Pergamon

Wat. Sci. Tech. Vol. 38, No. 4–5, pp. 323–330, 1998.
IAWQ

0273–1223/98 $19·00 + 0·00

PII: S0273-1223(98)00515-0

HEAVY METAL BIOSORPTION BY WHITE-ROT FUNGI

Ülkü Yetis*, Gülay Özcengiz**, Filiz B. Dilek*, Neslihan Ergen**, Alev Erbay* and Ayla Dölek*

** Biology Department, Middle East Technical University, 06531 Ankara, Turkey*
*** Environmental Engineering Department, Middle East Technical University, 06531 Ankara, Turkey*

ABSTRACT

In this study, heavy metal biosorption potentials of two white-rot fungi, *Polyporous versicolor* and *Phanarochaete chrysosporium*, which are commonly used in wastewater treatment were determined. Biosorption studies were performed for Cu(II), Cr(III), Cd(II), Ni(II) and Pb(II) at the same operational conditions and the effectiveness of both fungi at removing these heavy metals was compared. It was found that both *P. versicolor* and *P. chrysosporium* were the most effective in removing Pb(II) from aqeous solutions with maximum biosorption capacities of 57.5 and 110 mg Pb(II)/g dry biomass, respectively. With *P. versicolor*, the adsorptive capacity order was determined to be Pb(II)>Ni(II)>Cr(III)>Cd(II)>Cu(II) whereas the order was Pb(II)>Cr(III)>Cu(II)=Cd(II)>Ni(II) with *P. chrysosporium*. As a general trend, metal removal efficiency with these fungi decreased as the initial metal ion concentration increased.

KEYWORDS

Biosorption; *Polyporous versicolor*; *Phanarochaete chrysosporium*; sorptive capacity; heavy metals; white-rot fungi; waste sludge.

INTRODUCTION

There has been an increasing concern about heavy metal releases to the environment by technological activities because of their known toxicity and their tendency to accumulate throughout the food chain. A variety of suitable methods exist for the removal of heavy metals from industrial wastes when they are at high concentrations. These are chemical precipitation, electrode deposition, ion exchange, reverse osmosis, evaporative recovery and membrane technology application .

Using microorganisms as biosorbents for heavy metals is an attractive alternative to existing methods for toxicity reduction and recovery of valuable metals from industrial effluents, because of good performance and low cost of biosorbent material (Volesky, 1987; Gadd *et al.*, 1988; Brierley, 1990). Living, dead or resting cells of different species of bacteria, fungi and algae have served as biomass sources with varying metal binding capacities. The biosorption depends not only on the chemical composition of the cell or its components such as the wall, but also on external physico-chemical factors and the solution chemistry of the metal (Volesky, 1987).

The results of biosorption studies vary widely because of the different criteria used by the investigators to select a suitable biomass. In addition, the absence of uniform methodology often makes quantitative comparison impossible.

The use of waste biomass from pharmaceutical or food industries is one way to minimize the costs of the related bioprocesses since the additional costs associated with biomass generation or biomass pre-treatment are avoided (Niu *et al.*, 1993). On the other hand, the possibility of using waste biological sludge from industrial waste treatment facilities have not yet been investigated to date. In this regard, the white-rot fungi that hold great promise for degradation of chlorinated organics present in many industrial effluents (Prouty, 1990; Livernoche *et al.*, 1983, Galeno and Agosin, 1990) might be considered as potential candidates for heavy metal biosorption. Thus, the aim of the present study is to evaluate the effectiveness of two different white-rot fungi, *Polyporous versicolor* and *Phanerochaete chrysosporium*, which have been studied extensively for the treatment of pulp bleaching effluents, as biosorbents for the removal of five distinct heavy metals; Pb(II), Ni(II), Cu(II), Cr(III) and Cd(II).

EXPERIMENTAL

Biomass preparation

The cells of *P. versicolor* and *P. chrysosporium* grown on Sabaroud Dextrose Agar were suspended in sterile distilled water. Optical densities (A_{650}) of the suspensions were measured and adjusted to 0.5 using a Spectronic 20 spectrophotometer. This adjustment was needed to inoculate approximately the same amount of cells into each flask. To prepare the biomass, 3 mL aliquots from such cell suspensions were inoculated into 500 mL Erlenmayer flasks each containing 250 mL of the growth medium. The compositions of the liquid growth media used are given in Table 1.

