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Ca-alginate as a support for Pb(II) and Zn(II) biosorption with immobilized *Phanerochaete chrysosporium*

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Abstract

The basidio spores of *Phanerochaete chryosporium* were immobilized in alginate gel beads, and the immobilized spore containing alginate beads were incubated for the growth of fungus. The biosorption of Pb^{2+} and Zn^{2+} ions on alginate beads and both immobilized live and heat inactivated fungus was studied from artificial waste waters in the concentrations range of 30–600 mg l^{-1}. The surface charge density of the biosorbents varied with the pH of the medium and the maximum biosorption of heavy metal ions on the biosorbents was obtained between pH 5.0 and 6.0. The biosorption of Pb^{2+} and Zn^{2+} on the biosorbents increased as the initial concentration of Pb^{2+} and Zn^{2+} ions increased in the medium. Biosorption equilibrium was established about 1 h, the adsorbed heavy metal ions did not significantly change further with time. The maximum biosorption capacity (q_m) of alginate beads and both immobilized live and heat inactivated fungus were 230, 282 and 355 mg for Pb^{2+} and 30, 37 and 48 mg for Zn^{2+} per gram of dry biosorbents, respectively. The experimental biosorption equilibrium data for Pb^{2+}, and Zn^{2+} ions were in good agreement with those calculated by Langmuir model. The affinity order of heavy metal ions was $Pb^{2+} > Zn^{2+}$.

Keywords: Heavy metals; Alginate; Immobilization; Fungal biomass; Biosorption; *Phanerochaete chrysosporium*

1. Introduction

Polysaccharide gel immobilized microorganisms can be used to remove heavy metal ions from aqueous solutions, providing an alternative to physico-chemical technologies for waste water treatment (Harel, de La Queriere, Mignot, & Junter, 2000). Alginate is a linear polysaccharides composed of (1 → 4)-linked residues of α-L-guluronic acid (G) and β-D-mannuronic acid (M), and is found in many algal species especially brown algae and is also produced by certain bacteria (Stokke, Smidsrød, Bruheim, & Skjåk-Bræk, 1991). The overall composition and the sequence of monomers in the polysaccharide vary extensively depending on the origin. This polyelectrolyte is soluble in water but precipitates in the form of a coacervate in the presence of multivalent metal ions like Ca^{2+}, Co^{2+}, Fe^{2+}, Fe^{3+} and Al^{3+}. Ion binding involves homopolymeric sequences of guluronate residues (termed 'G blocks') (Draget, Steinsvåg, Onsøyen, & Smidsrød, 1998; Moe, Skjåk-Bræk, Elgsaeter, & Smidsrød, 1993). Carbohydrate polymers such as alginate, chitosan, chitin and carboxymethyl cellulose have been mostly used as the matrix for the immobilization of microbial cells via entrapment (Jianlong, Horan, Stentiford, & Yi, 2000). These polymers are also known to bind metal ions strongly. Entrapment of microbial cells in these polymers support could also enhance microbial cell performance and adsorptive capacity of the biosorbent system for heavy metal ions (Yan & Viraraghavan, 2001).

Fungi are important organisms that have been widely used in industry for the bioconversion of many valuable compounds and for the biodegradation of wastewater pollutants (Mittar, Khanna, Marwaha, & Kennedy, 1992). The biosorption of heavy metals using various live and heat treated fungi has been studied. These studies showed that the biosorption capacity of the heat treated cells might be greater, equivalent or less than that of their living counterparts (Kapoor, Viraraghavan, & Cullimore, 1999; Sağlam et al., 2002). However, the use of heat treated fungal biomass in industrial application may offer some advantages over living cells, such as lower, sensitivity to heavy metal ions concentration and adverse operating conditions (i.e. pH

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and temperature) (Huang, Westman, Huang, & Morehart, 1988; Tobin, White, & Gadd, 1994). Immobilization of microbial cells also provides additional advantages over freely suspended cells. These include ease of regeneration and reuse of the biomass, easier solid–liquid separation and minimal clogging in continuous flow systems (Kapoor & Viraraghavan, 1995; Valdman, Erijman, Pessoa, & Leite, 2001; Zulfadhly, Mashitah, & Bhatia, 2001).

The main concern of this work was the enhancement of the adsorptive capacity of Ca-alginate beads for the removal of heavy metals ion from aqueous solution by combining with microbial cells. Information is available on the use of immobilized *Phanerochaete chrysosporium* biomass for the removal of Cd^{2+} and Hg^{2+} ions (Kacar et al., 2002) but no study has been conducted on the use of the immobilized biomass for removal of other heavy metal ions. Therefore there is a need to study the performance of the immobilized fungus system with the other heavy metal ions. *Phanerochaete chrysosporium* spores were entrapped into Ca-alginate beads. After vegetation of the entrapped spore in the matrix, the immobilized live and/or heat inactivated fungus were used for the removal of Pb^{2+}, and Zn^{2+} ions from aqueous solutions in a batch system. The effect of pH on biosorption capacities was characterized by measuring the adsorption isotherms of heavy metals ions with both immobilized fungal preparations under pH-controlled conditions. The maximum adsorption capacity of the both immobilized preparations, based on dry weight, was determined by varying the concentration of the Pb^{2+} and Zn^{2+} ions in the artificial waste waters.

2. Materials and methods

2.1. Microorganism and media

Phanerochaete chrysosporium (ATCC-3454) is a white-rot fungus (belong to basidiomycete group) was maintained by subculturing on malt dextrose agar slants as previously described (Sağlam, Say, Denizil, Patir, & Arıca, 1999).

