ELSEVIER

Journal of Hazardous Materials B101 (2003) 285–300

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Biosorption of heavy metal ions on immobilized white-rot fungus *Trametes versicolor*

Gülay Bayramoğlu [a], Sema Bektaş [b], M. Yakup Arıca [a,*]

[a] *Biochemical Processing and Biomaterial Research Laboratory, Faculty of Science, Kirikkale University, 71450 Yahş ihan, Kirikkale, Turkey*

[b] *Department of Chemistry, Hacettepe University, 06532 Beytepe, Ankara, Turkey*

Received 14 February 2003; received in revised form 27 May 2003; accepted 29 May 2003

Abstract

Trametes versicolor mycelia were immobilized in carboxymethylcellulose, CMC, beads via entrapment, and the bead containing immobilized fungus spores were incubated at 30 °C for 3 days to attain uniform growth on the bead surface. After incubation, the live and heat inactivated immobilized fungus on the CMC beads were used for the biosorption of Cu^{2+}, Pb^{2+} and Zn^{2+} ions.

Plain CMC beads were used as a control system. The biosorption of Cu^{2+}, Pb^{2+} and Zn^{2+} ions by the CMC and both live and inactivated immobilized preparations increased as the initial concentration of Cu^{2+}, Pb^{2+} and Zn^{2+} ions in the medium increased. The maximum biosorption capacities for both immobilized live and heat inactivated *Trametes versicolor* were 1.51 and 1.84 mmol Cu^{2+}, 0.85 and 1.11 mmol Pb^{2+} and 1.33 and 1.67 mmol Zn^{2+} per g of dry biosorbents, respectively. Biosorption equilibrium was established in about 1.0 h and the equilibrium was well described by Langmuir and Freundlich isotherms. A temperature change in the range of 15–45 °C did not affect the biosorption capacity. The affect of pH was also investigated and the maximum adsorption of Cu^{2+}, Pb^{2+} and Zn^{2+} ions on the CMC and both live and inactivated immobilized fungal biomass was observed between pH 4.0 and 6.0. The CMC beads with the immobilized fungus can be regenerated using 10 mM HCl, with up to 97% recovery of the metal ions; the biosorbents reused up to five biosorption–desorption cycles without any major loss in the biosorption capacity.

Keywords: Cu^{2+}; Pb^{2+} and Zn^{2+}; Heavy metal; CMC beads; Biosorption; *Trametes versicolor*

* Corresponding author. Tel.: +90-318-357-2477; fax: +90-318-357-2329.
E-mail address: yakuparica@turk.net (M.Y. Arıca).

0304-3894/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0304-3894(03)00178-X

1. Introduction

The utilization of biosorption technology for the treatment of heavy metal contaminated wastewaters and for the recovery of precious metals from mining wastes or in metallurgical effluents is of importance. Many microorganisms are known to be capable of concentrating heavy metals from their aqueous environment [1–4]. Biomass materials are cheap and abundant. Biomass could be obtained as a waste by-product from large-scale fermentations such as *Sacchromyces cerevisiae* or be produced in large quantities as *Ascophyllum nodosum* [5]. These biologically produced materials could be used in a biosorption process. The term 'biosorption' has been used to describe the passive non-metabolically mediated process of metal ion binding by living or inactivated biomass [6,7].

There are several studies cited in the literature that point out the capability of fungal biomass to remove heavy metal ions from solution. Many fungal species, such as *Rhizopus arrhizus* [8], *Mucor miehei* [9], *Trametes versicolor* [10], *Lentinus sajur-caju* [11] and *Aspergillus niger* [12], have been extensively studied for their heavy metal biosorption capabilities and the metal removal mechanisms seem to be dependent upon species. Relatively few studies have been done using white-rot fungi, which are strong degrader of various xenobiotics to detoxify metal effluents [1]. These fungi could also be used to remove heavy metals from wastewaters by adsorbing the metals on their mycelia [13,14].

In industrial or technical operations, immobilized microbial cell systems could also provide additional advantages over freely suspended cells. These advantages include ease of regeneration and reuse of the biomass, easier solid–liquid separation and minimal clogging in continuous flow systems [15,16].

