Biotechnology Letters (2005) 27: 1319–1323
DOI 10.1007/s10529-005-0477-y

Production of fungal biomass immobilized loofa sponge (FBILS)-discs for the removal of heavy metal ions and chlorinated compounds from aqueous solution

M. Iqbal[1], A. Saeed[1], R.G.J. Edyvean[2], B. O'Sullivan[2] & P. Styring[2]
[1]*Environment Biotechnology Group, Biotechnology and Food Research Centre, PCSIR Laboratories Complex, 54600, Lahore, Pakistan*
[2]*Department of Chemical and Process Engineering, University of Sheffield, S1 3JD, Sheffield, UK*

Received 10 June 2005; Revisions requested 10 June 2005; Revisions received 23 June 2005; Accepted 28 June 2005

Key words: cadmium, 4-chloroanisole, fungal immobilization, loofa sponge, metal biosorption *Phanerochaete chrysosporium*

Abstract

A white rot basidiomycete, *Phanerochaete chrysosporium*, was immobilized on loofa sponge (FBILS) discs. It removed ca. 37 and 71 mg Cd (II) g^{-1} from 50 and 200 mg l^{-1} aqueous solutions and up to 89% of 4-chloroanisole from a 10 mg l^{-1} aqueous solution. FBILS are physically strong and chemically recalcitrant, resisting temperature, mechanical agitation, and variations in pH without alteration to shape, structure or texture.

Introduction

Pollution in water supplies and in waste-water discharge is a cause of increasing legislative and public concern. In many countries, industry is required to meet ever higher quality standards and has a need for improved, and environmentally sound, waste treatment methodologies for the removal of toxic chemicals from effluents. The use of biomass as biosorbents for pollutants offers an environmentally sound and potentially low cost alternative to existing technologies. Fungal biomasses have high affinities for toxic metals (Kratochvil & Volesky 1998) and organic chemicals (Perez *et al.* 1997, Reddy *et al.* 1998) in aqueous solution. Commercial application of such biomass has been hindered by problems associated mainly with physical manipulation (McHale & McHale 1994). Low mechanical strength and fragmentation of the biomass can cause difficulties in the contacting and separation of the effluent and biomass and this limits process design.

Immobilization technologies have been suggested to overcome these problems (Trujillo *et al.* 1995, Aloysius *et al.* 1999). Immobilization of microbial biomass in polymeric gel matrices is the most extensively studied method (Leenen *et al.* 1996, Arica *et al.* 2001). However, production of large amounts of gel beads needed for commercial applications is expensive and requires specialist equipment. Furthermore, the use of such polymeric matrices results in closed structures with restrictive diffusion and low mechanical strength (Hu & Reeves 1997).

The ideal immobilization matrix is strong and resistant and has an open structure. The plant-derived Loofa sponge is an inexpensive and easily available biological, and therefore renewable, matrix produced in most tropical and subtropical countries. The sponge is made up of interconnecting voids with an open network of fibrous support giving the potential for rapid contact of immobilized cells to the surrounding aqueous medium. Merits of the loofa biomatrix system include freedom from materials that might be toxic

to microbial cells, simple application and operation technique, and high stability during long-term repeated use.

The white rot basidiomycete, *Phanerochaete chrysosporium*, was chosen for this study as it has a known affinity for metal ions but this is the first report on the immobilization of *P. chrysosporium* on a biomatrix for the bioremediation of both inorganic and organic pollutants from aqueous solution. While the mechanisms for inorganic and organic removal are likely to be different, the inclusion of cadmium sorption experiments ensures continuity with previous work on biosorbents and to test the hypothesis that this form of immobilization does not affect metal uptake, and therefore surface reactivity of the fungal hyphae.

Materials and methods

Microorganism and culture medium

The white-rot basidiomycete, *Phanerochaete chrysosporium* ATTC 24725, was grown on (g l^{-1} distilled water); D-glucose, 10; KH_2PO_4, 2; $MgSO_4 \cdot 7H_2O$, 0.5; NH_4Cl, 0.1; $CaCl_2 \cdot H_2O$, 0.1; thiamine, 0.001; at pH 4.5.

Immobilizing materials and production of FBILS-discs

Loofa sponge for use as an immobilization matrix was obtained from the ripened dried fruit of *Luffa cylindrica*. The loofa was cut into discs of approximately 2.5 cm diam. and 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 discs were then oven dried at 70 °C and stored in a desiccator.

