake capacity by FBLS is better than both FFB and a naked loofa sponge. Moreover, the selectivity by the biosorbent was in the order of $Pb^{2+} > Hg^{2+} > Cd^{2+}$. One can conclude that the binding sites for metal ions are located on the *A. terreus* cell walls and immobilization does not alter the binding sites and binding ability of FBLS. Lower adsorption rates of metal uptake by FFB may be attributed to aggregated fungal hypha biomass, thus reducing their exposure surface area for sorption. Higher metal uptake by FBLS is due to hypha immobilization along the surface of the fibrous thread, no aggregate formation, and the open network of the loofa sponge. These all enhance the surface area and free access of metal ions to the sorption sites (Akhtar *et al.*, 2004). Increase in metal uptake by FBLS further indicates no limitations in the movement of metal ions or the masking of active sites by the loofa matrix, unlike other reported systems such as immobilization of a mixture of microorganisms from activated sludge on hydrogels where significant decreases in the rate of metal sorption occur (Gourdon *et al.*, 1990).

Biosorption in mixed-metal system. We tested the biosorption of metal ions by both FBLS and FFB

Table II
Biosorption capacity of biosorbent for Pb^{2+}, Hg^{2+} and Cd^{2+}; experiments were conducted in 100 ml single metal solution (Pb^{2+} = 6000 mg l^{-1}, Hg^{2+} = 800 mg l^{-1}, Cd^{2+} = 300 mg l^{-1}) at optimal condition (pH 5.0, temperature: 30°C, rotation speed: 200 rpm, amount of biosorbent: 100 mg)

Biosorbents	Metal adsorbed (mg/g biosorbents)		
	Pb^{2+}	Hg^{2+}	Cd^{2+}
FBLS	247.23	37.68	23.82
FFB	106.6	26.6	12.85
Loofa sponge	13.125	6.77	4.97

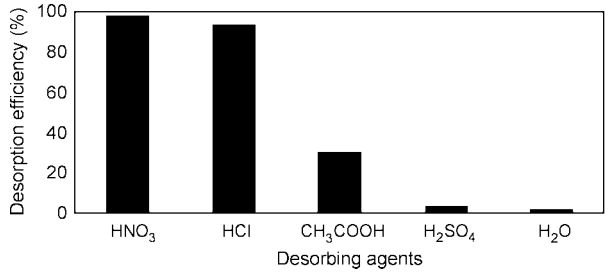


Fig. 8. Desorption efficiency of Pb^{2+} from FBLS by various desorbing agents.

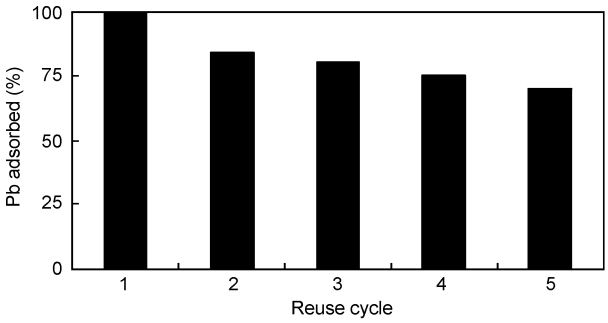


Fig. 10. Biosorption of Pb^{2+} by FBLS in five consecutive adsorption-desorption cycles.

in a mixed-metal system to determine the synergistic/antagonistic effect of cations present in the effluent metal solution. We tested metal solutions containing either one metal alone (6000 mg l^{-1}) or mixed with one another (3000 mg l^{-1} per metal) or two other metals (2000 mg l^{-1} per metal). Table III shows the metal removal percentage obtained with FBLS when mixed-metal solutions were used. The presence of other cations reduced the sorption capacity of Pb^{2+}, Hg^{2+} and Cd^{2+}. The milder influence of Hg^{2+} and Cd^{2+} on Pb^{2+} biosorption could be due to the greater atomic weight, electronegativity, and atom radius of these two metals

