Biosorption of Ni(ii) and Pb(ii) by *Phanerochaete chrysosporium* from a binary metal system - Kinetics

I Haluk Çeribasi and Ülkü Yetis*
Environmental Engineering Department, Middle East Technical University, 06531 Ankara, Turkey

Abstract

The biosorption kinetics of Ni(II) and Pb(II) by the resting cells of a lignolytic white-rot fungus, *Phanerochaete chrysosporium*, from a binary metal system were investigated. Kinetic studies revealed that biosorption takes place in two stages: a rapid surface adsorption, within the first 30 min, and a slow intracellular diffusion till the end of the 3 h contact time. In the first minutes of contact solution pH decreased sharply, parallel to the fast metal uptake, probably because of the protons released by the biosorbent. As sorption equilibrium was reached, solution pH also reached an equilibrium level. Metal biosorption capacities increased as the initial metal concentrations (C_i) increased, independent of initial pH (pH_i) and generally the metal with higher C_i had a higher uptake capacity. The results also show that some portion of the metal ions sorbed by *P. chrysosporium* was readily released to solution with a decrease in pH. At equilibrium, the maximum total metal uptake of *P. chrysosporium* was 109.5 mg/g and was reached at pH_i 5. Under these circumstances Ni(II) and Pb(II) uptake capacities were 55.9 mg Ni/g and 53.6 mg Pb/g, respectively.

Introduction

Heavy metal releases to the environment have been increasing continuously as a result of industrial activities and technological development, posing a significant threat to the environment and public health because of their toxicity, accumulation in the food chain and persistence in nature. It is therefore important to develop new methods for metal removal and recovery from dilute solutions (1 to 100 mg/l) and for the reduction of heavy metal ions to very low concentrations. The use of conventional technologies, such as ion exchange, chemical precipitation, reverse osmosis and evaporative recovery, for this purpose is often inefficient and/or very expensive (Chong and Volesky, 1995; Leusch et al., 1995; Spinti et al., 1995; Wilde and Benemann, 1993; Yu and Kaewsarn, 1999; Zhao et al., 1999).

In recent years, the biosorption process has been studied extensively using microbial biomass as biosorbents for heavy metal removal. In these studies, metal removal abilities of various species of bacteria, algae, fungi and yeasts were investigated (Chen and Yiacaumi, 1997; Guibal et al., 1992; Veglio and Beolchini, 1997; Yetis et al., 2000). Biosorption consists of several mechanisms, mainly ion exchange, chelation, adsorption, and diffusion through cell walls and membranes (Churchill et al., 1995; Kuyucak and Volesky, 1988), which differ depending on the species used, the origin and processing of the biomass and solution chemistry.

The exact mechanism by which micro-organisms take up metals is relatively unclear, but it has been demonstrated that both living and non-living fungal biomass may be utilised in biosorptive processes, as they often exhibit marked tolerance towards metals and other adverse conditions such as low pH (Gadd, 1990; Standberg et al., 1981; Volesky et al., 1993). Although metal removal from industrial effluents by means of biosorption has been studied extensively (Gadd, 1990; Volesky and Holan, 1995), very few studies have been carried out that examine the biosorbent characteristics of the white-rot fungi.

The purpose of this study is to investigate the use of *Phanerochaete chrysosporium* type white-rot fungus as the biosorbent, which is also employed for the treatment of industrial effluents containing chlorinated organics, such as the pulp and paper industry (Barr and Aust, 1994; Kirby et al., 1995; Mittar et al., 1992), for heavy metal removal from wastewaters having more than one metal in their constituents. A biological process of wastewater treatment by white-rot fungi, such as *P. chrysosporium*, continuously produces waste sludge of fungal mass, which needs to be appropriately disposed of. Thus, the main objective of selecting this type of fungus for studying biosorption is assessing the possibility of utilising the waste sludge for removal of heavy metals from industrial effluents, before disposal.

Multimetal biosorption systems would more closely represent the composition of industrial effluents, since these effluents generally include more than one metal. However, relatively few studies on multimetal systems have been reported, though multimetal competitive interactions in solution with the sorbent material are amongst the basic factors affecting the degree of metal removal by biosorption. With the help of multi-metal biosorption studies, these complex systems and their behaviour will be better understood. Accordingly, this study examines the biosorption of Pb(II) and Ni(II) from a binary metal system. Lead and Ni(II) are known environmental pollutants and are frequently encountered together in industrial wastewaters, such as from mine drainage, metal plating, paint and ink formulation and porcelain enamelling.