2.2. Preparation of biosorbents

The immobilization of *P. chrysosporium* basidio spore via entrapment was carried out as described previously (Arıca, Kacar, & Genç, 2001). Briefly, Na-alginate (2.0 g; from *Macrosytia pyrifera*, high viscosity, Sigma Chem. Co., USA) was dissolved in distilled water and it was then mixed with the spore suspension (10 ml, about 1×10^9 basidio spore ml^{-1}). The fungus spore immobilized beads (~4 mm in diameter) were cured in this solution for 1 h and then washed twice with 200 ml sterile distilled water. The beads with the immobilized spore were then transferred to the growth medium and were incubated on an orbital shaker (150 rpm) at 30 °C for 5 days. After 5-day incubation, the Ca-alginate beads with immobilized fungal mycelia were removed from the medium by filtration and washed twice with distilled water. This washed biomass is hereafter called 'immobilized live fungus'. At times immobilized live fungus was heated in 5 mM $CaCl_2$ solution at 90 °C for 10 min and it will be referred to as immobilized heat treated fungus.

2.3. Biosorption studies

The biosorption of Pb^{2+} and Zn^{2+}, on the alginate and on the immobilized live and heat inactivated fungus from artificial waste waters containing single metal ions was investigated in batch biosorption-equilibrium experiments. The effects of the medium pH and the initial concentrations of heavy metals ions on the biosorption rate and capacity were studied.

The effect of pH on the biosorption rate of the immobilized fungal biomass with Pb^{2+} and Zn^{2+} was investigated in the pH range 3.0–7.0 (which was adjusted with HCl or NaOH at the beginning of the experiment and not controlled after wards) at 25 °C. Each heavy metal ions (100 mg l^{-1}) was prepared in distilled water (25 ml) and biosorbent was transferred to this medium and agitated magnetically at 400 rpm.

The effect of the initial Pb^{2+} and Zn^{2+} ions concentration on the biosorption was studied at pH 6.0 as described above except that the concentration of each heavy metal ions species in the adsorption medium was varied between 30 and 600 mg l^{-1}.

After the desired incubation period (about 90 min) the aqueous phases were separated from the materials and the concentrations of the metal ions in these phase were measured by using an Atomic Absorption Spectrophotometer (AAS; Shimadzu AA 6800, Japan). Deuterium background correction was used and the spectral slit width was 0.5 nm. The working currents/wavelengths for Pb^{2+} and Zn^{2+} were 10 mA/283.3 and 8 mA/213.9 nm, respectively. The instrument response was periodically checked with known metal solution standards. For each set of data present, standard statistical methods were used to determine the mean values and standard deviations. Confidence intervals of 95% were calculated for each set of samples in order to determine the margin of error.

2.4. Data treatment

The amount of metal ions adsorbed per unit empty or fungus immobilized alginate preparations (mg metal ions g dry biosorbent^{-1}) was obtained by using the following expression

$$Q = [(C_o - C)\cdot V]/M \quad (1)$$

Q is the amount of metal ions adsorbed onto the unit amount of the biosorbents (mg g^{-1}). C_o and C are the concentrations of the Pb^{2+} or Zn^{2+} ions in the initial solution (mg l^{-1}) after

biosorption, respectively. V is the volume of the adsorption medium (l) and M is the amount of the biosorbent (g).

A known quantity of wet Ca-alginate or fungus immobilized preparations was used in the adsorption test. After the adsorption process, the beads were dried in an oven at 50 °C overnight and the dry weight of the preparations was used in the above equation. Each experiment was repeated three times and the results given are the average values.

In order to determine the reusability of the immobilized fungal preparations, consecutive adsorption–desorption cycles were repeated three times by using the same immobilized fungal preparations. Desorption of heavy metal ions was performed by 10 mM HNO_3 solution. The immobilized fungal preparations loaded heavy metal ions were placed in this desorption medium and stirred 400 rpm for 30 min at 25 °C. The final metal ion concentration in the aqueous phase was determined by using an AAS as described above. The desorption ratio was calculated from the amount of metal ions adsorbed on the immobilized preparation and the final metal ion concentration in the adsorption medium.

Desorption ratio was calculated from the following equation:

$$\text{Desorption ratio} = (\text{Amount of metal ions desorbed to the elution medium}) \times 100/(\text{Amount of metal ions adsorbed onto the beads}). \quad (2)$$

2.5. SEM studies

Sample of empty Ca-alginate and fungus immobilized beads were coated under vacuum with a thin layer of gold and examined by scanning electron microscopy (JEOL 5600).

3. Results and discussion

3.1. Properties of the alginate beads

In the present study, Ca-alginate beads and fungus immobilized form were prepared by liquid curing method in the presence of Ca^{2+} ions. Ca-alginate was used as an adsorbent and a support material for immobilization of *P. chrysosporium*; those were used for the removal of Pb^{2+} and Zn^{2+} from aqueous solution.

Alginate is a natural polymer and could be converted into hydrogels via crosslinking with divalent calcium cations. It was preferred over other materials because of its various advantages such as biodegradability, hydrophilicity, presence of carboxylic groups, and natural origin. The degradation of alginate by microorganisms in nature makes it a potential candidate for various uses in which it can be replaced polymers of petroleum origin. This is very important because polymers of petroleum origin are non-degradable and a major course of pollution. Heavy metal removal by polymeric ion-exchange resins is sensitive to the presence of Ca^{2+}, Mg^{2+} and K ions, whereas the fungal biomass does not adsorb ions such as Ca^{2+}, Mg^{2+} and K (Gadd, 1993). Thus, the use of immobilized fungus in alginate beads may be advantageous over the ion-exchange resins when Ca^{2+}, Mg^{2+} and K ions are present in an adsorption medium or industrial wastewater of high concentrations.