Natural polymers such as cellulose derivatives, alginate, chitosan and chitin have been used as the matrix for the immobilization of microbial cells via entrapment. These polymers are also known to bind metal ions strongly [17–19]. Immobilization of fungal cells in these polymer supports could also enhance fungal cell performance and adsorptive capacity of the biosorbent system for heavy metal ions [1,4]. Immobilized fungal cells were found to be far more stable during continuous operation in a bioreactor than the fungal cells in free forms [20].

The purpose of this research was to study the enhancement of the adsorptive capacity of CMC beads for the removal of heavy metal ions from aqueous solution by combining them with fungal cells. Information is available on the use of immobilized *T. versicolor* mycelia for removal of Cd^{2+} ions [21], but no study has been conducted on the use of the immobilized biomass of *T. versicolor* for removal of other heavy metal ions. Therefore, there is a need to study the performance of this immobilized fungus system with other heavy metal ions.

T. versicolor mycelia were immobilized using CMC as the natural polymeric matrix. After growth of the immobilized fungus on the matrix, live and heat inactivated forms were used for biosorption of Cu^{2+}, 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 of Cu^{2+}, Pb^{2+} and Zn^{2+} ions on both immobilized live and inactivated forms at different pH values. The maximum adsorption capacities of the both immobilized preparations, based on dry weight were determined by varying the concentration of the heavy metal ions in the

aqueous solutions. The effectiveness of a desorbing agent (HCl) in stripping adsorbed metal ions, from the CMC beads and both immobilized fungal preparations, was also investigated.

2. Experimental

2.1. Microorganism and media

White-rot fungus, *T. versicolor* was maintained by subculturing on malt dextrose agar slants. The pH of the medium was adjusted to 4.5. Inocula were obtained from 7 day old agar slant culture. The fungal mat (0.3 g) was removed and macerated aseptically in 5.0 ml of a sterile medium using a homogenizer. This preparation was used to inoculate 50 ml of medium in a 250 ml flask, and the flasks were incubated on a shaker (150 rpm) for 3 days at 30 °C. After this period, the biomass was harvested by filtration of the growth medium and washed several times with distilled water. The fungal biomass was obtained as discrete spherical clumps; it was homogenized with a commercial blender to destroy cell aggregates.

2.2. Immobilization of white-rot fungus "T. versicolor"

T. versicolor mycelia were immobilized using CMC beads via entrapment. Carboxymethylcellulose sodium salt (Na-CMC; high viscosity; 1.0% in H_2O at 25 °C: 700–1550 mPa; degree of substitution: 0.60–0.95; Sigma Chemical Co., St. Louis, MO) solution was prepared in distilled water (2%, 50 ml) and then mixed with the fungal mycelia (2.0 g in 50 ml saline solution). The mixture was introduced into a solution containing 0.1 M $FeCl_3$ through a nozzle (2.0 cm length, 1.0 mm i.d.) using a peristaltic pump, and the solution was stirred to prevent aggregation of the fungus mycelia immobilized CMC beads. The fungus-immobilized beads (∼2 mm diameter) were cured in this solution for 15 min and then washed twice with 200 ml sterile distilled water.

The CMC beads with immobilized mycelia were then transferred to the growth medium (50 ml) in 250 ml flask and were incubated on an orbital shaker (150 rpm) at 30 °C for 3 days. The mycelia growth in/on the beads was followed during the incubation period by using a microscope. After a 3 day incubation period, the CMC beads with immobilized fungal mycelia were removed from the medium by filtration and washed twice with distilled water. This washed biomass is called "immobilized live fungus". At times immobilized live fungus was heated in 5 mM $FeCl_3$ solution at 90 °C for 10 min, and it is referred to as immobilized heat inactivated fungus. The immobilized preparations were stored at 4 °C until use. The dry weight of the microbial growth in/on the immobilized preparations was determined by weighing (after drying in an oven at 50 °C overnight) the CMC beads before and after cell growth.

2.3. Biosorption studies

The biosorption of Cu^{2+}, Pb^{2+} and Zn^{2+} ions, on plain CMC beads and on the both immobilized live and heat inactivated *T. versicolor* was investigated in batch biosorption-

equilibrium experiments. The stock solutions of metal ions (i.e. Cu^{2+}, Pb^{2+} and Zn^{2+}: 1.0 g/l) were prepared using nitrate salts in double distilled water.

