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ABILITY OF LOOFA SPONGE-IMMOBILIZED FUNGAL BIOMASS TO REMOVE LEAD IONS FROM AQUEOUS SOLUTION

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Abstract

A new biosorbent was developed by immobilizing a white rot basidiomycete *Phanerochaete chrysosporium* within low cost and easily available matrix of loofa sponge. Fungal biomass immobilized on loofa sponge (FBILS) adsorbed Pb(II) very efficiently from aqueous solution and biosorption equilibrium was established in about 1 h. No loss to biosorption capacity of FBILS was found due to the presence of loofa sponge, indeed as compared to free fungal biomass (FFB) an increase of 24.27% was noted in the biosorption capacity of FBILS. Maximum biosorption capacities for FBILS and FFB were found as 136.75 and 110.04 mg Pb(II) g^{-1} biomass, respectively. Pb(II)-laden FBILS was regenerated using 50 mM HCl, with up to 99% recovery and reused in seven biosorption-desorption cycles without any significant loss in biosorption capacity. FBILS were found to very strong, both physically and chemically, and can resist a wide variation in pH, temperature and agitation without any visible change in shape, structure or texture. This study for the first time reports that FBILS have a high biosorption capacity to Pb(II) and can be used as an effective biosorbent for the removal of Pb(II) or other heavy metals from industrial wastewater.

Introduction

Release of heavy metals in the environment has received increased attention because of the threat to public health. The most commonly used technologies for heavy metal removal include chemical precipitation, ion exchange, activated carbon adsorption and membrane separation processes. Although these procedures tend to be efficient, they are generally expensive and require frequent service attention. Therefore, the need for economical, effective and safe methods for metal removal has resulted in the search for alternative technologies.

Biosorption by microbial biomass has emerged as a potential option for heavy metal removal (Kratochvil & Volesky, 1998). Commercial application of microbial biomass as a biosorbent, however, has been hindered by problems associated with physical characteristics of these materials such as small particle size with low density, poor mechanical strength and rigidity (McHale & McHale, 1994). Low mechanical strength of the biomass can cause difficulties in separation of the biomass from effluents which, in turn, contribute to limitations in process design. A further problem is associated with fragmentation of the biomass causing flow restrictions in continuous-flow contact vessels. Immobilization technologies may overcome many of these problems (Aloysius *et al.*, 1999; Iqbal & Edyvean, 2005).

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Several immobilization media, such as alginates, carrageenans and polacrylamide gel have been used for this purpose (Leenen *et al*., 1996). Immobilization based on these polymeric matrices, however, result in restrictive diffusion due to closed embedding structures with low mechanical strength (Hu & Reeves, 1997). The purpose of the present study, therefore, is to provide a new immobilized fungal biosorption process using a low cost, physically strong, ridged and highly porous immobilization matrix; loofa sponge. While it has been previously suggested as an immobilization matrix for plant, algal, bacterial and yeast cells (Iqbal & Zafar, 1993a,b; Iqbal & Zafar, 1994), the use of loofa sponge to immobilize fungal biomass for metal biosorption has not been reported. Thus, the objective of this study was to investigate the use of fungal biomass immobilized on loofa sponge (FBILS) as biosorbent for the removal of heavy metals using *Phanerochaete chrysosporium* and Pb(II) as the model organism and metal ion, respectively.

Materials Methods

Microorganism and culture medium: White-rot basidiomycete, *Phanerochaete chrysosporium* (ATTC 24725) was maintained by subculturing on potato dextrose agar slants. Spore suspensions for immobilization were prepared from 7-day old cultures, grown on potato dextrose agar slants at 30 ± 2°C. The growth medium consisted of D-glucose, 10.0; KH_2PO_4, 2.0; $MgSO_4.7H_2O$, 0.5; NH_4Cl, 0.1; $CaCl_2.H_2O$, 0.1; thiamine, 0.001 (g l^{-1} of distilled water).

Immobilizing material and preparation of FBILS: The loofa sponges were obtained from matured dried fruit of *Luffa cylindrica*. The sponge was cut into pieces of approximately 2.0-2.5x1.5-1.7 cm, 2-3 mm thick, soaked in boiling water for 30 min, thoroughly washed under tap water, and left for 24 h in distilled water, changed 3-4 times. The sponge discs were oven dried at 70° C and stored in desiccators till their further use.