Table 1. Compositions of growth media and cultivation conditions

Component, g/L or Condition	*P. chrysosporium* growth medium[a]	*P. versicolor* growth medium[b]
KH_2PO_4	2	2
$MgSO_4$	0.5	0.5
$CaCl_2$	0.1	0.1
NH_4Cl	0.12	0.12
Glucose	10.0	-
Dextrose	-	30
Thiamine	0.001	0.001
pH	4.5	5.5
T, °C	35	30
Shaking rate, rpm	200	180

[a]Prouty, 1990;[b]Livernoche *et al.*, 1983

As deduced from the growth curves, the logarithmic growth of both *P. versicolor* and *P. chrysosporium* lasted about for six days. Throughout the biosorption studies, the cells were harvested at the end of the logarithmic phase of growth, i.e. at 6th day (114th h) of incubation and subsequently used as biosorbent.

Biosorption experiments

The cells were harvested by filtering through glass fiber filter papers and 4.0 g of the wet biomass was added to 300 mL solution of metals at known initial concentrations. The corresponding dry weight of the biomass was determined at each batch of experiment by drying it at 105°C. Metal binding capacities were expressed as mg metal adsorbed by g dry weight of fungal biomass.

The metal salts of $NiCl_2.6H_2O$, $Pb(NO_3)_2$, $CuSO_4.5H_2O$, $Cr(NO_3)_3.9H_2O$, and $CdCl_2.H_2O$ were added to the biosorption flasks, to give various initial metal concentrations ranging from 10 to 200 mg/L. The flasks were shaked in an orbital shaken at an intensity of 150 rpm at 25°C. The initial pH values of metal solutions were adjusted to 6.0 in all biosorption experiments before adding the biomass. In kinetics studies, the samples taken at certain time intervals were filtered through 0.45 μm membrane filters and the filtrates collected were subjected to metal analyses. The analyses were performed using a Unicam Model 929 atomic absorption spectrophotometer. The metal ion concentration that remained steady for two successive readings was accepted as the equilibrium metal concentration. Whenever a partial release of metals was observed, the equilibrium time was taken as the time at which the maximum capacity was attained.

RESULTS AND DISCUSSION

Polyporous versicolor

The biosorptive capacity of resting cells of *P. versicolor* in adsorbing heavy metals, Cr(III), Cu(II), Cd(II), Pb(II) and Ni(II), were investigated under the above-mentioned operational conditions. The studies were concentrated on the time course of metal accumulation to determine the rate of biosorption as well as the equilibrium time. The equilibrium sorption isotherms were next prepared to describe metal sorption onto microbial surfaces.

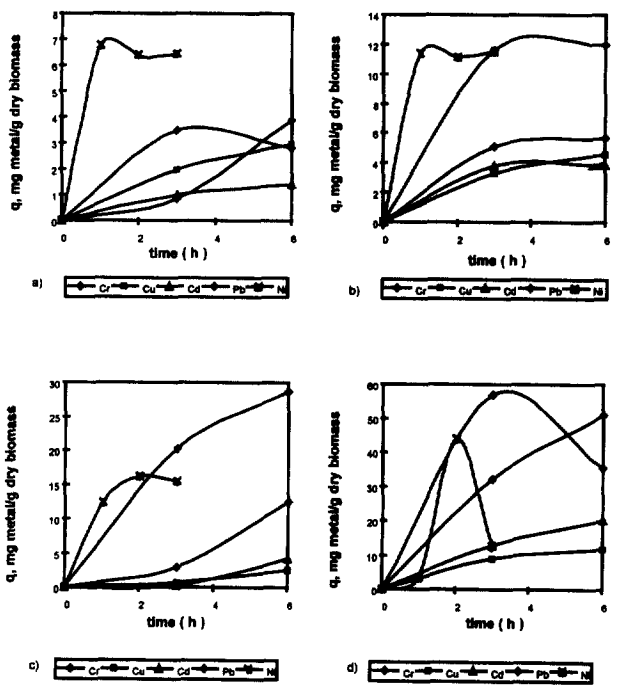

Figure 1. Adsorptive capacity of *P. versicolor* vs. time: a) 10 mg/L, b) 20 mg/L, c) 50 mg/L, d) 150 mg/L of initial metal concentration (T = 25°C, pH = 5.5-6.5, 150 rpm).