The SEM micrographs of the plain Ca-alginate beads and *P. chrysosporium* immobilized form were presented in Fig. 1 (A) and (B), respectively. The Ca-alginate bead is spherical shaped with a diameter about 4 mm. The SEM micrograph of fungus immobilized alginate beads were completely different from the plain beads and revealed a uniform fungal growth on the bead surface indicating that entrapment of basidio spore is not localized and led to a uniform growth on the beads surface. This uniform distribution is an important criterion for the proper biosorption of heavy metal ions on the whole surface area of the fungus immobilized beads. The amount of immobilized fungus in the support was 0.107 g gram

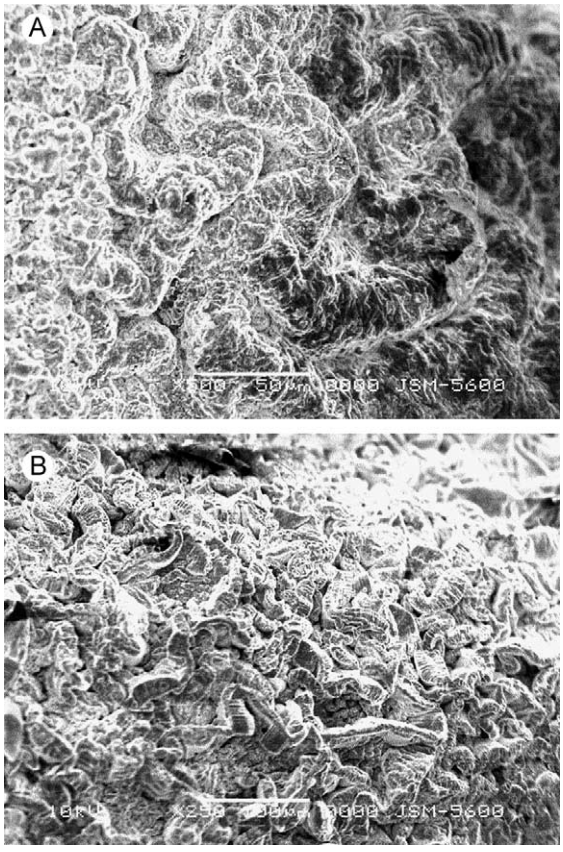

Fig. 1. SEM of Ca-alginate beads and fungus entrapped form. (A) Ca-alginate bead, (B) fungus entrapped form.

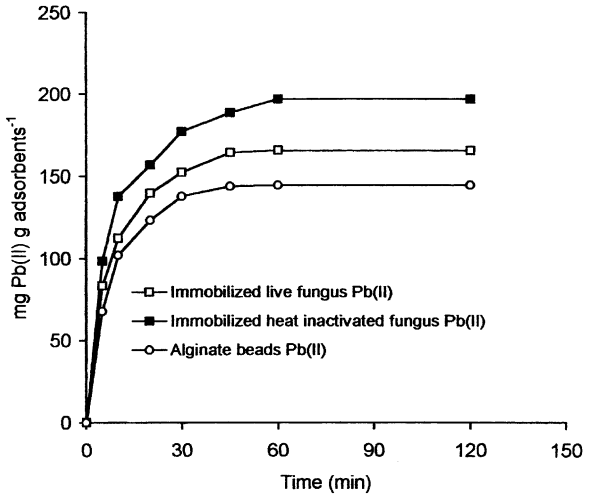

Fig. 2. Biosorption rates of Pb(II) on alginate and immobilized *Phanerochaete chrysosporium*:: pH 7.0, temperature, 25 °C.

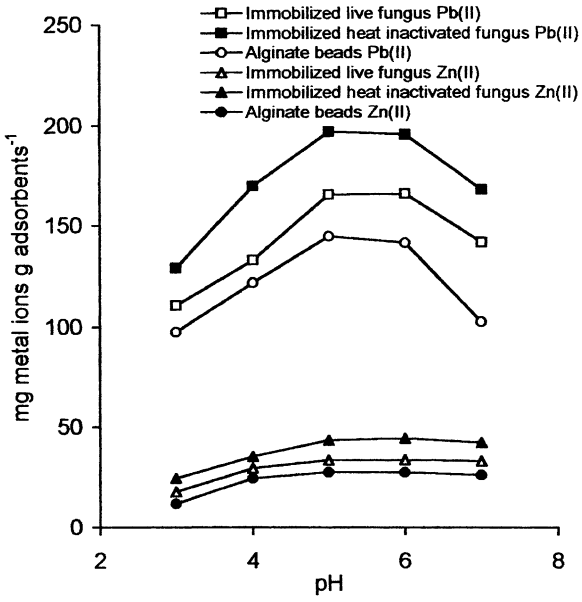

Fig. 3. Effect of pH on biosorption of Pb(II) and Zn(II) on alginate and immobilized *Phanerochaete chrysosporium*. Initial concentration of metal ions: 200 mg l^{-1}; temperature: 25 °C.

beads^{-1}. It was determined at the end of the five days cultivation period and no fungal biomass increase was detected after this period.