The immobilization of *P. chrysosporium* within sponge discs was carried out as follows: The spore suspension (0.5 ml) was inoculated into 250 ml Erlenmeyer flasks containing 100 ml of the growth medium and four pre-weighed loofa sponge discs as an immobilizing matrix. Culture flasks for free hyphal growth, with no loofa sponge discs in the medium, served as the controls. The inoculated flasks were incubated at 35°C and shaken at 100 rpm. After 8 days of incubation, both free and loofa immobilized biomass of *P. chrysosporium* (FBILS) were harvested from the medium, washed twice with distilled water and stored at 4°C until use. The dry weight of the fungal biomass entrapped within sponge discs was determined by weighing, after drying in an oven at 70°C overnight, the sponge discs before and after fungal growth.

Biosorption studies: The biosorption of Pb(II) on FBILS-discs from aqueous solutions was carried out in batch biosorption-equilibrium experiments. Desired concentrations of Pb(II) solution were prepared by diluting standard lead(II) stock solution ($Pb(NO_3)_2$, Merck) of concentration 1,000±2 mg l^{-1}. pH of the solution was adjusted to 5.0, unless otherwise stated using 0.1 M NaOH. Fresh dilutions were used for each biosorption study. The biosorption capacity of FBILS and FFB (100 mg) was determined by contacting 100 ml Pb(II) solution of known concentration in 250 ml flasks. The Pb(II) solution, incubated with biosorbent, was shaken on an orbital shaker at 100 rpm in tightly stopper flasks at room temperature (20 ± 2°C). FFB was removed from metal solution by

centrifugation at 5,000 rpm for 5 min., whereas FBILS were separated from the solution by simple decantation. Residual concentration of Pb (II) in the metal supernatant solutions was determined using atomic absorption spectrophotometer (UNICAM-969). Metal-free solution and fungal hyphal biomass-free metal solution containing only loofa sponge discs blanks were used as controls.

Desorption and reuse: In order to determine the reusability of the FBILS, consecutive adsorption-desorption cycles were repeated seven times by using the same biosorbent. Desorption of Pb(II) was performed by 50 mM HCl solution. FBILS and FFB loaded with Pb(II) ions were contacted with desorption medium at room temperature and agitated on orbital shaker at 100 rpm for 60 min. The biosorbent was removed and the supernatant was analysed for Pb(II) released into the solution by atomic absorption spectrophotometer.

Reproducibility and data analysis: Unless indicated, the data shown are the mean values from three separate experiments. Statistical analysis of the data was carried out using the Duncan's new multiple range test (Steel & Torrie, 1996).

Results and Discussion

Preparation of FBILS biosorbent: For producing FBILS biosorbent, *P. chrysosporium* were grown in the nutrient medium containing loofa sponge discs under batch cultural conditions for 8 d. The growth pattern of *P. chrysosporium* hyphae within the sponge discs, expressed as a function of biomass production (Fig.1). *P. chrysosporium* grown in nutrient medium without loofa sponge discs was used as control. Immobilization has a positive influence on the growth of *P. chrysosporium* as evident from 19.2% increase in biomass production by the immobilized as compared with FFB over a period of 8 d. No free hypal growth was observed in the culture flasks containing the immobilized system indicating no hyphal fragment release from the sponge discs. Rather, a single compact block of fungal mass developed (Fig. 2). Visual and microscopic examination of the loofa sponge discs revealed hyphal growth within the sponge matrix within 24 h of incubation. Complete coverage of the sponge disc with the hyphae of *P. chrysosporium* occurred within 5 d. However, biomass accumulation/loading was noted to continue until the attainment of stationary phase at day 7.

Stability of FBILS: The chemical and physical stability of FBILS are important considerations in determining their operational life for waste water treatment. Tests were therefore conducted to determine FBILS performance over repeated adsorption-desorption cycles in fixed bed column bioreactor. After 7 Pb(II) absorption-desorption cycles, no physical deterioration or change in shape and size were observed. The weight loss of immobilized biomass was ~ 1%. FBILS-discs were found to be very stable over the pH range 1-13 with no significant release of fungal biomass in 48 h exposure trials. In contrast, alginate beads, the most widely used polymeric matrix for microorganisms for metal biosorption, have been reported to be stable only at pH 6-9 (Hu & Reeves, 1997). Thus, the FBILS will perform better than hydrogel biosorbents in terms of reusability where a large amount of cell leakage has been reported during biosorption processing (Khoo & Ting, 2001).