The time course of biosorption at selected initial metal concentrations is shown in Fig. 1. There appeared a marked difference in the rate of adsorption of different metals. At all initial metal concentrations, except for 10 mg/L, the rates of adsorption of Pb(II) and Ni(II) were much higher than those of others. Ni(II) was found to be the metal that was adsorbed at the highest rate while Cd(II) or Cu(II) were generally adsorbed at the lowest rates. While 2 h was sufficient to reach equilibrium for Ni(II), it took 3 to 6 h for the other metals.

The rate of biosorptive Ni(II) uptake was relatively fast with 90 % of the total removal attained within the first hour of contact (Fig. 1a,b,c). However, for an initial Ni(II) concentration of 150 mg/L, this rapid adsorption was followed by desorption of the metal (Fig 1d). The same pattern was also observed for Pb(II) at the same concentration. The rate of Pb(II) biosorption was enhanced with increasing initial Pb(II) concentration. At the lowest concentration tested, Pb(II) was the metal which was adsorbed at the lowest initial rate. However, at a high concentration of 150 mg/L, this metal was adsorbed at the highest rate as compared to the other metals. Regarding the quantity adsorbed per unit mass of biomass, Pb(II) generally appeared to be the metal adsorbed at the highest capacity.

Cr(III) followed Ni(II) and Pb(II) in terms of both the capacity and rate of adsorption. As the initial Cr(III) concentration increased, the adsorption rate generally increased; the quantity adsorbed at the end of 6 h was about 3, 5.7, 12.5, and 52 mg Cr(III) per g of fungal biomass for the initial concentrations of 10, 20, 50 and 150 mg/L, respectively. At high initial concentrations, Cu(II) was obviously the one which was adsorbed with the lowest affinity by the fungus. The maximum adsorption capacity attained was only 12 mg/g biomass at 150 mg/L initial concentration. Similarly, Cd(II) was adsorbed at a low rate with a low affinity. Adsorptive capacities attained at various equilibrium metal concentrations are comparatively shown in Fig. 2. The maximum capacity with *P.versicolor* was detected with Pb(II), which was 57.5 mg/g, and the minimum capacity, as low as 12 mg/g, was seen with Cu(II), The order of sorption appeared as Pb(II)>Ni(II)>Cr(III) >Cd(II) >Cu(II).

The maximum Pb(II) adsorption capacity obtained with the cells of *P. versicolor* (57.5 mg/g of dry biomass) was generally less than those reported for this metal with other fungi. For example, with *Absidia orchidis,* 351 mg Pb(II)/g of dry biomass was attained by Holan and Volesky (1995). With another fungus, *Rhizopus nigricans*, the same authors reached a capacity of 166 mg Pb(II)/g dry biomass. Brierley *et al.* (1986), could obtain adsorptive capacities of 601 mg/g and 373 mg/g with *Bacillus subtilis* and fungal biomass, respectively by processing the biomass to improve biosorptive capacity. However, relatively lower capacities of 91 mg/g and 55 mg/g were also reported for *Rhizopus arrhizus* by Tobin *et al.* (1984) and Fourest and Roux (1992), respectively. On the other hand, the maximum adsorption capacity attained for Ni(II) with *P. versicolor* (45 mg/g) was higher than that obtained in previous studies with other species of fungi (Holan and Volesky, 1995; Fourest and Roux, 1992). A comparably high Ni(II) binding capacity has been reported by Holan and Volesky (1994) for a brown marine algae, *Sargassum natans*. Nevertheless, the differences in the operational conditions employed in above-mentioned studies should not be overlooked since the parameters like temperature, pH, mixing intensity, initial metal concentration and the state biomass may greatly vary from study to study.