There is more than one variable affecting the biosorption process, such as temperature, pH, agitation rate and metal concentration (Chen and Yiacaumi, 1997; Gadd, 1990; Veglio and Beolchini, 1997). Some of these factors (e.g. pH and metal concentration) have greater influence on metal removal by this process (Veglio and Beolchini, 1997; Volesky and Holan 1995; Yetis et al., 2000). In this study the effects of initial Pb(II) and Ni(II) concentrations (C_i(Pb) and C_i(Ni)) and pH (pH_i)on metal removal and sorptive capacity of microbial biomass have been investigated using the resting cells of the *P. chrysosporium* that are in the middle

* To whom all correspondence should be addressed.
E-mail: uyetis@metu.edu.tr
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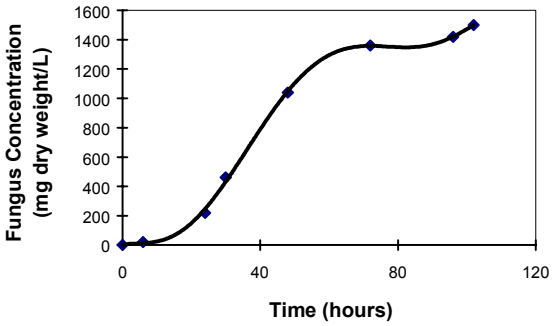

Figure 1
Growth curve of Phanerochaete chrysosporium

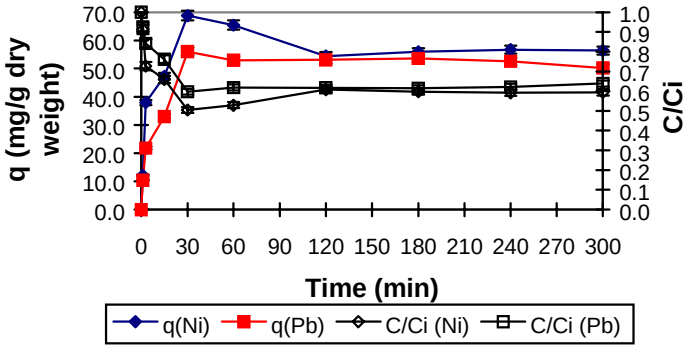

Figure 2
Time course of Pb(II) and Ni(II) sorption by P. chrysosporium
(C_i(Ni) = C_i(Pb) = 50 mg/l, pH_i = 5)

of the accelerated growth phase. The kinetics of metal removal were studied, and optimum conditions for Ni(II) and Pb(II) uptake by *P. chrysosporium* were determined.

Materials and methods

Biomass preparation

In order to prepare *Phanerochaete chrysosporium*, stocks grown on Sabaraud Dextrose Agar, as described by Prouty (1990), were used. *P. chrysosporium* inoculated stocks were treated with sterile water and homogenised under aseptic conditions. Optical density of the homogenised fungus solution was adjusted to 0.5 at 650 nm wavelength using a Jenway 6105 U.V./VIS. spectrophotometer to inoculate approximately the same amount of cells into each flask containing 250 ml growth media. The pH of the growth media was adjusted to 4.5 by using 1N H_2SO_4.

Following the inoculation, the flasks were incubated at an agitation rate of 200 $r.min^{-1}$ at 35°C for 41 h. This incubation time corresponds to the middle of the accelerated growth phase of the biomass (Fig. 1). The increase observed in micro-organism concentration after 90 h occurs because of the diauxic growth of fungal mycelium. Thus, the fungus growth consists of two exponential growth phases: Phase 1: 10 to 65 h and Phase 2: 100 to 150 h (Yetis et al., 2000). Selection of this 41 h cultivation period was based on the findings of the previous studies carried out with the same biosorbent for the removal of Pb(II) and Ni(II) from single-metal systems (Dolek, 1997), which indicated that the maximum biosorption capacities were attained after 41 h of incubation. Morphological observations revealed that cells have a somewhat spherical and bead-like shape with a diameter of about 1.5 to 2 mm.