A mycelium suspension of *P. chrysosporium*, 0.5 ml, was inoculated in 100 ml of autoclaved growth medium containing four pre-weighed loofa sponge discs in 250 ml Erlenmeyer flasks. Flasks, with no loofa sponge discs in the medium, were inoculated to provide free fungal biomass controls. The inoculated flasks were shaken at 100 rpm at 34 °C. After 8 days, both free and loofa immobilized biomass of *P. chrysosporium* (hereafter called FBILS – Fungal biomass immobilized loofa sponge) were harvested from the medium, washed twice with distilled water and stored at 4 °C until use. The dry weight of the fungal biomass was determined by weighing oven dried (70 °C overnight) sponge discs before and after fungal growth.

Results and discussion

Properties of loofa sponge

The successful use of immobilized biosorbents requires that the immobilization matrix provides a high surface contact area and is stable to adverse chemical and physical treatments. Neither autoclaving (10 times for 20 min), nor pH (2.0–12 for 24 days) produced any change in the shape and structure of the sponge. Table 1 shows the lowest and highest values of physical parameters of loofa sponge discs. These results indicate that loofa sponge discs can be repeatedly reused in adverse conditions.

Production and properties of FBILS biosorbent

Microscopic examination shows hyphal growth in the sponge matrix within 24 h of incubation. Complete coverage of the sponge disc with the hyphae of *P. chrysosporium* occurs within 5 days (Figure 1a–c) and growth continues until the attainment of stationary phase at day 7. While the immobilized hyphal biomass is packed tightly within the sponge there remain large numbers of micro-channels for free movement of solute during the biosorption process (Figure 1c). In contrast, the free hyphal growth was compact and pelleted. At day 8, immobilized *P. chrysosporium* had a 21% increase in biomass over the freely growing control with biomass levels reaching 1900 mg l^{-1}. Such an increase is unusual in immobilized systems.

Table 1. Some physical characteristics of loofa (*Luffa cylindrica*) sponge.

Physical properties	
Structural nature	Fibrous network
Porosity (%)	85–95
Density (g/cm^3)	0.018–0.05
Specific pore volume (cm^3/g)	26–34

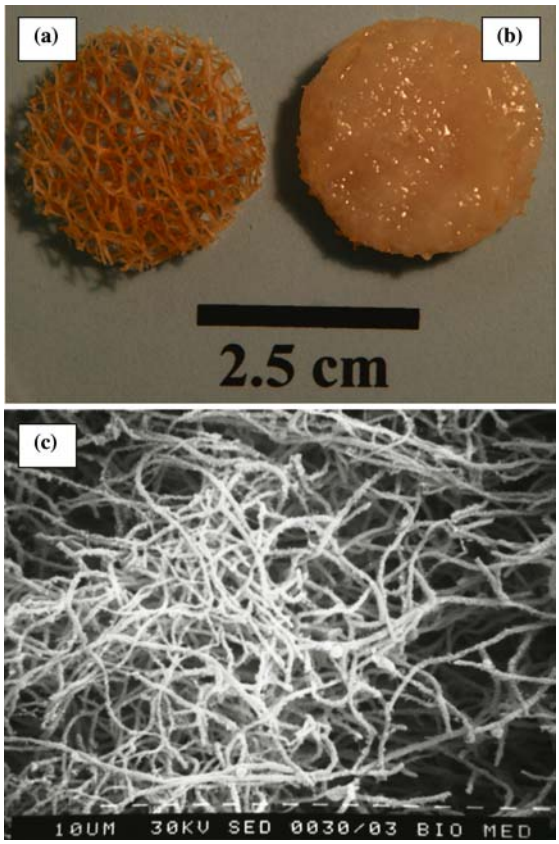


Fig. 1. Immobilization of *Phanerochaete chrysosporium* within loofa sponge discs: (a) loofa sponge disc; (b) loofa sponge disc covered with *P. chrysosporium* hyphal biomass; (c) scanning electron micrographs of immobilized *P. chrysosporium* showing micro-channel and void volume for free solute movement. White scale bars at bottom of micrograph = 10 μm.