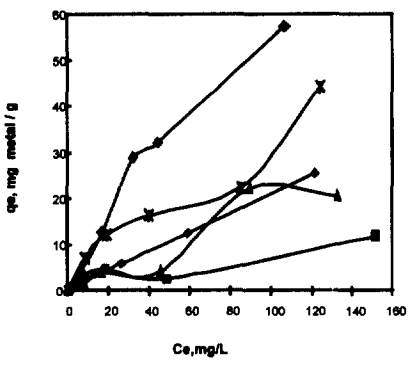

Figure 2. Adsorption isotherms for various heavy metals with *P. versicolor.*

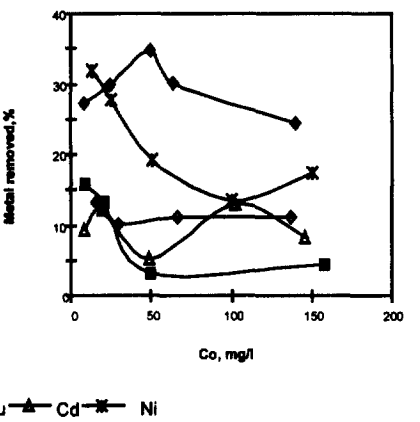

Figure 3. Effect of initial metal concentration on heavy metal removal efficiency by *P. versicolor.*

A decrease in metal removal efficiency was generally observed with increasing initial metal ion concentration. Among the five distinct metals tested, the sorption of Ni(II) by *P. versicolor* was affected by the initial metal concentration to the largest extent; and the fractional removal has ranged from 32 to about 13% while the initial Ni(II) concentration changed from 10 to 100 mg/l. However, Cr(III) sorption was not seriously affected by the initial Cr(III) concentration and only a 3 percentage change in the removal efficiency was observed with an increase in the initial Cr(III) concentration from 10 to 140 mg/L. Cd(II) removal efficiency was found to oscillate with the initial metal concentration (Fig. 3).

Phanerochaete chrysosporium

Time courses of heavy metal biosorptions at different initial metal ion concentrations are shown in Figure 4. The rate of metal adsorption by *P. chrysosporium* varied greatly with different metals. The highest biosorption rate was observed with Pb(II) and the lowest biosorption rate generally with Ni(II). Adsorption rate of Pb(II) increased with increasing initial metal ion concentration whereas the adsorption rates of Cr(III) and Ni(II) were relatively speaking not much dependent on initial metal ion concentration.

Adsorptive capacities for the metals tested were also generally induced by increasing initial metal ion concentration, but as can be easily seen, there was a partial metal release at high initial concentrations for Cr(III), Cd(II) and Ni(II) (Fig. 4). Partial release of adsorbed metals was also recorded in a previous study (Pighi *et al.*, 1989). Thirty two different fungi from a culture collection were tested for their ability to accumulate various metal ions from aqueous solutions. Bivalent ions such as Cu(II), Cd(II), Ni(II) and Pb(II) were partially released after about 50 min. The authors postulated that the metal stress increased fermentation with liberation of organic acids whose complexation effects might cause desorption.

