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Full Length Research Paper

Biosorption of Cr, Mn, Fe, Ni, Cu and Pb metals from petroleum refinery effluent by calcium alginate immobilized mycelia of *Polyporus squamosus*

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Two samples of wastewater were collected and initial concentrations of heavy metals and other elements in wastewater were determined using X-ray fluorescence spectroscopy. Exponentially growing mycelia of the white rot fungus *Polyporus squamosus* immobilized on calcium alginate was applied to 500 ml of wastewater samples. Biosorption investigations were conducted in a stirring bioreactor. Results obtained showed significant increments in the quantity of heavy metals sequestered by immobilized live mycelia of *P. squamosus* as compared with empty ca-alginate beads. pH played an important role in the biosorption capability of the immobilized *P. squamosus*, with the fungal biomass having maximum adsorption for cationic metal ions at pH 4 - 6. The results of this investigation could provide a basis for applying the white rot fungi for an environmentally friendly and economically feasible decontamination of pollutants.

Key words: Biosorption, white rot fungi, heavy metals, fungal biomass.

INTRODUCTION

The ability of microorganisms to take up metals has been demonstrated for some time (Filipovic-kovacevic et al., 2000; da Costa and Duta, 2001; Yalcinkaya et al., 2001; Hussein et al., 2004; Preetha and Viruthagiri, 2005). Microbial biomass can be used to decontaminate metal bearing wastewaters as well as to concentrate metals. The nature of biological surfaces is such that different functional groups form complexes with metal ions (Huang et al., 1994), resulting in chemical complexation as an uptake mechanism. Metal uptake can also be due to physical sorption or bioaccumulation.

Majority of toxic pollutants are waste products of Industrial and metallurgical processes. The concentrations of these metals need to be reduced to meet ever-changing legislative standards. According to the World Health Organization (WHO, 1984), the metals of most immediate concern are cadmium, chromium, cobalt, copper, lead, nickel, mercury and zinc. The presence of such metals (>5 g cm^3, Mahavi, 2005) in aquatic environments cause severe damage to aquatic life, killing microorganisms during biological water purification process. Moreover, these metals have exacting consequences on humans such as brain damage, reproductive failures, nervous system failures, tumour formation, etc (Hamman, 2004; Mahavi, 2005).

Conventional processes for removal of metals from industrial wastewaters include chemical precipitation, oxidation-reduction, filtration, electrochemical techniques and other sophisticated separation procedures using membranes. These processes are expensive when metals are found in relatively moderate concentrations, such as 1 - 100 mg/L. Biological methods such as biosorption or bioaccumulation strategies for the removal of metals ions may provide an attractive alternative to existing technologies (Kapoor and Viraraghavan, 1995; da Costa and Duta, 2001; Yalcinkaya et al., 2002; Hussein et al., 2004; Preetha and Viuthagiri, 2005). So far, the biomass from filamentous fungi such as *Aspergillus niger* and *Rhizopus oryzae*, yeast-like *Saccharomyces cerevisiae*, algae such as *Chlorella regularis* and unicellular bacteria such as *Zoogloea ramigera* and *Pseudomonas aeruginosa,* have demonstrable capability for the uptake or binding of several metal ions. Brierley et al. (1986) has suggested that a metal loading capacity greater than 15% of biomass could be used as an economic threshold for practical ap-

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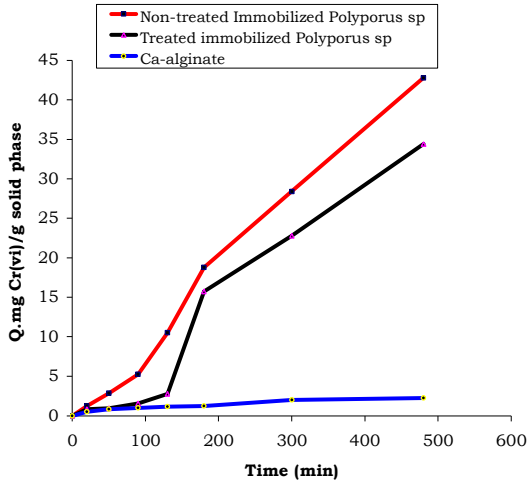

Figure 1. Biosorption capability of immobilized *P. squamosus* and plain Ca-alginate for Cr(VI) ions in refinery effluent.

plications of biosorption as compared with alternative techniques.

