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Full Length Research Paper

Effect of pretreatment on Cd^{2+} biosorption by mycelial biomass of *Pleurotus florida*

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The effect of pretreatment on the Cd^{2+} biosorption capacity of mycelial biomass of *Pleurotus florida* was investigated. For this purpose, the biomass was subjected to physical treatments such as heat, autoclaving and freeze drying and chemical treatments using acids, alkali and organic solvents. All the pretreatment methods improved the biosorption of Cd^{2+} in comparison with live biomass of *P. florida*. Among physical treatments, freeze drying showed significant improvement in Cd^{2+} sorption capacity. Pretreatment of biomass with NaOH showed maximum cadmium biosorption followed by formaldehyde and methanol treatment.

Key words: Pleurotus, pretreatment, biomass, biosorption, cadmium.

INTRODUCTION

Fungi like other microorganisms can absorb heavy metals from their external environment by means of physico-chemical and biological mechanisms. Biosorption is a process by which living and dead microbial cells are able to remove heavy metal ions from aqueous solutions (Pighi et al., 1989; Siegel et al., 1990). Living cells are likely to be more sensitive to metal ion concentration and adverse operating conditions of pH and temperature. Furthermore, a constant nutrient supply is required for using living cells. Recovery of metals and regeneration of biosorbent is complicated for living cells. In addition, non viable cells frequently exhibits a higher affinity for metal ions compared with viable biomass probably due to absence of competing protons produced during metabolism. So, non viable biomass is preferred than the viable cells. The living cells can be inactivated by physical pretreatments using heat treatment (Galun et al., 1983a; Siegel et al., 1986; Townsley et al., 1986a), autoclaving and vacuum drying (Tobin et al., 1984; Huang et al., 1988a) or treating the biosorbent with chemicals like acids, alkali, detergents, organic solvents (Muzzarelli et al., 1980a, b; Huang et al., 1988a; Azab and Peterson, 1989; Rao et al., 1993; Brady et al., 1994) or by mechanical disruption (Tsezos and Volesky, 1981; Yakubu and Dudeney, 1986). These types of pretreat-ments modify the cell surface which is essential for biosorption either by removing or masking the groups or exposing more metal binding sites (Gupta et al., 2000). The aim of this study was to investigate the effect of physical and chemical pretreatment of mycelial biomass of *Pleurotus florida* on biosorption of Cd^{2+}.

MATERIALS AND METHODS

Preparation of biomass

Tissues from the fruit bodies of *P. Florida* were cultured on Potato Dextrose Agar (PDA) media. After 7 days of incubation, the biomass was aseptically transferred into a mineral medium of following composition: glucose (20 g); KH_2PO_4 (1 g); $MgSO_4.7H_2O$ (0.5 g); $(NH_4)_2SO_4$ (0.5 g); distilled water (1000 ml) with a pH of 6.5. The inoculated liquid medium was then agitated in a rotary shaker at room temperature for 7 days at 125 rpm. For the biosorption studies, the mycelial biomass was harvested by filtering the medium through Whatman No. 1 filter paper.

Pre-treatment of biomass

5 g of wet biomass harvested by filtration was pretreated in different ways:

Physical treatments:

(M1) Only dried at 60°C for overnight.
(M2) Autoclaved for 15 min at 121°C and 15 psi.
(M3) Freeze-dried.

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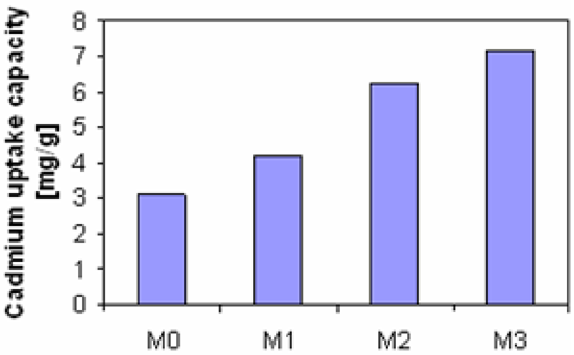

Figure 1. Effect of physical pretreatment on biosorption of cadmium (II) by *Pleurotus florida*. M0- untreated biomass; M1- oven dried biomass; M2- Autoclaved biomass; M3- Freeze dried biomass.