No change in the shape, size or weight of FBILS was observed during exposure to various pH in the range 2–12 and were stable when exposed to acid (HCl), alkali (NaOH) and salt (NaCl) solutions for 5 days. FBILS retain 99% of the immobilized biomass within the loofa sponge when shaken for 7 days at 150 rpm in distilled water. In contrast to the results for FBILS, significant cell leakage has been reported to occur during biosorption from systems immobilized using polymer gel systems (Hu & Reeves 1997). These results show that, in contrast to the polymer gel immobilization method which requires more sophisticated equipment involving high costs, the more robust FBILS system can be made simply by adding the microbial cell/hyphal/spore suspensions to a growth medium containing the inexpensive sponge discs without any prior chemical treatment.

Metal removal studies

Figure 2 shows the efficiency of the removal of Cd (II) from solution by FBILS, free fungal biomass and loofa sponge control. While other authors have reported reductions in metal ion

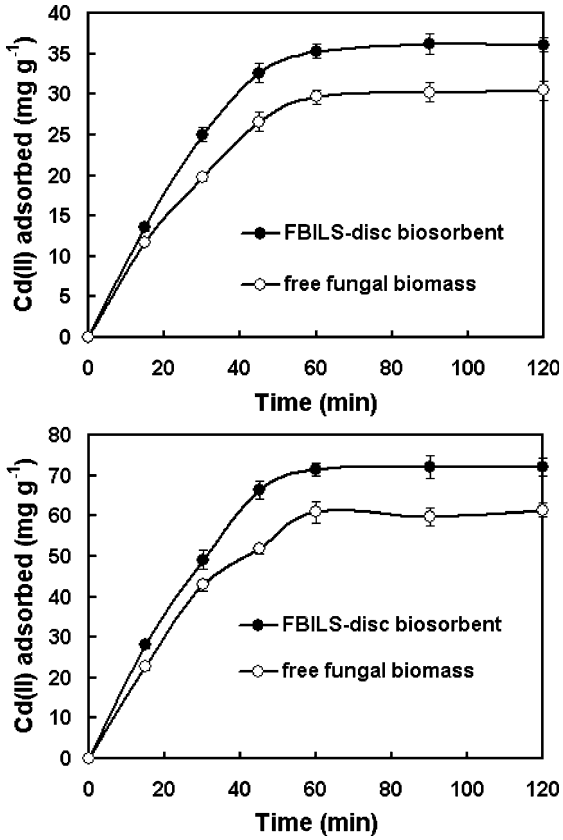


Fig. 2. Biosorption of Cd (II) from (a) 50 mg l^{-1} and (b) 200 mg l^{-1} solutions by free or immobilized *Phanerochaete chrysosporium*. Cd (II) solutions were prepared from $Cd(NO_3)_2$ and adjusted to pH 5 using 0.1 M NaOH. Fresh dilutions were used for each experiment. Hundred milligram of free or immobilized (FBILS-discs) fungal biomass was contacted with 100 ml Cd (II) solution in 250 ml flasks shaken at 100 rpm at 20 ± 2 °C. Free fungal biomass was separated by centrifugation at $3500 \times g$ for 5 min, whereas FBILS-discs were separated by simple decantation. Residual concentrations of Cd (II) in the supernatant were determined using an atomic absorption spectrophotometer. Metal-free and fungal biomass-free solutions were used as controls. Statistical analysis of the data was carried out according to the Duncan's new multiple range test (Steel & Torrie 1996).

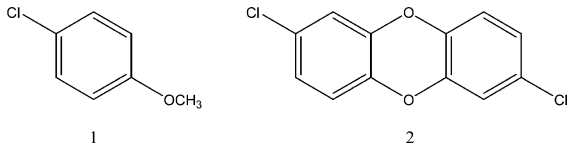
uptake when biomass is immobilized, these results show that this form of immobilization does not affect metal uptake capacity of the fungal hyphae. Mahan and Holcombe (1992) reported a 40% reduction in the sorption of Pb (II) when *Stichococcus bacillaris* was immobilized on silica gel and Lopez *et al.* (2002) report a 60% decrease in metal sorption by *Pseudomonas fluorescens* cells immobilized in agar beads, both in comparison with free cells. The statistically significant lower uptake of Cd (II) by free hyphal biomass found in this work may be due to a reduction in the surface area available for sorption due to hyphal aggregation and pelletization. Such reductions due to aggregation have been found for yeast cells as well as fungal hyphae (de Rome & Gadd 1987, Plette *et al.* 1996, Aloysius *et al.* 1999). The findings presented here indicate no diffusional limitations and demonstrate that FBILS are better suited for biosorption and other reactions than either free hyphal biomass or polymeric gel immobilization. From 50 mg l^{-1} metal solution uptake reached 36.8 ± 0.7 mg g^{-1} fungal biomass for FBILS but only 29.7 ± 0.9 mg g^{-1} free fungal biomass. For 200 mg l^{-1} metal solution, uptake reached 71.3 ± 1.3 mg g^{-1} fungal biomass for FBILS but only 59.9 ± 1.5 mg g^{-1} free fungal biomass.