3.2. Biosorption time of Pb^{2+} and Zn^{2+}

Fig. 2 shows the exemplified biosorption time of Pb^{2+} on Ca-alginate and on the both immobilized live and heat treated preparations from solutions containing 200 mg l^{-1} of Pb^{2+} ions. As seen in the figure, the saturation levels were obtained after about 60 min. Note that in such a biosorption process, there are several parameters which determine the biosorption rate, such as the structural properties both of the support and biosorbent (e.g. protein and carbohydrate composition and surface charge density, topography and surface area). The amount of biosorbent, initial concentration of metal ions and existence of other ions (which may compete with the ions of interest for the active biosorption sites) also affect the biosorption rate. All these studies published in the literature have been carried out under different experimental conditions (Kaewsarn & Yu, 2001; Say, Denizli, & Arıca, 2001). Hence, it is difficult to compare them with the equilibrium adsorption times reported here.

3.3. Effect of pH and temperature on the biosorption capacity

Biosorption of heavy metal ions onto microbial biomass is affected by several factors, in term of the specific surface properties of the microbial cell wall and the physico-chemical properties of the adsorption medium such as metal ions concentration, temperature, pH and the amount of biomass (Gadd, 1993; Sağlam et al., 1999). The medium pH affects the solubility of metal ions and the ionisation state of the functional groups (i.e. carboxylate, phosphate, and amino groups) on the fungal cell wall (Kapoor et al., 1999; Sağlam et al., 2002; Tobin et al., 1994). The carboxylate and phosphate groups carry negative charges that allow the microbial cells to be potent scavengers of metal ions (Kapoor & Viraraghavan, 1997). The experimental results are presented in Fig. 3. In all cases the maximum heavy metal species adsorption occurred between pH 5.0 and 6.0. The amount of adsorbed heavy metal ions (Pb^{2+} and Zn^{2+} at 200 mg l^{-1}) on the biosorbents at pH 5.0 were found to be 144.4 and 21.5 mg g^{-1} for Ca-alginate, 165.2 and 34.2 for immobilized live fungus and 194.3 and 36.9 for inactivated immobilized preparation, respectively. There was an increase in metals ions adsorption per unit weight of biosorbents with increasing pH from 3.0 to 5.0, and seems to level off at pH greater than 6.0. At acidic pH (pH ≈3), protonation of the cell wall component adversely effect the biosorption capacity of the adsorbents, but its effect becomes minor with increasing pH of the aqueous medium. It has been proposed that the functional groups on the fungal cell walls responsible for metal binding are carboxyl groups, which have pK_a's between 3.0 and 4.0 (Esposito, Pagnanelli, Lodi, Solisio, & Vegilo, 2001). On the other hand, the pK_a values for the carboxylic groups of the α-L-guluronic acid and β-D-mannuronic acid residues of the alginate have been reported as 3.65 and 3.38, respectively (Haug, Larsen, & Smidsrød, 1974) are very close to the fungal cell walls. It should be noted that the metal ion binding properties of an alginate is primarily determined by its monomeric composition, and the length of the G-blocks (Draget, Skjåk-Bræk, & Smidsrød, 1994). The interaction of both heavy metal ions with Ca-alginate and immobilized fungus preparations could be primarily with the carboxylate groups of Ca-alginate and the cell wall component of the mycelia. Several

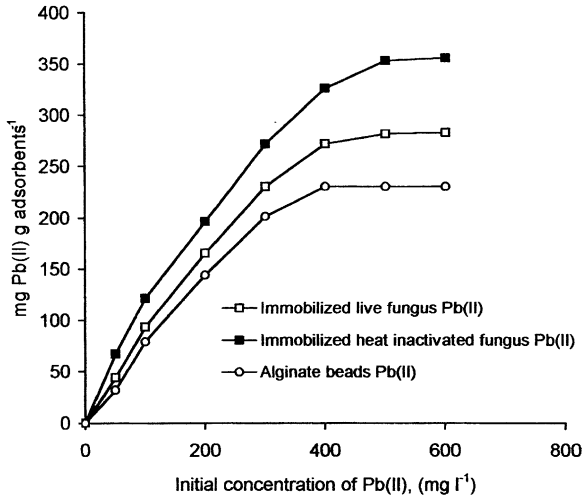

Fig. 4. Effect of initial Pb(II) ions concentration on biosorption capacity of alginate and immobilized *Phanerochaete chrysosporium*: pH 7.0; temperature, 25 °C.

researchers have investigated the effect of pH on biosorption of heavy metals by using different kind of microbial biomasses. For example, the biosorption of Cu^{2+} heat inactivated *Saccharomyces cerevisiae* was pH dependent and maximum biosorption was obtained in the pH range of 5.0–7.0 (Huang et al., 1988). The optimum pH for Zn^{2+} uptake was 6.0 for immobilized *Zoogloea ramigera* cells (Park, Jim, & Chang, 1999). The biosorption of Zn^{2+} and Cu^{2+} on the algal surface was pH dependent and the pH values for both heavy metals are of the same order of magnitude (Xue, Stumm, & Sigg, 1988).

The biosorption of Pb^{2+} by Ca-alginate and both immobilized live and heat treated fungus forms appears to be temperature independent in the temperature range between 15 and 45 °C and the maximum binding capacity values were almost the same for all the tested biosorbents.