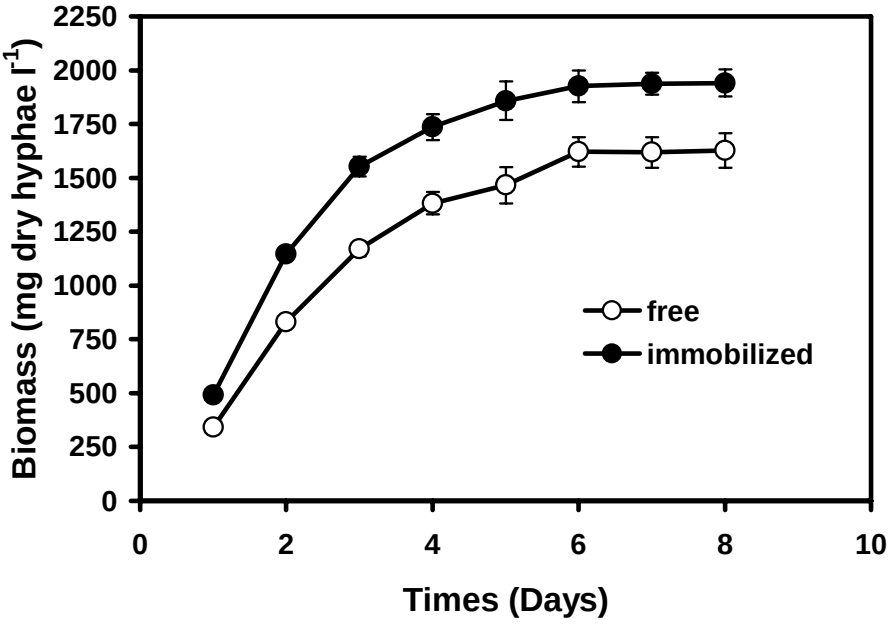

Fig. 1. Growth of *P. chrysosporium* immobilized in loofa sponge and in suspension cultures.

Fig. 2. Immobilization of *P. chrysosporium* within loofa sponge pieces: (a) loofa sponge pieces; (b) loofa sponge pieces covered with *P. chrysosporium* hyphal biomass.