Analytical methods

Biomass measurements were made gravimetrically, by filtration of the cell suspension samples through 0.45 µm membrane filters. Metal analyses were performed using a Unicam Model 929 atomic absorption spectrophotometer. The detection limits for Pb(II) and Ni(II) were 3 to 25 mg/l, and 0.5 to 6 mg/l, respectively, and measurement results were expressed as three significant digits. All experiments were run in duplicate and the arithmetic averages of the results were considered in data analysis. Metal uptake (q) was calculated from; q (mg/g dry biomass) = $V(C_i-C_t)/m$, where "V" is the sample volume (L), "C_i" and "C_t" are the initial and final metal concentrations (mg/l), respectively, and "m" is the amount of dry biomass (g) (Volesky and May-Philips, 1995).

Kinetics of metal uptake by resting cells

Kinetic studies were carried out in batch reactors. Resting cells of 90 mg/g dry weight, which were prepared and measured as described previously, were added to 250 ml metal solutions in 500 ml sorption flasks. These were incubated at the same temperature (35°C) and agitation rate (200 r/min) at which the biomass had been grown. The initial pH of the metal solutions with varying Ni(II) and Pb(II) concentrations (between 10 and 50 mg/l) was adjusted to pH values ranging from 4 to 6 using dilute HNO_3 and NaOH before adding the biomass. Thus, the effects of initial metal concentrations and pH on the biosorptive capacity of the biomass and the metal removal rates were investigated. For preparation of the metal solutions, the metal salts of $NiCl_2.6H_2O$ and $Pb(NO_3)_2$ (Merck) were used throughout the study.

Samples (10 ml) were taken from these flasks at different time intervals such as, 15, 30 and 60 min and acidified with HNO_3 to a pH of less than 2 and stored at 4°C in the dark for at most two days. The samples were then subjected to metal analysis. At each sampling, the solution pH was also measured in order to follow the change of pH during sorption. In addition, to determine the metal uptake of the biomass, metal solutions were filtered after the last sample had been taken (after 5 h of sampling). The biomass was retained and the filter paper was dried to constant weight and digested by the addition of 5 ml of concentrated HNO_3 at 700°C. These digested samples were resuspended in 25 ml of de-ionised water and stored under the conditions specified above for metal analyses.

Results and discussion

Kinetic studies revealed that maximum biosorption capacities and metal removal efficiencies for Ni(II) and Pb(II) were achieved generally in the first 30 min of contact. Metal removal and sorption were also rapid during this period. Figure 2 shows the time course of biosorption, when the initial pH (pH_i) was 5 and the initial metal concentrations (C_i) were 50 mg/l. In the first 5 min, sorption takes place very rapidly and then it continues at a relatively slower rate up to maximum sorption. Equilibrium was reached in a contact time of 3 h.

This figure also verifies that sorption takes place in two stages: a very rapid surface adsorption and a slow intracellular diffusion. Similar results were reported by Chen and Yiacaumi (1997), Sing and Yu (1998), and Volesky and Holan (1995), while in some other studies single-step uptake was suggested for different biosorbents (Huang et al., 1990).

Some portion of the metal ions adsorbed to the surface of the biosorbent during the first rapid sorption phase were released to solution till equilibrium was reached. Thus, the equilibrium metal removal and sorption capacities were generally less than the maximum values reached during the first phase and the contact time required for the maximum total metal removal was generally 30 min.

It can be seen that maximum initial sorption rates of both metals were maximum at pH_i 4 (Table 1). The initial sorption rate of Pb(II) was maximum (2.45 mg Pb/g-min), when C_i(Ni) was 20 mg/l and C_i(Pb) was 50 mg/l. When initial concentrations of both metals were 50 mg/l, maximum initial sorption rate for Ni(II) (2.6 mg Ni/g-min) was observed. At all pH_i levels, initial sorption rates increased with increasing metal concentrations. In addition, when C_i(Pb) and pH_i were constant, Ni(II) sorption rates increased with increasing C_i(Ni).

At all pH_i levels, maximum Pb(II) removal efficiencies were observed when C_i(Pb) was 10 mg/l. The highest removal was at pH_i 6, when initial metal concentrations were 10 mg/l. Under these conditions, 70.7% of Pb(II) and 46.9 % of Ni(II) removal was achieved. Nickel removal from the solution was maximum, when C_i(Ni) was 10 mg/l, independent of pH_i. The maximum removal rate was 70.6 % and reached at pH_i 6, when C_i(Pb) was 50 mg/l. Lead removal was 47.3 % under these conditions. Total metal removal efficiency was almost the same for both of these cases.