From isotherm studies the maximum uptake levels are 75.9 ± 1.7 mg g^{-1} for FBILS and 63.7 ± 1.5 mg g^{-1} for free fungal biomass from Cd (II) concentrations of 250 mg l^{-1} and above.

Chlorinated aromatic organic compound removal studies

Chlorinated aromatic organic compounds pose severe environmental and health hazards. In particular, methods for the removal of poly-chlorinated dioxins and dibenzofurans are of widespread interest. Because of the hazardous properties of these materials, 4-chloroanisole in aqueous solution was chosen as a model compound as it can be regarded as being structurally similar to half a dichlorodioxin molecule (Figure 3).

After 7 days, contact at 34 °C, FBILS removed 84%, 78% and 69% of 4-chloroanisole from a 5, 10 and 20 mg l^{-1} aqueous solutions (Table 2). No removal was detected in the 4-chloroanisole control using untreated loofa sponge. Hundred percent of 4-chloroanisole was removed from 250 ml of 10 mg solution in 8 days (Figure 4). There was no evidence in the chromatograms for the presence of aromatic deg-



1 2

Fig. 3. Structures of 4-chloroanisole (1) and dichlorodioxin (2).

Table 2. Removal of different concentrations of 4-chloroanisole by fungus biomass immobilized in loofa sponge (FBILS) discs after 7 days.

4-chloroanisole, initial concentration (mg l^{-1})	4-chloroanisole removed (mg g^{-1} FBILS disc)
5	14.1 ± 0.9
10	25.4 ± 1.2
20	45.8 ± 2.8

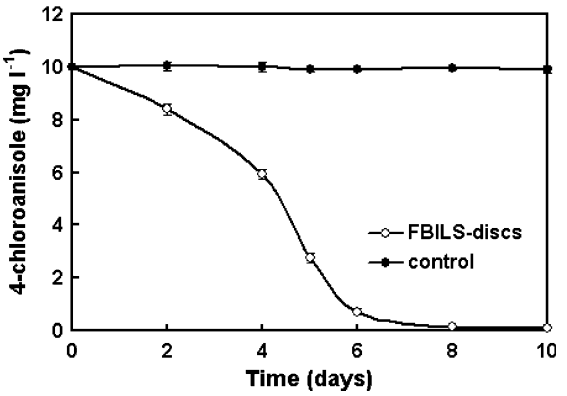


Fig. 4. Effect of contact time on the removal of 4-chloroanisole from aqueous solution. FBILS-discs were shaken at 100 rpm for 10 days in an aqueous solution of 10 mg l^{-1} 4 chloroanisole at 35 °C. This concentration (and the others used) is well below the maximum solubility of 4-chloroanisole in water (237 mg l^{-1}, Lun *et al.* 1995). FBILS were removed after different periods of contact by simple decantation. Hundred millilitre of the decanted culture medium was mixed with an equal volume of diethyl ether in a separating funnel. The organic layer was removed, reduced to 10% volume and analysed for 4-chloroanisole by GC using a WCOT (Wall Coated Open Tubular) fused silica capillary column, 30 m × 0.25 mm ID. Concentrations of 4-chloroanisole were determined against calibration standards using accurately obtained solutions of the organic material in diethyl ether. Dry weight of fungal biomass was determined after drying in an oven at 70 °C overnight.

radation intermediates such as anisole or phenols, however, the control studies show that this removal does not occur by an adsorption process. It is known that *P. chrysosporium* will degrade the aromatic ring structure of chlorinated aromatic compounds to carboxylic acids and carbon dioxide (Valli *et al*. 1992), so this is the likely pathway. Such compounds would not be detected using the current experimental technique. These results clearly demonstrated the ability of FBILS to remove the chlorinated organic compound from aqueous solution. Furthermore, the absence of aromatic degradation products demonstrates added value in the process, as degrading chlorodioxins simply to dioxins would only lead to a small decrease in toxicity. Degradation of the whole structure to carboxylic acids represents a much cleaner overall remediation process.