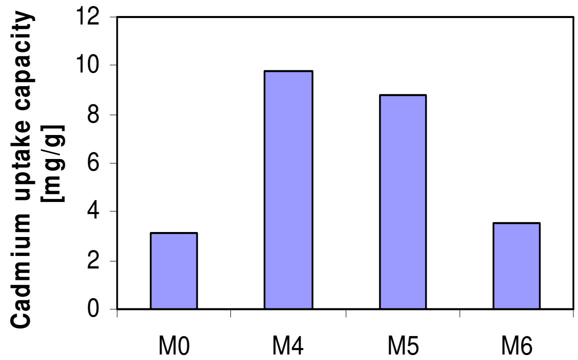

Figure 2. Effect of alkali pretreatment on biosorption of cadmium (II) by *Pleurotus florida.* M0- untreated biomass; M4- 0.5 N NaOH treated biomass; M5- 0.1 N $NaHCO_3$ treated biomass; M6- 0.5 N Na_2CO_3 treated biomass.

Chemical treatments

Pre-treatment with alkali: (M4) Boiled for 15 min in 50 ml of 0.5 N sodium hydroxide (NaOH). (M5) Boiled for 15 min in 50 ml of 0.1 N sodium bicarbonate ($NaHCO_3$). (M6) Boiled for 15 min in 100 ml of 0.5 N sodium carbonate (Na_2CO_3).

Pretreatment with acids: (M7) Boiled for 15 min in 100 ml of 10% (v/v) glacial acetic acid (CH_3COOH). (M8) Added to 7 ml of 0.1 M oxalic acid ($C_2H_2O_4.2H_2O$) and dried at 60˚ C for overnight. (M9) Treated with 20 ml of 10% (v/v) ortho-phosphoric acid (H_3PO_4) and agitated for 30 min at 125 rpm.

Pretreatment with organic solvents: (M10) Treated with 10 ml of 10% (v/v) methanol (CH_3OH) and agitated for 3 h. (M11) Boiled for 15 min with 20 ml of 50% (v/v) dimethyl sulfoxide (C_2H_6SO). (M12) Treated with 100 ml of 10% (v/v) formaldehyde (HCHO) and agitated for 3 h.

All the pretreated biomass were washed with distilled water until the pH of the wash solution was in near neutral range (6.8 - 7.2) and then subjected to oven drying at 60°C for overnight.

Biosorption studies

Biosorption experiments were carried out using Cd^{2+} containing solutions added in the form of $Cd(NO_3)_2.4H_2O$ in distilled water. All the dried biomasses were powdered. Each type of pretreated biomass (0.1 g) was added to 100 ml of 10 mg of Cd^{2+}/L at pH 5. The flasks were kept on a rotary shaker at room temperature and 125 rpm. After 2 h of contact time, the biomass was separated by centrifugation of reaction mixture for 15 min at 10,000 rpm and the supernatant were analyzed for metal concentration.

Measurement of metals

Cadmium concentration in the solution was measured by using Varian AA- 240 Atomic Absorption spectrophotometer. Before measurement, the solutions containing Cd^{2+} were appropriately diluted with deionized water to ensure that the Cd^{2+} concentration in the sample was linearly dependent on the absorbance detected. Biosorption experiments were conducted in triplicate and average values were used in the analysis. The amount of Cd^{2+} (mg) biosorbed per gram of dried biomass was calculated using the following equation:

$$Q = [(C_o-C)/m] \times V$$

Where, Q = amount of metal ion biosorbed per gram of biomass (mg g^{-1}); C_o = initial metal ion concentration (mg L^{-1}); C = final metal ion concentration (mg L^{-1}); m = weight of biomass in the reaction mixture (g); and V = volume of the reaction mixture.