Other workers have previously described biosorption using immobilized fungal spores in or on different matrices (Lovely, 1995; Kapoor and Viraraghavan, 1999; Lloyd and Lovely, 2001). Immobilization techniques provides for high cell concentrations, making the separation of treated water from biomass easy. In this work, the exponentially growing hyphal cells of the fungus *Polyporus squamosus* were entrapped using sodium-alginate beads as the natural polymeric matrix. This was used for biosorption of metals from petroleum refinery effluents in a batch system.

MATERIALS AND METHODS

Cell immobilization

The spores of *P. squamosus* were obtained by the procedure Anderson et al. (1973). The procedure for obtaining the pure culture of *P. squamosus* culture was as previously described by Wuyep et al. (2003). The method of James (1992) for cell immobilization, as modified by Shide et al. (2004) was used. The cells of exponentially growing mycelia of the culture were harvested aseptically into a 1 L capacity blender (Master Chef 650, UK), using a spatula. The harvested cells were homogenized and about 17.5 ml of the homogenate was measured into a 250 ml conical flask containing 87.5 ml distilled water and mixed thoroughly. The mixture was allowed to settle, and after 10 min, exactly 3.063 g of sodium alginate (Hopkins and Williams limited, England) was weighed into the supernatant (concentrated cells). The mixture was subsequently pumped through a 5 ml syringe drop wise, into a flask containing sterilized 100 ml of 0.12 M calcium chloride solution (May and Baker Limited, England). The reaction, which was almost instantaneous, was allowed a retention time of 1 h for complete precipitation that formed spherical beads. The immobilized cells were removed and stored until use at 4^{o}C in 5 mM $CaCl_2$ solution.

Biosorption studies

The samples were labeled as treated and non-treated, respectively. Biosorption of all wastewater samples was carried out in a batch system in a 1 L micro carrier bioreactor (µCBR, 1965 - 00500, M3622129, Bellco, U.S.A.). The samples and beads of immobilized cells were placed into the bioreactor and placed on a magnetic stirrer hot plate regulator (Gallenkamp, England) which induced a stirring effect of about 400 rpm. A known quantity of beads of immobilized cells was maintained in the bioreactor containing 500 ml of wastewater for 3, 5 and 8 h, after which samples were withdrawn for analysis. Another set-up with only plain Ca-alginate beads was maintained as control. Effect of pH on the rate of biosorption of the heavy metals was also determined. HCl or NaOH was used to adjust the pH.

Elemental analysis

Samples were analyzed using X-ray fluorescence (XRF). Prior to biosorption, 100 ml of the sample, both treated and untreated is measured and nitrified using 2 - 3 drops of nitric acid (BDH limited England), then 0.1 g of APDC Pyrrolidin-1-dithiocarboxylic acid ammonium salt (Schuchardt, 8011 Hihebbrumn Munchen) diluted with 0.5 ml of distilled water was added to the nitrified sample and stirred. The sample was then filtered through a Millipore filter paper. The residue on the filter paper was allowed to dry. The filter paper was then placed on the Na/Li detector (Canberrra, U.S.A) which uses cadmium as its source of X-rays. The quantitative and qualitative analysis of the elements present was done using Axil and Maestro softwares, respectively. At the end of biosorption study, samples were collected prepared and analyzed in the same manner. The amount of metal ions adsorbed per unit mass of both empty and fungus immobilized alginate beads (mg metal ions/g dry beads) was obtained using the equation:

$$Q = (Co-C)V/M \qquad (1)$$

where Q is the amount of metal ions adsorbed onto the unit mass of the absorbent (mg/g), Co and C are the concentrations of the metal ions before and after biosorption (mg/L), V is the volume (L) of the aqueous phase, and M is the amount (g) of the adsorbent (Gadd and White, 1993).