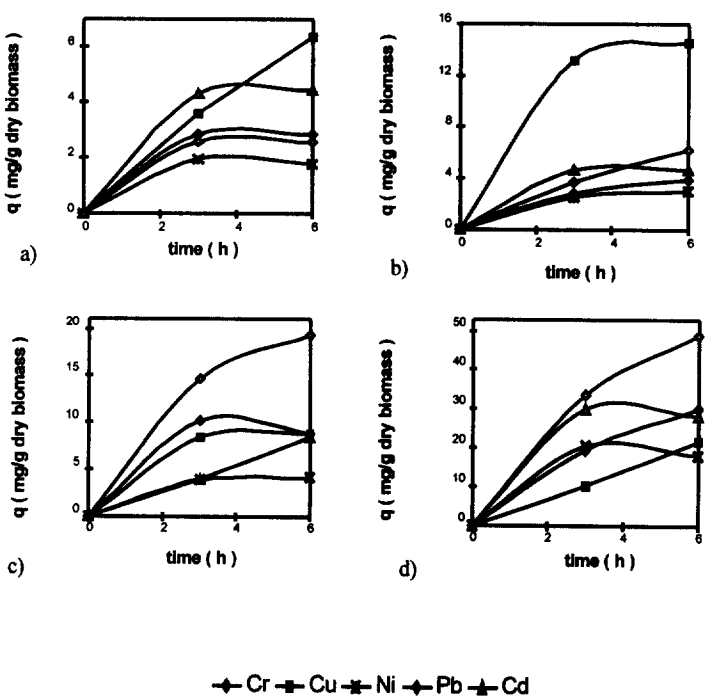

Figure 4. Change in adsorptive capacity of *P. chrysosporium* with time with different metals at initial metal ion concentrations of: a) 10 mg/L, b) 20 mg/L, c) 50 mg/L, d) 150 mg/L (T = 25°C, pH = 5.5-6.5, 150 rpm).

Adsorption isotherms for metals are shown in Fig. 5. Pb(II) biosorption capacity of *P. chrysosporium* is better than the others. Cu(II) biosorption seemed to reach its maximum adsorptive capacity in dilute

solutions, whereas Pb(II) biosorption could reach its maximum at the equilibrium concentration of 275 mg/L. For the other metals, the maximum biosorption capacities were attained at equilibrium concentrations above 100 mg/L.

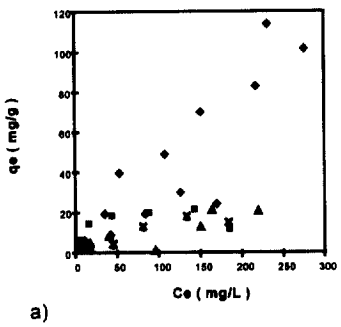

Figure 5. Adsorption isotherms for various heavy metals with *P. chrysosporium*.

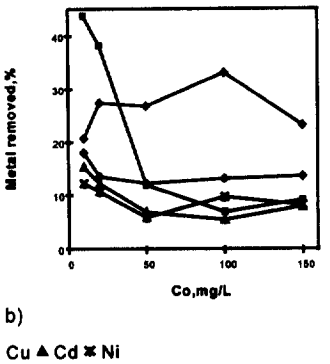

Figure 6. Effect of initial metal concentration on heavy metal removal efficiency by *P. chrysosporium*.

With regard to the extent of metal removal, the highest removal obtained with Pb (II) fluctuated with the initial metal concentration. By contrast, for all other metals, removal decreased with increasing initial metal concentration (Fig. 6).

As deduced from the results, the order of equilibrium biosorption capacity was: Pb(II)>Cr(III)>Cu(II)=Cd(II)> Ni(II). Adsorption capacity order is quite specific to the type of biomass employed. For example, the order was reported to be Ag(I)>Cr(III)> Pb(II)>Cu(II)>> Zn(II)>Cd(II)>Co(II)>=Ni(II) with *Streptomyces noursei* biomass (Mattuschka and Straube, 1993). Nakajima and Sakaguchi (1986) showed that Pb and Cu as well as Hg were more readily accumulated by actinomycetes than Zn, Mn, Co, Ni and Cd. This order is somewhat similar to that obtained in our study; yet our adsorption capacities were higher. Pighi *et al.* (1989) reported that *Penicillium chrysogenum* cells bound metal ions in relative capacities of Ag(I)>Cu(II)>Pb(II)>Ni(II)>Cd(II). It therefore appears that Ni(II) biosorption is rather poor regardless of the type of biomass.

Although Pb (II) was the element to which *P. chrysosporium* had the best adsorptive capacity, the value of 113 mg/g was lower than those attained previously by using the other species of fungi (Holan and Volesky, 1995; Brierley *et al.*, 1986).