Table III
Effect of other ions on biosorption of Pb^{2+}, Hg^{2+} and Cd^{2+} by FBLS; 100 ml metal solutions containing either one metal alone (6000 mg l^{-1}) or mixed with one other (3000 mg l^{-1} per metal) or two others (2000 mg l^{-1} per metal) were contacted with 100 mg of FBLS for 90 min at 30°C, 200 rpm

Interfering ions	%[a] metal recovered on FBLS when the interfering ions were present		
	Pb	Hg	Cd
None	100	100	100
Pb	–	30	13
Hg	99	–	49
Cd	98	62	–
Pb + Hg	–	–	30
Pb + Cd	–	57	–
Hg + Cd	97.5	–	–

[a] Mean of 3 determinations

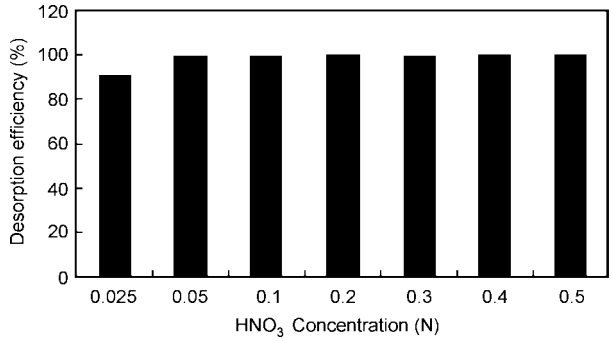


Fig. 9. Effect of HNO_3 concentrations on the desorption efficiency of Pb^{2+} from FBLS.

(Tsekova and Petrov, 2002). When all the three metals were presented, FBLS adsorbed the metal ions in the following order: $Pb^{2+}>Hg^{2+}>Cd^{2+}$. Our study shows that *A. terreus* greatly favors Pb^{2+} adsorption.

Desorption and regeneration. We conducted desorption tests to determine the suitable eluant and its applicable concentration. We tested five desorbing agents, 0.1 N sulfuric acid, 0.1 N acetic acid, 0.1 N hydrochloric acid, 0.1 N nitric acid and deionized water. The portions of Pb^{2+} desorbed from the metal-saturated FBLS by the various desorbing agents are shown in Fig. 8. It is clear that nitric acid has a better desorption efficiency than the others. Therefore, we selected it as the desirable eluant for the studies. We conducted other desorption experiments to determine optimum concentrations for the desorbing agent HNO_3. For practical applications, the concentration of acid solution used for the recovery of metals should be as low as possible. Biomass loaded with Pb^{2+} was contacted with HNO_3 solutions of different concentrations for 60 min. We determined the amount of Pb^{2+} released back into the HNO_3 solution and expressed it as desorption efficiency. The results show that increasing the concentrations of HNO_3 increased the desorption efficiency (Fig. 9). Nitric acid with the concentration of 0.05 N or higher can remove more than 99.0% of adsorbed Pb^{2+}. Therefore, we used 0.05 N nitric acid as the eluant in the subsequent studies.

The purpose of regenerating the biosorbent is to cut down operating costs. We examined the feasibility of regenerating metal-adsorbed FBLS by 0.05 N nitric acid by repeating the adsorption-desorption process for five consecutive cycles. Results demonstrate that FBLS retained its metal adsorption capacity after five regenerating cycles (Fig. 10). The deterioration in sorption capacity of FBLS for Pb^{2+} after five cycles remained at 78%, which suggests that FBLS has the potential to adsorb Pb^{2+} ions repeatedly from aqueous solution. The deterioration effect on metal adsorption may be due to the physical damage of the FBLS caused by the desorbing agent used in the regeneration cycles.

To evaluate the potential of FBLS for the removal of Pb^{2+} from a real industrial wastewater, we placed the FBLS into a flask containing the industrial effluent (Pb^{2+}: 236.8–255.8 mg l^{-1}). The Pb^{2+} biosorption capacity of the FBLS in the batch experiment (contact at pH 5.0, 200 rpm and 30°C for 90 min) was 183.6 mg Pb^{2+}/g FBLS. The results show that FBLS has potential for commercial applications.

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