3.4. Effects of initial concentration of metals ions on the adsorption capacity

The biosorption capacities of Pb^{2+} and Zn^{2+} onto the Ca-alginate and both live and inactive immobilized *Phanerochaete chrysosporium* are given in Figs. 4 and 5, respectively, as a function of the initial concentration of heavy metals ions within the aqueous phase. The biosorption capacity of the biomass increased first with increasing of the initial concentration of metals ions and reached a saturation value. These saturation values are around 500 mg l^{-1} for Pb^{2+} and 300 mg l^{-1} for Zn^{2+}. As seen in this figure, the amount of Pb^{2+} and Zn^{2+} ions was adsorbed on the plain alginate beads, which was 230.0 and 28.9 mg g dry alginate beads^{-1}, respectively. While, entrapment of fungus in the Ca-alginate beads led to a significant increase adsorption capacity of the sorbent up to 282.5 and 37.1 mg g^{-1} dry biosorbent, these were further increased up to 355.6 and 47.7 mg for Pb^{2+} and Zn^{2+} ions per g biosorbent when immobilized heat treated fungus used in the adsorption test, respectively. All the tested biosorbents showed a high affinity for Pb^{2+} ions than that of Zn^{2+} ions. Similar results have been reported in the literature. For example, Foster (1976) reported that Pb^{2+} ions was found to bound to alginates more strongly than any other metal ions. In a previous study, the biosorption capacities of the alginate based biosorbents for Hg^{2+} and Cd^{2+} ions were less than that of the presented results (Kacar et al., 2002). No definitive conclusions can be drawn for the high Pb^{2+}

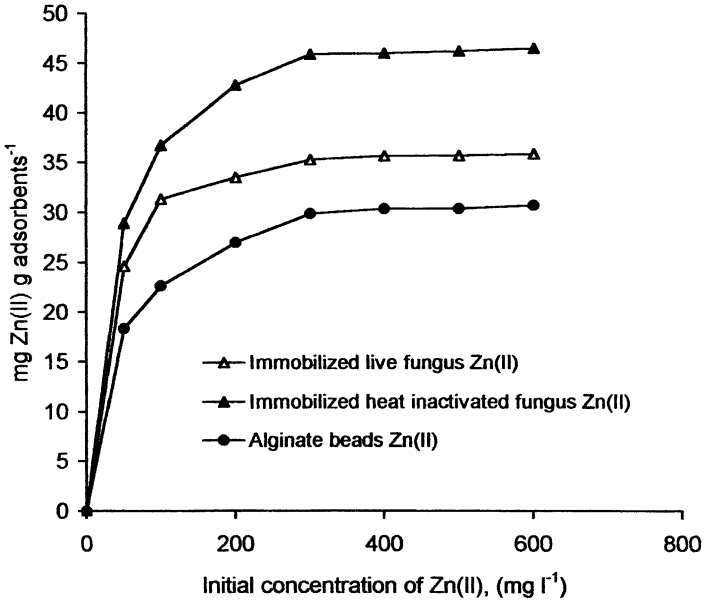

Fig. 5. Effect of initial Zn(II) ions concentration on biosorption capacity of alginate and immobilized *Phanerochaete chrysosporium*: pH 7.0; temperature, 25 °C.

biosorption capacity from these above finding and further studies are necessary to explain the biosoption mechanism.

The dead form of the immobilized fungus had a higher adsorption capacity for Pb^{2+} and Zn^{2+} ions than the living immobilized form. This might be due to an increase in the surface area for adsorption of Pb^{2+} and Zn^{2+} ions because of cell rupture upon death. Similar observations have been reported for other biomasses, including fungus and yeast (Gadd, 1993) and can be devoted to a variety of resistance mechanisms. These mechanisms include extracellular complexation with metal binding proteins such as metallothioneins and phytochelatins (which are proteins that contain large amounts of cysteine and bind heavy metal ions) (Tobin et al., 1994) and efficient pumping out if metal ions enter the cell. Compared with living cells, the dead cells may be stored or used for extended periods. They do not require a nutrient supply as well as cultural maintenance. They stand up harsh operation conditions such as high pH and temperature. Therefore, their operation and regeneration could be easily performed and also the dead cells could be effectively used as a biosorbents for the removal of heavy metals ion from water streams.

The adsorption capacity of the immobilized heat treated fungus reached 355.6 mg g^{-1} dry biosorbent with Pb^{2+} ions is comparable with the values reported in the previous studies. For example, the adsorption capacity of *Rhizopus arrhizus* was 78 mg for Fe^{3+}, 71 mg for Pb^{2+} and 62 mg for Zn^{2+} per g dry biomass (Özer, Ekiz, Özer, Kutsal, & Caglar, 1997). The biosorption capacity of *Aspergillus flavus* was 46 mg for uranium per g dry biomass (Hafez, Abdel-Razek, & Hafez, 1997). The living *Aureobasidium pollulans* and *Saccharomyces cerevisiae* were used for removal of Pb^{2+} the adsorption capacities were about 170.6 and 95.3 mg per g dry biomass, respectively (Suh & Kim, 2000). *Mucor rouxii* was immobilized in polysulphone matrix, the adsorption capacities of the biosorbent system for Pb^{2+} and Zn^{2+} ions were 4.06 and 3.76 mg per g biosorbent, respectively (Yan & Viraraghavan, 2001).