Cu(II) uptake capacity for *P. chrysosporium* was found to be 20 mg/g. Of fungi, yeast and bacteria examined as biosorbents for Cu(II) adsorption to date, the highest adsorption capacity of 152 mg/g was obtained with pretreated *Bacillus subtilis*. (Beveridge, 1986; Brierley *et al.*, 1986; Brierley and Brierley, 1993). Adsorption capacities obtained ranged between 0.4 and 18 mg/g by using filamentous fungi. Gadd *et al.* (1988) obtained 18 and 10 mg/g with *Cladosporium resinae* and *Rhizopus arrhizus*, respectively. By using *Penicillium chrysogenum*, Niu *et al.* (1993) obtained the adsorptive capacity as 9 mg/g. With *Penicillium spinulosum*, *Aspergillus niger* and *Trichoderma viride*, uptake capacities were ranging between 0.4 and 2 mg/g (Townsley *et al.*, 1986).

When compared to the adsorptive capacity values which have been so far reported for fungi, our maximum Cd(II) sorption value (25 mg/g) with *P. chrysosporium* can be accepted to be good. For example, Holan and Volesky (1995) and Niu *et al.* (1993) used *Penicillium chrysogenum* as biosorbent and reported 56 and 11 mg/g Cd(II) uptake, respectively. Another fungus, *Rhizopus arrhizus* displayed an adsorption capacity of 30 mg/g (Tobin *et al.*, 1984 and Fourest and Roux, 1992). Holan and Volesky (1995) obtained 19 mg/g and Townsley *et al.* (1986) 0.4 mg/g by using *Rhizopus nigricans* and *Penicillum spinulosum*, respectively.

Of several microorganisms examined, *Bacillus* biomass had the highest adsorptive capacity for Cr(III) with 118 mg/g (Brierley and Brierley, 1993). On the other hand, other bacteria such as *Streptomyces noursei* had a very low uptake capacity of 1.8 mg/g (Mattuschka *et al.*, 1993). The adsorptive capacity of *Rhizopus arrhizus* was 31 mg/g (Tobin *et al.*, 1984). The adsorptive capacity of *P. chrysosporium* for this metal was approaching to that of *R. arrhizus*.

In the literature, the Ni(II) binding capacity reported by Holan and Volesky (1994) for brown marine algae *Sargassum natans* was around 44 mg/g. For fungus species *Rhizopus nigricans*, *Absidia orchidis*, *Rhizopus arrhizus* and *Candida tropicalis* as yeast, adsorption capacity values were 5, 5, 16 and 20 mg/g, respectively (Holan and Volesky, 1995; Fourest and Roux, 1992; Mattuschka *et al.*, 1993). Ni(II) uptake capacity of *P. chrysosporium* (19 mg/g) was acceptable as compared to these previously reported values.

CONCLUSIONS

The following conclusions were drawn from the present study.

1. When tested for different heavy metals, *P. versicolor* displayed an adsorptive capacity order of Pb(II)>Ni(II)>Cr(III)>Cd(II)>Cu(II) whereas the order appeared as Pb(II)>Cr(III)>Cu(II)=Cd(II)>Ni(II) for *P. chrysosporium*.

2. Both organisms had a selective affinity to Pb(II) though the maximum adsorptive capacity of *P. chrysosporium* was two times higher than that of *P. versicolor*. With the former organism, the highest biosorption rate was observed with Pb(II) and the lowest biosorption rate was recorded with Ni(II). *P.versicolor*, on the other hand, exhibited the highest biosorption rate with Ni(II) and the lowest rate with Cd(II) and Cu(II).

3. Ni(II) biosorptive capacity of *P. versicolor* was higher than those obtained in previous studies with different fungi, as well as than that obtained with *P. chrysosporium*.

4. In the case of Pb(II) removal, biosorption by both *P. versicolor* and of *P. chrysosporium* was enhanced by increasing initial Pb(II) concentration.

5. In these fungi, adsorptive capacities for the metals tested were generally induced by increasing initial metal ion concentration, but there was a partial metal release at high initial concentrations for Cr(III), Cd(II) and Ni(II).

REFERENCES

Brierley, J. A. (1990). Production and application of *Bacillus*-based product for use in metals biosorption. In: *Biosorption of Heavy Metals*, Volesky, B. (ed). CRC Press, Boca Raton, FL, 305-312.