TABLE 1
Change of initial sorption rates with the initial metal concentrations and pH

	Initial sorption rate (mg Pb/g-min)			Initial sorption rate (mg Ni/g-min)		
pHi = 4 Ci(Pb) (mg/l)Ci(Pb) (mg/l)						
C_i(Ni) (mg/l)	10	20	50	10	20	50
10	0.29	0.65	0.58	0.18	0.05	0.20
20	0.33	0.55	2.45	0.61	0.87	0.78
50	0.55	0.80	1.86	1.15	2.27	2.60
pH_i = 5 C_i(Pb) (mg/l)C_i(Pb) (mg/l)						
C_i(Ni) (mg/l)	10	20	50	10	20	50
10	0.57	0.32	0.68	0.41	0.38	0.40
20	0.45	0.44	0.91	0.79	0.83	0.75
50	0.12	0.39	1.87	1.82	1.81	2.29
pH_i = 6 C_i(Pb) (mg/l)C_i(Pb) (mg/l)						
C_i(Ni) (mg/l)	10	20	50	10	20	50
10	0.65	1.18	2.10	0.44	0.18	0.65
20	0.64	0.60	1.63	0.76	0.83	0.80
50	0.44	0.84	1.84	1.45	1.55	1.56

Nickel and Pb(II) sorption capacities of *P. chrysosporium* reached their maximum levels in the first 30 min of contact when sorption was rapid. The maximum capacity for both metals was observed at pH_i 4. Nickel sorption capacity was 77.96 mg/g, when initial metal concentrations were 50 mg/l. The highest sorption capacity for Pb(II) was 73.56 mg/g, at an initial Ni(II) concentration of 20 mg/l and Pb(II) concentration of 50 mg/l. Figure 3 presents the change of metal uptake capacities with pH_i, at the initial metal concentrations that provided the maximum capacities. Generally, biosorption capacities for both of the metals increased with increasing initial metal concentration. When initial Pb(II) concentration increased, while initial Ni(II) concentration was constant, the Ni(II) sorption capacity also increased at all pH_i levels studied. However, the opposite was only valid at a pH_i of 5.

In multimetal systems initial metal concentrations and pH are the most important factors affecting the biosorption apart from the characteristics of the biosorbent (Gadd, 1990; Volesky et al., 1993; Chong and Volesky, 1995). It is a fact that pH of the solution influences both the ionic forms of metals and the ligands for binding of metals at the cell surface (Guibal et al., 1992; Zhou and Kill, 1991), strongly affecting the biosorption (Huang et al., 1990; Mameri et al, 1999; Sing and Yu, 1998; Yetis et al., 2000). These factors affected the removal of metals from the Ni(II)-Pb(II) binary metal system by *P. chrysosporium* in various interrelated ways.

At the pH_i levels of concern, Ni(II) and Pb(II) uptake capacities of *P. chrysosporium* were directly proportional to the initial metal concentrations. Generally, metals with a higher initial concentration have a higher sorption capacity. Initial pH affected the sorption of Ni(II) and Pb(II), but depended on the initial concentrations of metals.

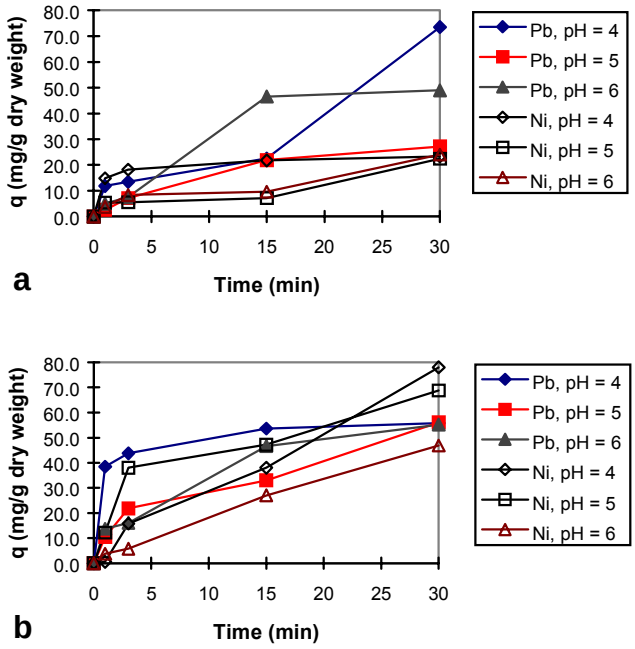

Figure 3
Change of metal sorption capacities with pH_i in the first 30 min of contact at initial metal concentrations of a) C_i(Ni) = 20 mg/l, C_i(Pb) = 50 mg/l, b) C_i(Ni) = C_i(Pb) = 50 mg/l

The highest equilibrium sorption capacities for Ni(II) and Pb(II) were observed at pH_i 4 and 5 for Ni(II) and Pb(II), respectively.