3.5. Langmuir and the Freundlich adsorption isotherms

Out of the several isotherm equations, the two most commonly used adsorption isotherms for biosorption studies (the Langmuir and the Freundlich adsorption isotherms) were investigated, which are widely used to analyse data for water and wastewater treatment applications. The Langmuir equation, which is valid for monolayer adsorption on to a surface, a finite number identical sites and the model described by the following equation:

$$q_{eq} = q_m C_{eq}/(k_d + C_{eq}) \quad (3)$$

where C_{eq} and q_{eq} show the residual metal concentration and the amount of metal adsorbed on the adsorbent at equilibrium, respectively, $k_d = k_2/k_1$ is the Langmuir constant of the system. The semi-reciprocal plot of C_{eq}/q_{eq} versus C_{eq} was employed to generate the intercept of K_d/q_m and the slope of $1/q_m$ (Figs. 6 and 7).

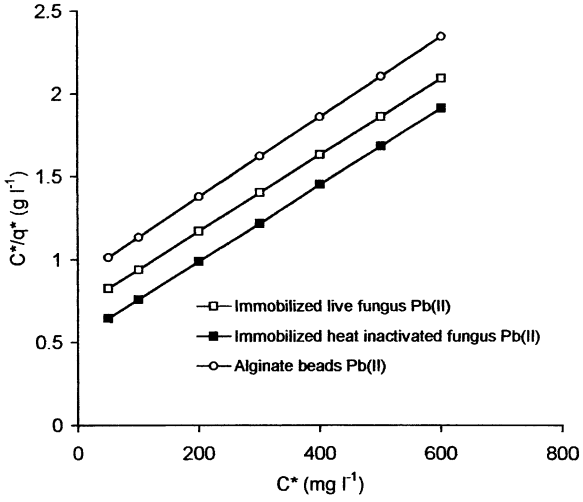

Fig. 6. Langmuir adsorption isotherm of Pb(II) ions on the adsorbents.

In addition, there may be interactions between adsorbed molecules, a phenomenon referred to as *cooperativity*. A molecule attached to a surface may make it more, or less, difficult for another molecule to become attached to a neighbouring site, and this would lead to a deviation from the ideal adsorption equation. The empirical Freundlich equation based on the amount of a substance adsorbed (q_{eq}) is related to the concentration C_{eq} by the equation:

$$q_{eq} = K_F(C_{eq})^{1/n} \quad (4)$$

where K_F and n are the Freundlich constants characteristic of the system. K_F and n are indicators of adsorption capacity and adsorption intensity, respectively. The slope and intercept of the linear Freundlich equation are equal to $1/n$ and ln K_F, respectively.

Figs. 6 and 7 show the Langmuir plot for Pb^{2+} and Zn^{2+} ions by Ca-alginate and both immobilized live and heat treated form, respectively. The Langmuir constants

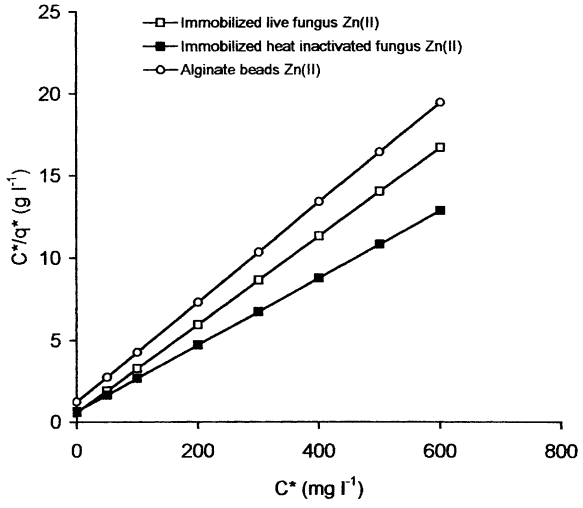

Fig. 7. Langmuir adsorption isotherm of Zn(II) ions on the adsorbents.

Table 1
Langmuir and Freundlich constants and correlation coefficients for biosorption of Pb(II) and Zn(II) ions on alginate and immobilized fungus (live or heat inactivated form)

Biosorbent	Langmuir			Freundlich		
	q_m (mg g^{-1})	k_d (M) ($\times 10^7$)	R^2	K_F	n	R^2
Alginate Pb(II)	264	2.33	0.995	0.42	1.47	0.951
Immobilized live fungus Pb(II)	330	7.87	0.997	0.67	1.61	0.954
Immobilized heat inactivated fungus Pb(II)	395	5.61	0.998	0.98	1.86	0.954
Alginate Zn(II)	33	4.91	0.999		3.27	0.984
Immobilized live fungus Zn(II)	39	4.53	0.999		3.67	0.991
Immobilized heat inactivated fungus Zn(II)	52	6.10	0.999		3.09	0.998

(q_m and k_d) along with correlation coefficients (R^2) have been calculated from the plots (Figs. 5 and 6) for biosorption of Pb^{2+} and Zn^{2+} ions on the biosorbents and the results are presented in Table 1. The maximum capacity q_m determined from the Langmuir isotherm defines the total capacity of the biosorbents for Pb^{2+} and Zn^{2+} ions. The order of maximum capacity (q_m) for the biosorbents for Pb^{2+} and Zn^{2+} removal was found as: immobilized heat treated fungus > immobilized live fungus > Ca-alginate (Table 1). The entrapment of fungus in Ca-alginate beads led to a significant increase in the q_m value with respect to Ca-alginate beads. It is clear that this increase in the q_m value is due to in an increase in the adsorptive sites on the biosorbents.