RESULTS AND DISCUSSION

Pre-treatment of living biomass (M0) using oven drying (M1), Autoclaved (M2) and freeze dried (M3), NaOH (M4), $NaHCO_3$ (M5), Na_2CO_3 (M6), Glacial acetic acid (M7), oxalic acid(M8), ortho-phosphoric acid (M9), methanol (M10), dimethyl sulfoxide (M11) and formaldehyde (M12) resulted in an improvement in cadmium biosorption in comparison with living biomass. Figure 1 shows the effect of physical pretreatment of mycelial biomass of *P. florida* on biosorption of cadmium. It was observed that Q values obtained for all the physically pretreated biomasses were high in comparison with living biomass (from 3.21 to 7.13 mg/g). Freeze dried biomass showed the maximum improvement on cadmium sorption. The bioabsorption capacity of oven dried and autoclaved biomass increased in comparison with live biomass may be attributed to the exposure of latent binding sites.

Figure 2 shows the effect of pretreatment with alkali chemicals. Pretreatment of biomass with NaOH showed maximum increase on biosorption of Cd^{2+} by approximately three times in comparison with living biomass (from 3.21 to 9.76 mg/g). In a study by Galun et al. (1987), NaOH treated biomass of *Penicillium digitatum* also showed enhancement of Cd (II) bioadsorption. Removal of surface impurities, rupture of cell membrane and exposure of available binding sites for metal bioadsorption after pretreatment may be the reason for the increase in metal biosorption.

Figure 3 shows the effect of pretreatment with acids. Oxalic acid and orthophosphoric treatment significantly

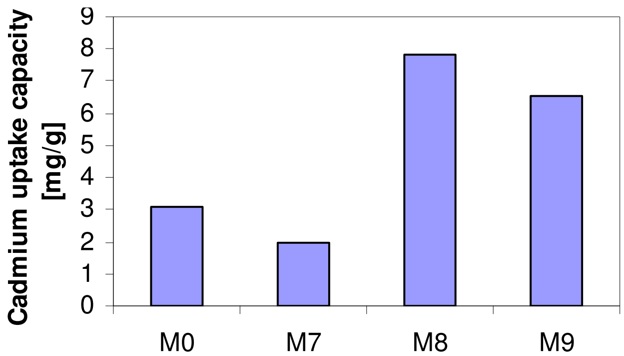

Figure 3. Effect of acid pretreatment on biosorption of cadmium (II) by *Pleurotus florida.* M0- untreated biomass; M7- 10% v/v glacial acetic acid treated biomass; M8- 0.1 M oxalic acid treated biomass; M9- 10% v/v ortho-phosphoric acid treated biomass.

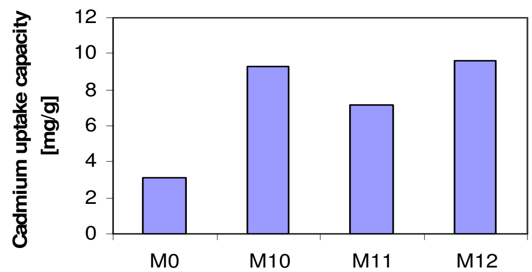

Figure 4. Effect of pretreatment with organic solvents on biosorption of cadmium (II) by *Pleurotus florida*. M0- untreated biomass; M10- 10%v/v methanol treated biomass; M11- 50% v/v dimethyl sulfoxide treated biomass; M12- 10%v/v Formaldehyde treated biomass.

increased the biosorption of cadmium, which is in agreement with the observation of Huang and Huang (1996) who reported that the acid pretreatment can strongly enhance the adsorption capacity of *Aspergillus oryzae.* The pretreatment with glacial acetic acid decreased the biosorption of cadmium compared to living biomass which is in agreement with the observation of Kapoor and Viraraghavan (1998) in case of *Aspergillus niger*. The polymeric structure of biomass surface exhibits a negative charge due to ionization of organic groups and inorganic groups. The higher the biomass electronegativity, greater is the attraction and adsorption of heavy metal cations. Thus, the remaining H^+ ions on the acid pretreated biomass may change the biomass electro-negativity, resulting in a reduction in bioadsorption capacity.

Figure 4 shows the pretreatment of biomass with organic solvents viz. methanol, dimethyl sulfoxide and formaldehyde which resulted significant improvement in Cd^{2+} biosorption. Maximum improvement was noted in case of formaldehyde treatment. Similar response was reported by Kapoor and Viraraghavan (1998*).* They reported that biosorption of cadmium on *A. niger* was more efficient after formaldehyde treatment. However, Huang and Huang (1996) suggested that when biomass was pretreated with formaldehyde, methylation of the amino groups present in the cell wall significantly reduced biosorption capacity because the biomass was boiled during formaldehyde treatment. But in the present experiment biomass was simply mixed with formaldehyde, it was not boiled. So the difference in result may be due to the specific treatment.