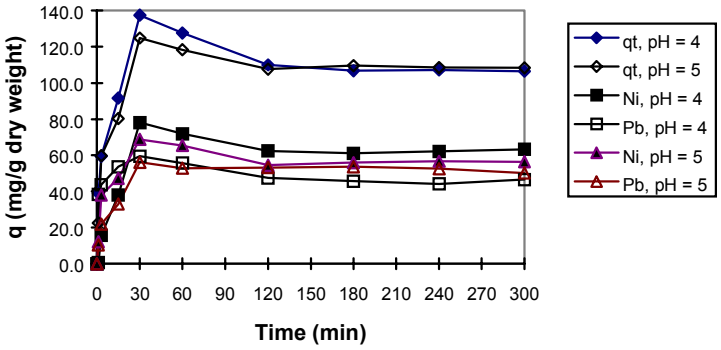

Figure 4
Kinetics of Ni(II), Pb(II) and total metal uptake with respect to initial pH (C_i(Ni) = C_i(Pb) = 50 mg/l)

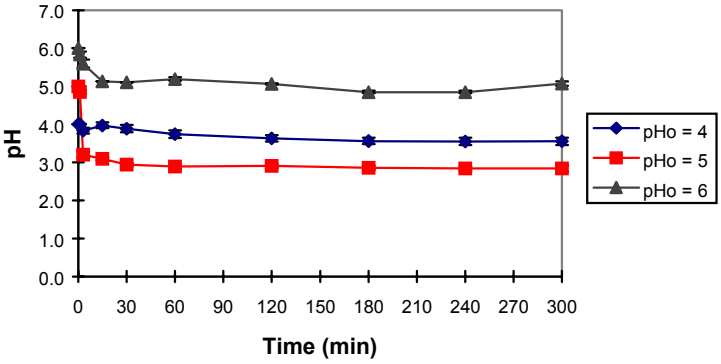

Figure 5
Change of solution pH with time during biosorption with respect to initial pH levels (C_i(Ni) = C_i(Pb) = 50 mg/l)

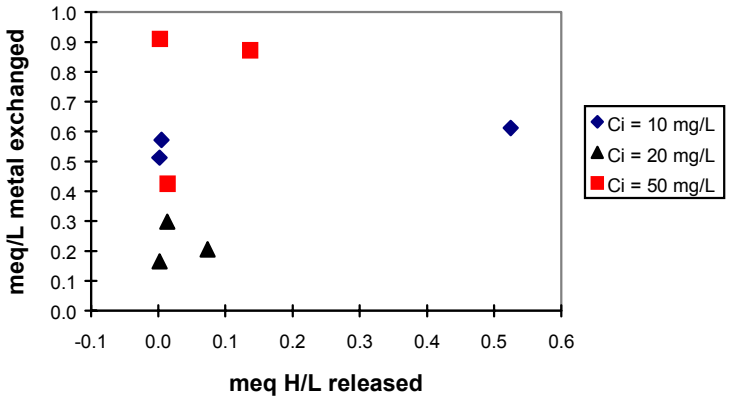

Figure 6
Quantity of total metal sorbed vs. protons released with respect to initial Ni(II) and Pb(II) concentrations

The highest equilibrium uptake capacities were: 64.13 mg Ni/g and 53.58 mg Pb/g. The highest capacity for Ni(II) was determined at C_i(Ni) = 50 mg/l and C_i(Pb) = 20 mg/l, while maximum Pb(II) sorption was observed when initial concentrations of both metals were 50 mg/l. It should be noted that when C_i of both metals was 50 mg/l, Ni(II) sorption capacity was 61.15 mg/g (pH_i = 4), which was very close to the maximum capacity observed. On the other hand, equilibrium sorption capacities determined at pH_i 6 was almost half of these maximum capacities (26.9 mg Ni/g and 26.99 mg Pb/g). This decrease may be because of the hydrolysis of metals and complex formation (Fourest and Roux, 1992). Also, microprecipitation of Ni(II) and Pb(II) was reported above pH_i 5 and 6, respectively, by Veglio and Beolchini (1997), and Volesky and Holan (1995).