The apparent Langmuir constant (k_d) estimated from the intercept is a measure of the stability of the complex formed between metal ions and adsorptive surface layer of the biosorbents under specified experimental conditions. For example, a small k_d value indicates that the metal ion has a high binding affinity for the biosorbent. The (k_d) values are presented in Table 1. Although the apparent k_d values for the three biosorbents for Pb^{2+} and Zn^{2+} ions are of the same order of magnitude. The entrapment of fungus in alginate beads resulted about more than 3.0 fold reduction in the k_d values. Also, the heat treatment of the immobilized fungus lead to a further reduction in the k_d value. This can be due to an increase in the adsorptive sites by combining fungal cells with Ca-alginate leading to better affinity for heavy metal ions than that of the Ca-alginate.

The Freundlich plots for Pb^{2+} and Zn^{2+} adsorption by Ca-alginate and both immobilized live and heat treated form is presented in Figs. 8 and 9, respectively. The magnitude of K_F and n (Freundlich constants) show easy separation of metal ions from aqueous medium and indicate favourable adsorption. The intercept K_F value is an indication of the adsorption capacity of the adsorbent; the slope $1/n$ indicates the effect of concentration on the adsorption capacity and represent adsorption intensity. The magnitude of K_F and n values showed easy uptake of Pb^{2+} and Zn^{2+} from aqueous medium with the biosorbents (i.e. immobilized live and heat treated fungus) and resulted a high adsorption capacity than that of the Ca-alginate. As seen from Table 1 for all the experimentally tested biosorbents, n values were found high enough for separation.

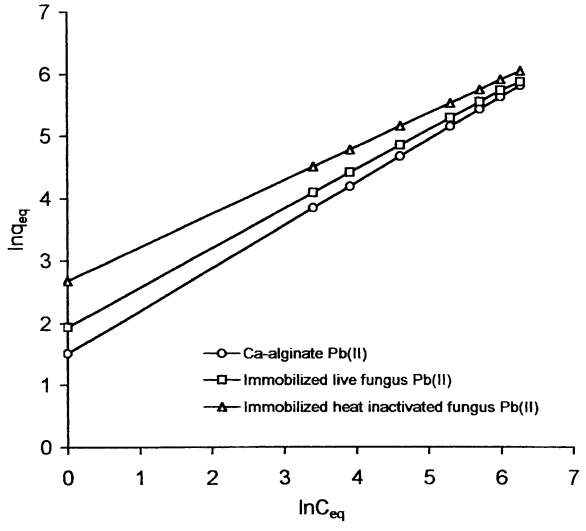

Fig. 8. Freundlich plot of Pb(II) ions on the alginate and immobilized *Phanerochaete chrysosporium*.

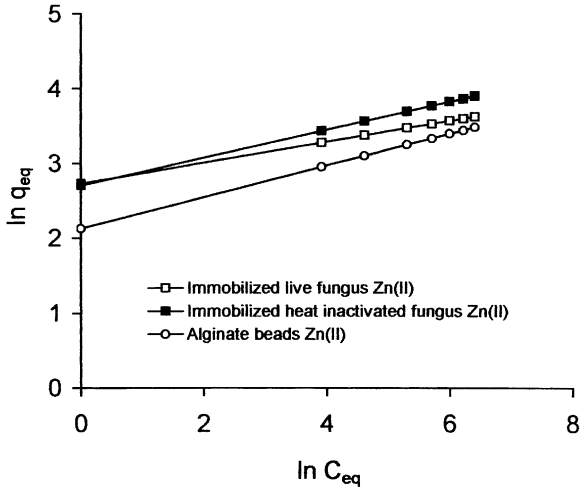

Fig. 9. Freundlich plot of Zn(II) ions on the alginate and immobilized *Phanerochaete chrysosporium*.

4. Conclusion

The results shows that Ca-alginate beads and both immobilized live and heat inactivated *P. chrysosporium* can be used as biosorbents for the effective removal of heavy metal ions from aqueous solutions. The mechanism and the kinetics of Pb^{2+} and Zn^{2+} ions biosorption on the biosorbents depend on the experimental conditions particularly medium pH and metals ion concentration. The time required to reach an equilibrium state was not significantly changed as the initial Pb^{2+} concentration increased. The biosorption capacity of the immobilized fungus was enhanced greatly when biosorption took place following heat treatment. As the pH increased, the metal biosorption capacity increased significantly up to pH 5.0. The distribution of Pb^{2+} ions between liquid phase and solid phase was analysed by Langmuir and the Freundlich isotherm models. The biosorbents can be regenerated and reused by acid treatment.

References

Arıca, M. Y., Kacar, Y., & Genç, 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 Technology*, *80*, 121–129.

Draget, K. I., Skjåk-Bræk, G., & Smidsrød, O. (1994). Alginic acid gels: The effect alginate chemical composition and molecular weight. *Carbohydrate Polymers*, *25*, 31–388.

Draget, K. I., Steinsvåg, K., Onsøyen, E., & Smidsrød, O. (1998). Na- and K-alginate; effect on Ca^{+2}-gelation. *Carbohydrate Polymers*, *35*, 1–6.

Esposito, A., Pagnanelli, F., Lodi, A., Solisio, C., & Veglio, F. (2001). Biosorption of heavy metals by *Sphaerotilus natants*: An equilibrium study at different pH and biomass concentration. *Hydrometallurgy*, *60*, 129–141.

Foster, P. (1976). Concentrations and concentration functions of heavy metals in brown algea. *Enviromental Pollution*, *10*, 45–56.

Gadd, G. M. (1993). Interaction of fungi with toxic metals. *New Phytology*, *124*, 25–60.