According to the results of the present experiment, it is obvious that the biomass of *P. florida* pre-treated physically or chemically is able to remove cadmium ions from aqueous solution. It may be advantageous to use this novel fungal biomass after chemical pretreatment especially with NaOH. Thus the fungal biomass of *P. florida* may be applied as potent biosorbent for removing cadmium ions from effluents.

REFERENCES

Azab M, Peterson SPJ (1989). The removal of cadmium from water by the use of biological sorbents. Water Sci. Technol. 21: 1705-1706.

Brady D, Stoll A, Duncan JR (1994). Biosorption of heavy metal cations by non viable yeast biomass. Environ. Technol. 15: 429-438.

Galun M, Keller P, Malki D (1983a). Removal of uranium (VI) from solution by fungal biomass and fungal wall related biopolymers. Science 219: 285-286.

Galun M, Galun E, Siegel BZ , Keller P, Lehr H, Siegel SM (1987). Removal of metal ions from aqueous solutions by *Penicillium* biomass: kinetic and uptake parameters. Water. Air Soil Pollut. 33: 359-371

Gupta R, Ahuja P, Khan S, Saxena RK, Mohapatra H (2000). Microbial biosorbents: meeting challenges of heavy metal pollution in aqueous solutions. Curr. Sci. 78(8): 967-973.

Huang CP, Westman D, Quirk K, Huang JP (1988a). The removal cadmium (II) from dilute solutions by fungal adsorbent. Water Sci. Technol. 20: 369-376.

Huang C, Huang CP (1996). Application of *Aspergillus oryzae* and *Rhizopus oryzae* for Cu(II) removal. Water Res. 9: 1985-1990.

Kapoor A, Viraraghavan T (1998). Biosorption of heavy metals on *Aspergillus niger*: Effect of pretreatment. Bioresour. Technol. 63: 109-113.

Muzzarelli RAA, Tanfani F, Scarpini G (1980a). Chelating, film forming, and coagulating ability of the chitosan-glucan complex from *Aspergillus niger* industrial wastes. Biotechnol. Bioeng. 22: 885-896.

Muzzarelli RAA, Tanfani F, Scarpini G, Tucci E (1980b). Removal and recovery of cupric and mercuric ions from solutions using chitosan-glucan from *Aspergillus niger*. J. Appl. Biochem. 2: 54-59.

Pighi PL, Pumpel T, Schinner F (1989). Selective accumulation of silver by fungi. Biotechnol. Lett. 11: 275-280.

Rao CRN, Iyengar L, Venkobachar C (1993). Sorption of Copper(II) from aqueous phase by waste biomass. J. Environ. Eng, Div. Am. Soc. Civ. Engr, 119: 369- 377.

Siegel SM, Galun M, Siegel BZ (1990). Filamentous fungi as metal biosorbents: a review. Water, Air Soil Poll. 53: 335-344.

Siegel SM, Keller P, Galun M, Lehr H, Siegel B, Galun E (1986). Biosorption of lead and chromium by *Penicillium* preparations. Water Air Soil Poll. 27: 69-75.

Townsley CC, Ross IS, Atkins AS (1986a). In: Fundamental and Applied Biohydrometallurgy. (ed. Lawrence RW, Branion RM, Enner HG) Elsevier, Amsterdam, pp. 279-289.

Tobin JM, Cooper DG, Neufeld RJ (1984). Uptake of metal ions by *Rhizopus arrhius* biomass. Appl. Environ. Microbiol. 47: 821-824.

Tsezos M, Volesky B (1981). Biosorption of Uranium and Thorium, Biotechnol. Bioeng. 23: 583-604.

Yakubu NA, Dudeney AWL (1986). *In:* Immobilisation of ions by biosorption. (ed. Eccles HH, Hunt S) Horwood, Chichester, UK, pp. 183- 200.