Conclusions

Loofa sponge is an effective immobilization matrix for the entrapment of fungal hyphae to produce the fungal biomass immobilized loofa sponge (FBILS). FBILS have a high capacity to remove both the toxic metal and chlorinated organic compounds from aqueous solution. High bioremoval capacity, good mechanical strength, ease of handling, high porosity and low cost availability of the immobilization matrix are features which lend this system to practical applications for the removal of metals and chlorinated aromatic compounds from industrial effluents.

References

Aloysius R, Karim MIA, Ariff AB (1999) The mechanism of cadmium removal from aqueous solution by non-metabolizing free and immobilized live biomass of *Rhizopus oligosporus*. *World J. Microbiol. Biotechnol.* **15**: 571–578.

Arica MY, Kacar Y, Gene O (2001) Entrapment of white rot fungus *Trametes versicolor* in ca-alginate beads: preparation and biosorption kinetic analysis for cadmium removal from aqueous solution. *Bioresource Technol.* **80**: 121–129.

de Rome L, Gadd GM (1987) Copper adsorption by *Rhizopus arrhizus*, *Cladosporium resinae* and *Penicillium italicum*. *Appl. Microbiol. Biotechnol.* **26**: 84–90.

Hu MZC, Reeves M (1997) Biosorption of uranium by *Pseudomonas aeruginosa* strain CUS immobilized in a novel matrix. *Biotechnol. Prog.* **13**: 60–70.

Kratochvil D, Volesky B (1998) Advances in the biosorption of heavy metals. *Trends Biotechnol.* **16**: 291–302.

Leenen EJTM, Dos Santos VAPM, Grolle KCF, Tramper J, Wijffels RH (1996) Characteristics of and selection criteria for cell immobilization in wastewater treatment. *Water Res.* **30**: 2985–2996.

Lopez A, Lazaro N, Morales S, Marques AM (2002) Nickel biosorption by free and immobilized cells of *Pseudomonas fluorescens* 4F39: a comparative study. *Water Air Soil Poll.* **135**: 157–172.

Lun R, Shiu WY, Mackay D (1995) Aqueous solubilities and octanol water partition coefficients of chloroveratroles and chloroanisoles. *J. Chem. Eng. Data* **40**: 959–962.

Mahan CA, Holcombe JA (1992) Immobilization of algae cells on silica gel and their characterization for trace metal preconcentration. *Anal. Chem.* **64**: 1933–1939.

McHale AP, McHale S (1994) Microbial biosorption of metals: potential in the treatment of metal pollution. *Biotechnol. Adv.* **12**: 647–652.

Perez RR, Benito GG, Miranda MP (1997) Chlorophenol degradation by *Phanerochaete chrysosporium*. *Bioresource Technol.* **60**: 207–213.

Plette ACC, Benedetti MF, Riemsdjik WH (1996) Competitive binding of protons, calcium, cadmium and zinc to isolated cell walls of a gram-positive soil bacterium. *Environ. Sci. Technol.* **30**: 1902–1910.

Reddy GVB, Gelpke MDS, Gold MH (1998) Degradation of 2,4,6-trichlorophenol by *Phanerochaete chrysosporium*: involvement of reductive dechlorination. *J. Bacteriol.* **180**: 5159–5164.

Steel RGD, Torrie JH (1996) *Principles and Procedures of Statistics: A Biometrical Approach*, 3New York:McGraw-Hill.

Trujillo EM, Sprinti M, Zhuang H (1995) Immobilized biomass: a new class of heavy metal ion exchangers. In: Senguptal AK, ed. *Ion Exchange Technology: Advances in Pollution Control*, Pennsylvania: Technomic Publishing Company Inc, pp. 225–271.

Valli K, Wariishi H, Gold MH (1992) Degradation of 2,7-dichlorodibenzo-*p*-dioxin by the lignin-degrading basidomycete *Phanerochaete chrysosporium*. *J. Bacteriol.* **174**: 2131–2137.