Hafez, N., Abdel-Razek, A. S., & Hafez, M. B. (1997). Accumulation of some heavy metals on *Aspergillus flavus*. *Journal of Chemical Technology and Biotechnology*, *68*, 19–22.

Harel, P., de La Queriere, G., Mignot, L., & Junter, G.-A. (2000). Mechanical properties of sugar beet Ca-pectate gel usable for cell immobilization and heavy metal accumulation. *Industrial Crops and Products*, *11*, 259–264.

Huag, A., Larsen, B., & Smidsrød, O. (1974). Uronic acid sequence in alginate from different sources. *Carbohydrate Research*, *32*, 217–225.

Huang, C.-P., Westman, D., Huang, C., & Morehart, A. L. (1988). The removal of Zn(II) from dilute aqueous solutions by fungal biosorbent. *Water Science and Technology*, *20*, 369–376.

Jianlong, W., Horan, N., Stentiford, E., & Yi, Q. (2000). The radial distribution and bioactivity of *Pseudomonas* sp immobilized in calcium alginate gel beads. *Process Biochemistry*, *35*, 465–469.

Kacar, Y., Arpa, C., Tan, S., Denizli, A., Genç, O., & Arıca, M. Y. (2002). Biosorption of Hg(II) and Cd(II) from aqueous solutions: Comparison of biosorptive capacity of alginate and immobilized live and heat inactivated *Phanerochaete chrysosporium*. *Process Biochemistry*, *37*, 601–610.

Kaewsarn, P., & Yu, Q. (2001). Cadmium(II) removal from aqueous solutions by pre-treated biomass of marine alga *Padina* sp. *Environmental Pollution*, *112*, 209–213.

Kapoor, A., & Viraraghavan, T. (1995). Fungal biosorption—An alternative treatment option for heavy metal bearing wastewaters: A review. *Bioresource Technology*, *53*, 195–206.

Kapoor, A., & Viraraghavan, T. (1997). Heavy metal biosorption sites in *Aspergillus niger*. *Bioresource Technology*, *61*, 221–227.

Kapoor, A., Viraraghavan, T., & Cullimore, D. R. (1999). Removal of heavy metals using the fungus *Aspergillus niger*. *Bioresource Technology*, *70*, 95–104.

Mittar, D., Khanna, P. K., Marwaha, S. S., & Kennedy, J. F. (1992). Biobleaching of pulp and paper mill effluents by *Phanerochaete chrysosporium*. *Journal of Chemical Technology and Biotechnology*, *53*, 81–92.

Moe, S. T., Skjåk-Bræk, G., Elgsaeter, A., & Smidsrød, O. (1993). Swelling of covalently crosslinked alginate gels: Influence of ionic solutes and nonpolar solvents. *Macromolecules*, *26*, 3589–3597.

Özer, A., Ekiz, H. I., Özer, D., Kutsal, T., & Çaglar, A. (1997). A staged purification process to remove heavy metal ions from wastewater using *Rhizopus arrhizus*. *Process Biochemistry*, *32*, 319–326.

Park, J. K., Jim, Y. B., & Chang, H. N. (1999). Reusable biosorbents in capsules from *Zooogloea ramigera* cells for cadmium removal. *Biotechnology and Bioengineering*, *63*, 116–121.

Sağlam, N., Say, R., Denizli, A., Patır, S., & Arıca, M. Y. (1999). Biosorption of inorganic mercury and alkylmercury species on to by *Phanerochaete chrysosporium* mycelium. *Process Biochemistry*, *34*, 725–730.

Sağlam, A., Yacınkaya, Y., Denizli, A., Arıca, M. Y., Genç, O., & Bektas, S. (2002). Biosorption of mercury by carboxycellulose and immobilized *Phanerochaete chrysosporium*. *Microchemical Journal*, *71*, 73–81.

Say, R., Denizli, A., & Arıca, M. Y. (2001). Biosorption of cadmium(II), lead(II) and copper(II) with the filamentous fungus *Phanerochaete chrysosporium*. *Bioresource Technology*, *76*, 67–70.

Stokke, B., Smidsrød, O., Bruheim, P., & Skjåk-Bræk, G. (1991). Distribution of uronate residues in alginate chains in relation to alginate gelling properties. *Macromolecules*, *24*, 4637–4645.

Suh, J. H., & Kim, D. S. (2000). Comparison of different sorbents (inorganic and biological) for removal of Pb(II) from aqueous solutions. *Journal of Chemical Technology and Biotechnology*, *75*, 279–284.

Tobin, J. M., White, C., & Gadd, G. M. (1994). Metal accumulation by fungi: Application in environmental biotechnology. *Journal of Industrial Microbiology*, *13*, 126–130.

Valdman, E., Erijman, L., Pessoa, F. L. P., & Leite, S. G. F. (2001). Continuous biosorption of Cu and Zn by immobilized biomass *Sargassum* sp. *Process Biochemistry*, *36*, 869–873.

Xeu, H. B., Stumm, W., & Sigg, L. (1988). The binding of heavy metal to algal surface. *Water Research*, *22*, 917–922.

Yan, G., & Viraraghavan, T. (2001). Heavy metal removal in a biosorption column by immobilized *M. rouxii* biomas. *Bioresource Technology*, *78*, 243–249.

Zulfadhyl, Z., Mashitah, M. D., & Bhatia, S. (2001). Heavy metals removal in fixed bed column by the macro fungus *Pycnoporous sanguineus*. *Environmental Pollution*, *112*, 463–470.