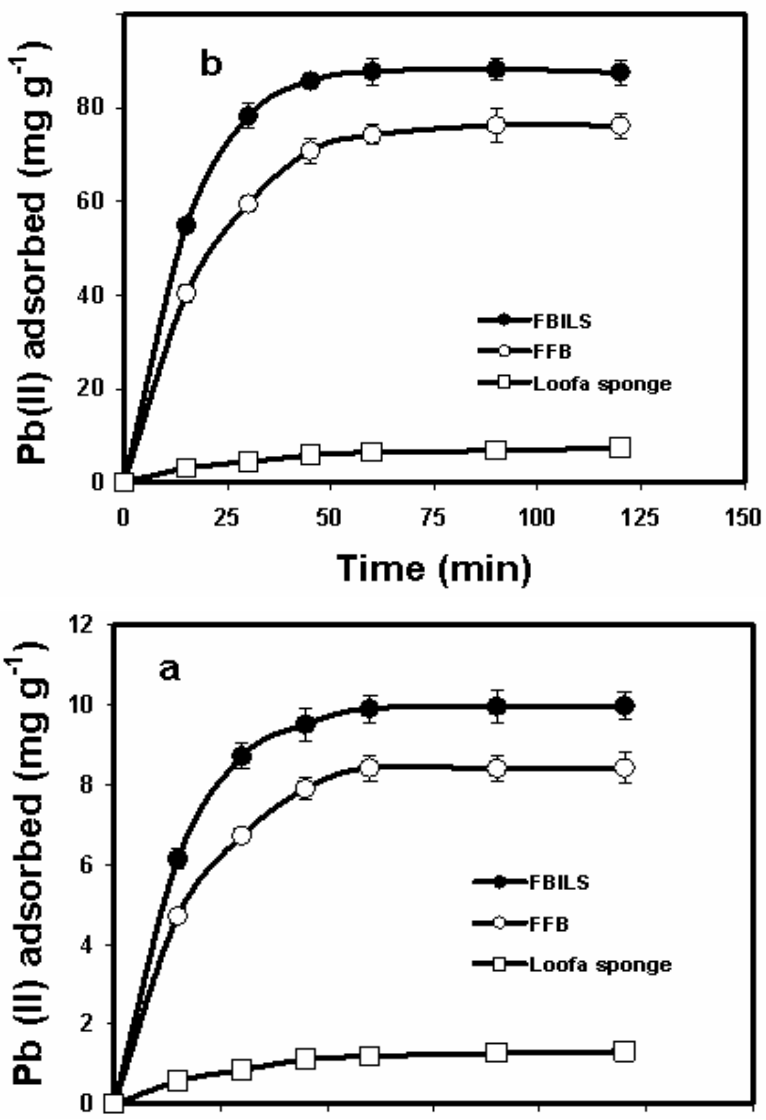

Fig. 3. Biosorption of Pb(II) from (a) 10 mg l^{-1} and (b) 100 mg l^{-1} solutions, pH 5.0, by 1 g l^{-1} of *P. chrysosporium* free (FFB) or immobilized in loofa sponge (FBILS) as related to the time of contact during orbital shaking at 100 rpm at 25 ^{o}C.

Biosorption studies: To demonstrate the metal binding capacity of FBILS as a new biosorption system, the FBILS were exposed to aqueous metal solution having concentration of 10 and 100 mg l^{-1} of Pb(II) in batch experiments. FBILS removed the Pb(II) very efficiently (Fig. 3), with uptake level of about 9.94 and 86.27 mg g^{-1} biomass, respectively while FFB exhibited uptake of about 8.29 and 70.68 mg g^{-1} biomass, respectively. From figures 3a & b it is evident that loofa sponge discs without fungal

biomass adsorbed the Pb(II) far less than that of either FFB or FBILS, suggesting the role of immobilized fungal biomass within the discs in the biosorption of Pb(II) from the solution. Adsorption isotherms (Fig. 4) also demonstrated that Pb(II) was adsorbed to higher levels by FBILS than by FFB, with a maximum uptake level of 136.75 mg g^{-1} and 110.04mg g^{-1} for FBILS-disc biosorbent and FFB, respectively.

The statistically significant lower uptake of Pb(II) by FFB may be attributed to their aggregation, in the form of pellet, due to electrostatic interaction, thus reducing the surface area for sorption. Metal sorption efficiency of free mass of yeast cells and fungal hyphae has been reported to decrease with a reduction in distance between the hyphae or cells, resulting in intracellular linkages between their reactive groups (de Rome & Gadd, 1987; Trujillo *et al.*, 1995). The structural microbarrier so created limits accessibility of the metal to the binding sites for adsorption through reduced diffusion (Plette *et al*., 1996).

In comparison with FFB, the higher rate of Pb(II) removal by FBILS further indicates that no diffusional limitations were presented as noted with immobilization of a mixture of organisms from activated sludge in hydrogels in which case significant decrease in the rate of metal sorption occurred (Gourdon *et al*., 1990). In another study, 40% reduction in the sorption of Pb (II), in comparison with free cells was noted when *Stichococcus bacillaris* was immobilized on silica gel (Mahan & Holcombe, 1992). Lopez *et al*., also noted about 60% decrease in the rate of metal sorption by *Pseudomonas fluorescens* cells immobilized in agar bead, as compared with free cells. Immobilization of *P. chrysosporium* within the loofa sponge provides better contact of biomass, through micro-channels present between the hyphal network, to metal ions in aqueous solution. It is therefore better suited for biosorption than enclosed or beaded immobilization in polymeric gel structures.

The regeneration of the biosorbent is likely to be a key factor in accessing the potential of the biosorbent for commercial application. The capacity of the FBILS to adsorb Pb(II) was therefore, determined by repeating the adsorption experiments in seven consecutive cycles. HCl (50 mM) solution was used as a desorption agent. Higher than 98% desorptions were obtained after seven adsorption-desorption cycles (Fig. 5). The FBILS-discs undergoing successive adsorption-desorption processes retained good metal adsorption capacity even after seven cycles. The total decrease in sorption efficiency of FBILS biosorbent after the seven cycles was only about 4.49 which show that FBILS biosorbent has good potential to adsorb metal ions from aqueous solution and can be used repeatedly.