Prior to this, a known weight of Ca-alginate or fungus immobilized beads was dried in an oven at 50^{o}C overnight and the dry weight used in the calculation. The quantity of heavy metal ion uptake (percentage) was determined by comparing the amount of heavy metal ion sequestered by the biosorbent (Q from equation 1) with the initial concentration of heavy metals in the refinery effluent before biosorption studies. This had been determined earlier by X-ray fluorescence (XRF) technique. The percentage was calculated as follows:

$$q = (qmax/Co) \times 100 \qquad (2)$$

Where q is the fungal adsorption efficiency (%), qmax is the maximum uptake capacity by unit mass of the absorbent (mg/g dry wt) and Co is the concentration of the metal ions before biosorption (mg/L).

RESULTS AND DISCUSSION

Figures 1 - 6 illustrates the changes in the amount of heavy metal ions biosorbed with time in both treated and non-treated refinery effluents. Values were obtained based on equation 1. There was significant increaments in

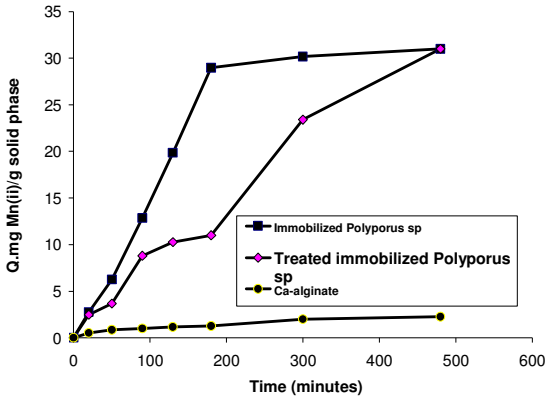

Figure 2. Biosorption capability of immobilized *P. Squa-mosus* and plain Ca-alginate for Mn(II) ions in refinery effluent

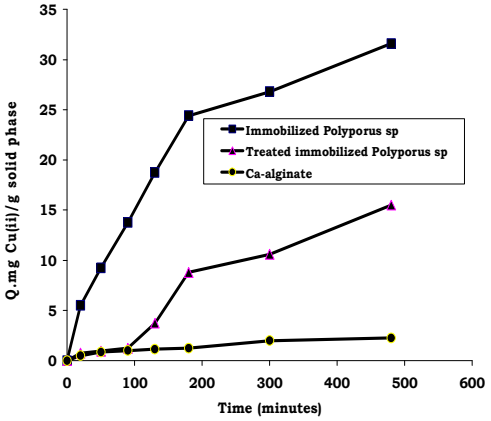

Figure 5. Biosorption capability of immobilized *P. squa-mosus* and plain Ca-alginate for Cu(II) ions in refinery effluent

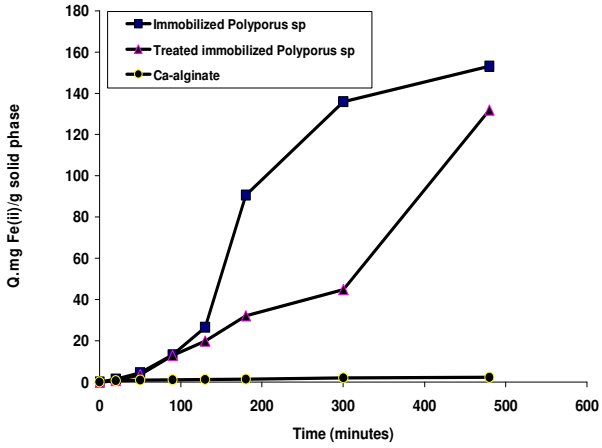

Figure 3. Biosorption capability of immobilized *P. squamosus* and plain Ca-alginate for Fe(II) ions in refinery effluent

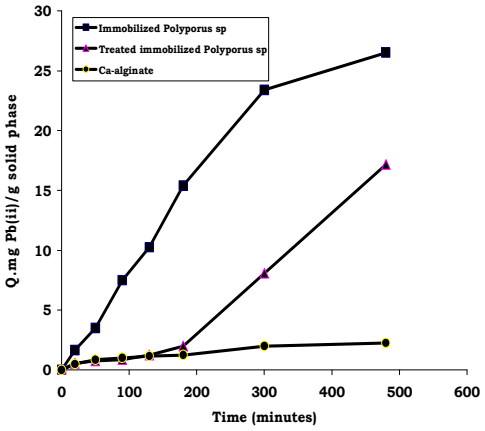

Figure 6. Biosorption capability of immobilized *P. squamosus* and plain Ca-alginate for Pb(II) ions in refinery