Total equilibrium metal uptake capacities determined at pH_i 4 and 5 were very close with values of 106.8 and 109.5 mg/g dry weight, respectively. These capacities were observed when initial metal concentrations were 50 mg/l, and the equilibrium capacities, at pH_i 5, for Pb(II) and total metal uptake were the maximum capacities observed. Figure 4 shows the kinetics of Ni(II), Pb(II) and total metal uptake with respect to initial pH and similar trends were observed for the sorption of these components. Therefore, it can be seen that both Ni(II) and Pb(II) sorption by *P. chrysosporium* evolves in a similar way and none of these metals have a relatively greater effect on metal uptake of *P. chrysosporium*.

In the first 15 min of contact, during which sorption was very rapid, solution pH decreased sharply and generally reached equilibrium in 1 h of contact. The pH levels reached at equilibrium varied form 3 to 5 depending on initial pH and metal concentrations. The change of solution pH with time at various initial pH levels is presented in Fig. 5 for the initial metal concentrations that provided the highest capacities.

The probable reason for this rapid decrease in solution pH is the release of protons by the biosorbent during the sorption of Ni(II) and Pb(II). A comparison between the quantities of Ni(II), Pb(II) and total metal sorbed as well as the protons released was made to determine the extent of ion exchange (Fig. 6). It can be seen that total metal uptake by the biosorbent was much higher than the quantity of protons released. Only at pH_i 6, when initial concentrations of both metals were 10 mg/l, protons released accounted for about 80 % of the total metal uptake. However, at higher initial metal concentrations (Fig. 7), released protons were only comparable with the amount of Pb(II) removed to a limited extent. These results imply that ion exchange is not the major mechanism of biosorption, though metal-binding mechanisms seem to involve the release of protons into solution (thus ion exchange), to some extent, as biosorption proceeds. Thus, some other mechanisms are involved in the release of protons during biosorption.

The studies carried out at pH_i 4 with the same initial concentrations of Ni(II) and Pb(II) revealed a higher sorption capacity for Ni(II), when compared with Pb(II). On the other hand, at pH_i 5 and 6, equilibrium sorption capacities determined for Ni(II) and Pb(II) were very close. Thus, it can be said that at pH_i 4, Ni(II) sorption is relatively more preferred by *P. chrysosporium*, while at other pH_i levels neither of the metals were better sorbed than the other. In addition, Ni(II) was sorbed before Pb(II) at pH_i 4 and 5, while at pH_i 6, Pb(II) sorption precedes Ni(II) sorption (Fig. 8). These findings show that as initial pH rises, metal sorption precedence of the biosorbent changes from Ni(II) to Pb(II).

A sorption-desorption experiment was also conducted to investigate the extent of intracellular accumulation by *P. chrysosporium* resting cells. In this experiment the initial pH was 5 and the initial metal concentrations were 20 mg/l. Following the metal sorption, fungi were harvested and subjected to acid treatment and a rapid

release of some portion of the sorbed metal was observed. The results showed that about 45% of Ni(II) and 40% of Pb(II) sorbed remained within the biomass cells (Fig. 9). This observation revealed that intracellular accumulation plays a significant role in metal removal from this binary system.

Conclusions

Biosorption was rapid in the first 15 min of contact, which implied chemisorption. Maximum metal uptake capacities were encountered in the first 30 min, which were higher than equilibrium capacities, and equilibrium was reached at the end of 3 h. Thus, biosorption is a two-stage process for both metals; a fast surface adsorption and relatively slow intracellular diffusion. Solution pH, which is one of the most significant operating parameters affecting the biosorption process, decreased sharply in the first 15 min of biosorption and generally reached equilibrium in a contact time of 1 h. The probable reason for this rapid decrease in solution pH is the release of protons by the biosorbent as biosorption proceeds.