Conclusions

FBILS is shown to have an excellent biosorption capacity for the removal of Pb(II) ions from aqueous solution. The recovery of adsorbed Pb(II) from the FBILS was also excellent (<98.7%). The results demonstrate that loofa sponge is an effective immobilization carrier for the entrapment of fungal hyphae to produce the FBILS biosorbent. Biosorption studies on this newly developed system show that FBILS have a promising potential for practical applications to remove heavy metals from industrial effluents. High metal loading capacity, good mechanical strength, ease of handling, high porosity, regeneration ability and low cost availability of the immobilization matrix are the key features of this biosorbent.

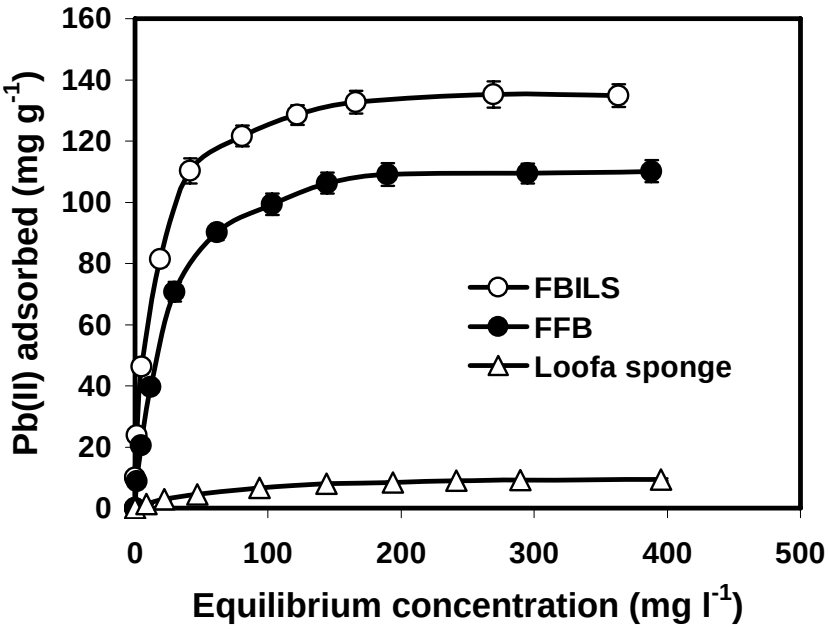

Fig. 4. Biosorption isotherm of *P. chrysosporium* biomass free (FFB) and immobilized within loofa sponge (FBILS) and plain loofa sponge pieces for Pb(II) ions; 100 ml of Pb(II) solution (10-500 mg l^{-1}, pH 5.0) was mixed with each biosorbents at 100 rpm and 25 ^{o}C for 60 min.

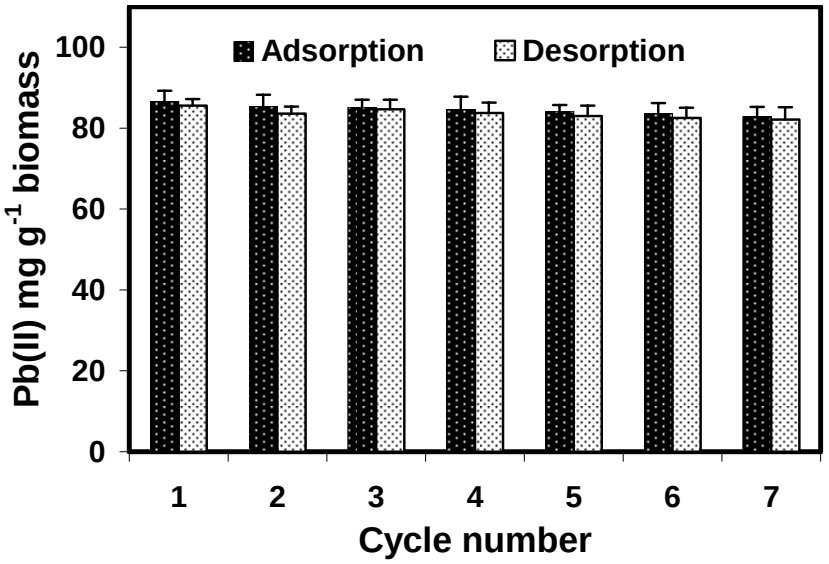

Fig. 5. Biosorption-desorption of Pb(II) by FBILS biosorbent in seven consecutive cycles.

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