Brierley, J. L., Brierley, C. L. and Goyak, G. M. (1986). A new wastewater treatment and metal recovery technology. *Amt-Bioclaim*. In: *Fundamental and Applied Biohydrometallurgy*, Lawrence, R. W., Branion, R. M. R. and Ebner, H. G. (eds). Elsevier, Amsterdam, 291-304.

Brierley, C. L. and Brierley, J. A. (1993). Immobilization of biomass for industrial application of biosorption. In: *Biohydrometallurgical Technologies*, Torma, A. E., Apel, M. L. and Brierley, C. L. (eds). The Minerals, Metals & Materials Society: Warrendale, PA, **2**, 35-44.

Beveridge, T. J. (1986). The immobilization of soluble metals by bacterial walls. In: *Biotechnology and Bioengineering Symposium No. 16 Biotechnology for the Mining, Metal-Refining, and Fossil Fuel Processing Industries*, Ehrlich, H. L. and Holmes, D. S. (eds). J. Wiley Interscience, New York, 127-140.

Fourest, E. and Roux, J. C. (1992). Heavy metal biosorption by fungal mycelial by-products: mechanism and influence of pH. *Appl. Microbiol. Biotechnol.* **37**, 399-403.

Gadd, G. M., White, C. and de Rome, L. (1988). Heavy metal and radionuclide uptake by fungi and yeasts. In: *BioHydroMetallurgy: Proceeding of the International Symposium*, Norris, P. R. and Kelly, D. P. (eds). Sci. Tech. Letters, Kew, Surrey, UK, 421-436.

Holan, Z. R. and Volesky, B. (1994). Biosorption of lead and nickel by biomass of marine algae. *Biotechnol. Bioeng.* **43**, 1001-1009.

Holan, Z. R. and Volesky, B. (1995). Accumulation of Cd(II), Pb(II) and Ni(II) by fungal and wood biosorbents. *Appl. Biochem. Biotechnol.*, **53**(2), 133-146.

Livernoche, D., Jurasec, L., Desrocher, M. and Dorica, J. (1983). Removal of color from kraft mill wastewaters with cultures of white-rot fungi and immobilized mycelium of *Coriolus versicolor. Biotechnol. Bioeng.*, **15**, 2055-2065.

Niu, H., Xu, X. S., Wang, J. H. and Volesky, B. (1993). Removal of Pb(II) from aqueous solutions by *Penicillium* biomass. *Biotechnol. Bioeng.* **42**, 785-787.

Mattuschka, B. and Straube, G. (1993). Biosorption of metals by waste biomass. *J. Chem. Tech. Biotechnol.* **58**, 57-63.

Mattuschka, B., Junghaus, K. and Straube, G. (1993). Biosorption of metals by waste biomass. In: *Biohydrometallurgical Technologies*, Torma, A. E., Apel, M. L. and Brierley, C. L. (eds). The Minerals, Metals & Materials Society , Warrendale, PA, **2**, 125-132.

Nakajima, A. and Sakaguchi, T. (1986). Selective accumulation of heavy metals by microorganisms. *Applied Microbial Biotechnol.* **24**, 59-64.

Pighi, L., Pumpel, T. and Schinner F. (1989). Selective accumulation of silver by fungi. *Biotech. Lett.* **11**, 275-280.

Prouty, A. L. (1990). Bench-scale development and evaluation of a fungal bioreactor for color removal from bleach effluents. *Appl. Microbiol. Biotechnol.* **32**, 490-493.

Tobin, J. M., Cooper, D. G. and Neufeld, R. J. (1984). Uptake of metal ions by *Rhizopus arrhizus* biomass. *Appl. Environ. Microbiol.* **47**, 821-824.

Townsley, C. C., Ross, I. S. and Atkins, A. S. (1986). Biorecovery of Metallic residues from various industrial effluents using filamentous fungi. In: *Fundamental and Applied Biohydrometallurgy*, Lawrence, R. W., Branion, R. M. R. and Ebner, H. G. (eds). Elseviver, Amsterdam, 279-289.

Volesky, B. (1987). Biosorbents for metal recovery. *Trends Biotechnol.* **5**, 96-100.