Metals with higher C_i generally sorbed better by *P. chrysosporium* and higher sorption capacities were observed. In addition, as initial pH rises, metal sorption precedence of the biosorbent changes from Ni(II) to Pb(II). As initial metal concentration of both or one of the metals increases, metal sorption capacity also increases. These results show that *P. chrysosporium* is a potential biosorbent for the removal of metals from aqueous wastes including both Ni(II) and Pb(II), and optimum operating pH was 5 for biosorption of these metals.

The metal sorption mechanism seems to involve the release of protons into solution and ion exchange plays a role, to some extent, in the biosorption of metals by *P. chrysosporium* from this binary metal system. However, this is not expected to be the principle mechanism. After metal sorption, when fungi were subjected to acid treatment, some portion of the sorbed metal was rapidly released, indicating that acids can be used as effective desorbing agents.

This study provided a basis for the batch equilibrium studies, which will have an important contribution to the understanding of the biosorption mechanisms. In addition, the information obtained related to the kinetic behavior of this complex system would be useful in continuous flow sorption studies and thus, in the design of this treatment process for practical applications.

Acknowledgements

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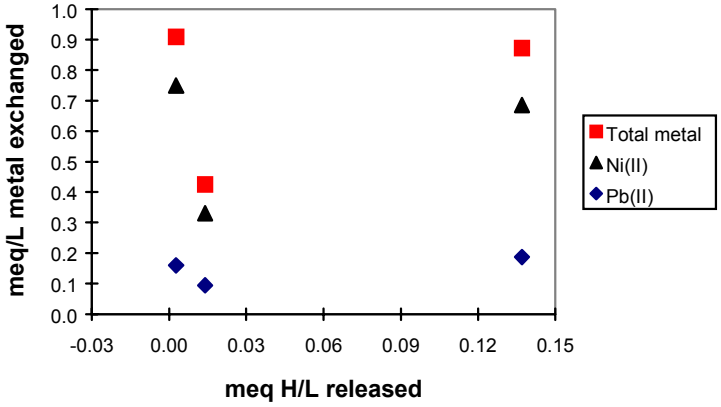

Figure 7
Quantity of Ni(II), Pb(II) and total metal sorbed vs. protons released (C_i(Ni) = C_i(Pb) = 50 mg/l

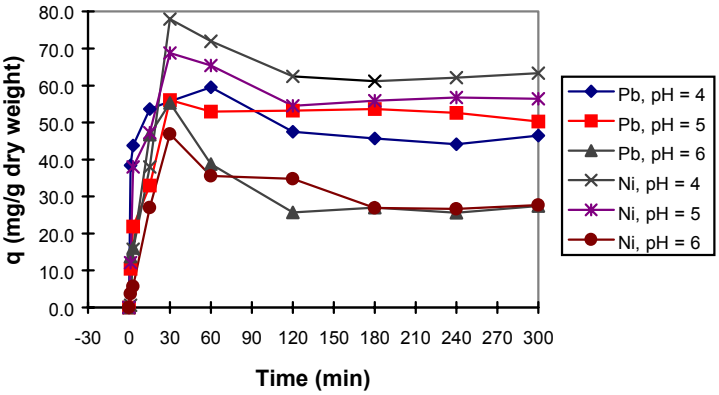

Figure 8
The effects of pH on the course of Ni(II) and Pb(II) sorption (C_i(Ni) = C_i(Pb) = 50 mg/l)

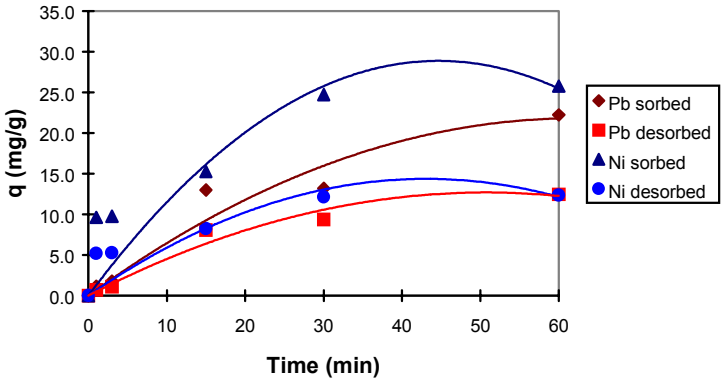

Figure 9
Sorption-desorption kinetics of Ni(II) and Pb(II) removal by P. chrysosporium *resting cells (pH_i = 5, C_i(Ni) = C_i(Pb) = 50 mg/l)*

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