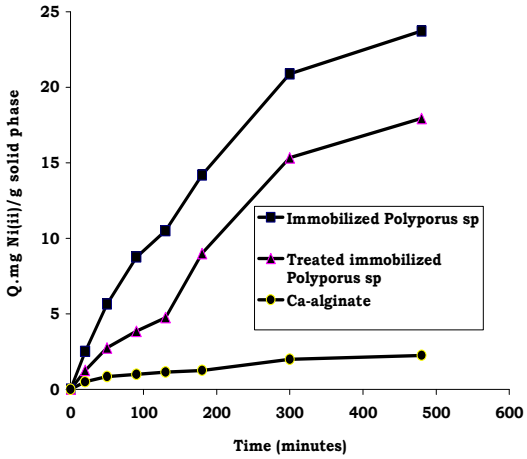

Figure 4. Biosorption capability of immobilized *P. squamosus* and plain Ca-alginate for Ni(II) ions in refinery effluent

in the biosorption rates for the all the heavy metal ions known to be associated with pollution. An increasing uptake pattern was observed for all the metals. The biosorption rate for Cr(VI) ions was steady throughout the period of biosorption with immobilized *P. squamosus* for both treated and non-treated effluent, when compared with plain Ca-alginate as shown in Figure 1. The amount of Cr (VI) ions adsorbed on the immobilized *P. squamosus* beads was 42.8 ± 1.52 mg/g for non-treated and 34.4 ± 1.52 mg/g for treated, while that for plain Ca-alginate was 2.05 ± 1.05 mg/g. Mn (II) ions adsorption was 29.0 ± 1.45 mg/g for non-treated and 23.4 ± 1.23 mg/g for treated (Figure 2). Similar observations were made for Fe(II), Ni(II), Cu(II) and Pb(II) (Figure 3) having the amount of adsorbed ions as 153.04 ± 5.78 and 131.092 ± 5.13 mg/g, 23.72 ± 0.78 and 17.94 ± 0.97 mg/g, 31.6 ± 1.29 and 15.5 ± 0.67 mg/g, and 26.52 ± 1.10 and 17.18 ± 0.69 mg/g, respectively for non-treated and treated wastewater, respectively, as show in Figure 4 - 6.

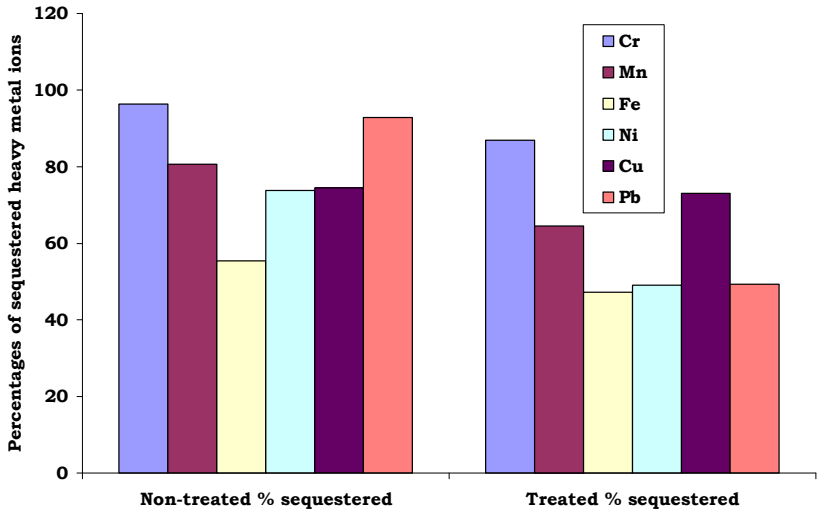

Figure 7. Percentages of heavy metal ions sequested by immobilized *P. squamosus*.

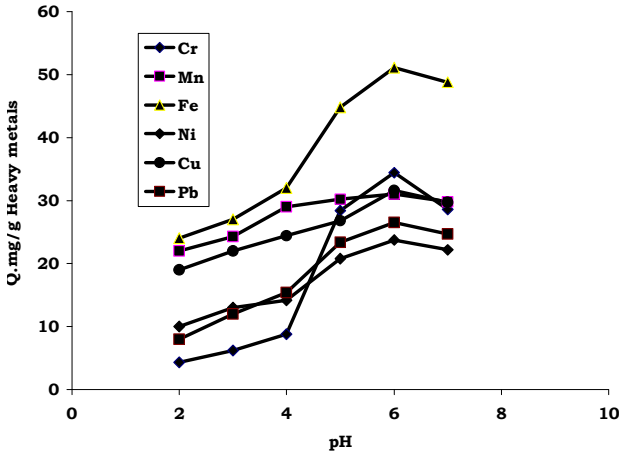

Figure 8. The effect of solution pH on the biosorption of Heavy metals non-treated effluent by immobilized *P. squamosus*.

From the results described above, the amount of metal ions sequestered by immobilized *P. squamosus* was higher in non-treated effluent than in treated effluent and plain Ca-alginate. This is as expected, because non-treated effluent has not undergone any form of treatment prior to biosorption with the immobilized *P. squamosus*. Saturation of biomass by metals as is the case with other models was not observed, suggesting the availability of biosorption sites. Classical adsorption equations were not used, because uptake in our investigation probably was not restricted to surface phenomena. Viable cells were used, and metabolic activity could be in action during uptake.

It was also observed that the biosorption capability of the plain Ca-alginate was not significant for heavy metal ions, considering the rates of adsorption on the sorbent. Yalcinkaya et al. (2002) reported that immobilized Ca-alginate possessed significant advantage over non-immobilized alginate, especially when Ca^{2+}, Mg^{2+}, and K^{+} were present as ion-exchange resins. Our investigation have shown that, for immobilized and plain Ca-alginate, the amounts of K, Ca, Mg, adsorbed were higher than those of the heavy metal ions (data not shown).

It was also observed that the amount of metal ions adsorbed per unit mass of the biosorbent increased in both non-treated and treated effluent medium by immobilized *P. squamosus*. Parameters like stirring rate of the aqueous phase, the structural properties of biosorbent such as its protein and carbohydrate composition, surface charge and density, topography and surface area made comparative investigation of biosorption rates of individual heavy metal ions in the refinery effluent difficult. Nevertheless, the amount or quantity of heavy metal ion uptake was determined as a measured of the amount of individual metal ions sequestered from the non-treated and treated refinery effluent. The results obtained as shown in Figure 7 indicate that white rot fungi have biosorption capabilities and are able to sequester significant amounts of heavy metals found in liquid phase, when immobilized on Ca-alginate matrix.

Fourest et al. (1994) and Lovely (1995) reported that sorption of heavy metals in aqueous solutions lies in the properties of the adsorbent, and molecules of the adsorbate transferred from the solution to the solid phase. In addition, the biosorption capability of heavy metals by fungi is strongly pH dependent, such that biosorption increases with increase in pH. Our investigation shows that fungal biomass has maximum sorption capability for cationic metal ions at pH values of 4 to 6, as shown in Figure 8. At pH below 3, uptake of heavy metal ions were negligible, because of cation competition effects with hydronium ion H_3O^{+}.

Conclusion

Our results show that *P. squamosus,* a white rot fungus has biosorption capability, by being able to sequester subtantial amounts of heavy metals from effluents. Previously, the ability of white rot fungi to degrade recalcitrant pollutants has been demonstrated. This ability is probably largely due their elaborate ligninolytic enzymes. It is difficult to say whether these heavy metals were biodegraded. However, their accumulation in the fungal biomass suggests that the mycelia of these fungi is able to entrap the heavy metals as they occur in the aqueous phase. Our results could establish a basis for evaluating the role of white rot fungi in the search for an environmentally friendly approach to dealing with pollutants in aqueous phase.

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