The effects of the medium pH and the initial concentration of Cu^{2+}, Pb^{2+} and Zn^{2+} ions on the biosorption rate and capacity were studied. The effect of pH on the biosorption rate was investigated in the pH range of 3.0–7.0 (which was adjusted with HCl or NaOH at the beginning of the experiment and not controlled afterwards) at 25 °C. Solution containing 200 mg/l of Cu^{2+}, Pb^{2+} and Zn^{2+} ions and each CMC, live or heat inactivated fungus immobilized in CMC beads were combined and the samples were stirred at 400 rpm.

The effect of temperature on the biosorption capacity of the biosorbent was determined at pH 6.0 when the metal ion concentration was 200 mg/l.

The effect of the initial Cu^{2+}, Pb^{2+} and Zn^{2+} ions concentration on biosorption was studied at pH 6.0 as noted above except that the concentration of Cu^{2+}, Pb^{2+} and Zn^{2+} ions in the adsorption medium was varied between 30 and 400 mg/l.

The competitive biosorption of Cu^{2+}, Pb^{2+} and Zn^{2+} ions from their mixture was investigated in the same manner. The medium containing 100 mg/l of each metal ion was incubated with the biosorbents in batch fashion.

2.4. Analytical procedure

A known weight of biosorbent was transferred into the metal ion solution (25 ml). After a 60 min incubation period, the aqueous phases were separated from the biosorbent and the concentration of Cu^{2+}, Pb^{2+} and Zn^{2+} ions determined by an atomic absorption spectrophotometer (AAS; Shimadzu AA 6800, Japan) with an air–acetylene flame. Lead or copper–zinc hollow cathode lamps were used. The instrument response was periodically checked with known Cu^{2+}, Pb^{2+} and Zn^{2+} ions 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.

The amount of metal ions adsorbed per unit CMC beads and both fungus-immobilized CMC preparations (mg metal ions/g dry beads) was obtained by using the following expression:

$$Q = \frac{[(C_0 - C)V]}{M} \tag{1}$$

where Q is the amount of metal ions adsorbed onto the unit amount of the adsorbents (mg/g); C_0 and C are the concentrations of the metal ions in the solution (mg/l) before and after biosorption, respectively; V is the volume of the aqueous phase (l); and M is the amount of the adsorbent (g).

2.5. Desorption

In order to determine the reusability of the CMC beads and the immobilized fungal forms, consecutive adsorption–desorption cycles were repeated five times by using the same biosorbents. Desorption of Cu^{2+}, Pb^{2+} and Zn^{2+} ions was accomplish by using a 10 mM HCl solution. The CMC beads and both immobilized fungal forms loaded with

Cu^{2+}, Pb^{2+} and Zn^{2+} ions were placed in the desorption medium and stirred at 400 rpm for 60 min at 25 °C. The final Cu^{2+}, Pb^{2+} and Zn^{2+} concentration in the aqueous phase was determined by using the AAS as described above. The desorption ratio was calculated from the amount of metal ions adsorbed on the immobilized preparations and the final Cu^{2+}, Pb^{2+} and Zn^{2+} ion concentration in the adsorption medium.

The desorption ratio was calculated utilizing the following equation:

$$\text{Desorption ratio} = \frac{(\text{amount of metal ions desorbed}) \times 100}{\text{amount of metal ions adsorbed}} \quad (2)$$

2.6. SEM studies

Sample of CMC and fungus-immobilized beads were coated under vacuum with a thin layer of gold and examined by scanning electron microscopy (Jeol, model JMS 5600).

3. Results and discussion

3.1. Properties of the CMC-based biosorbents systems

CMC is a water-soluble derivative of natural polymer cellulose. Carboxymethylation provides functional carboxylic groups on the cellulose derivative for cross-linking via trivalent metal ions. CMC can easily be converted into hydrogels via cross-linking with trivalent metal ions such as ferric chloride and aluminium chloride [22]. It is preferred over other materials because of its various advantages such as biodegradability, hydrophilicity, presence of functional carboxylic groups, and natural origin. These are very important properties because polymers of petroleum origin are non-degradable, making them a major cause of pollution.

In this research, CMC gel beads were used both as an adsorbent and a support material for the entrapment of a white-rot fungus *T. versicolor*. These materials were used for the removal of Cu^{2+}, Pb^{2+} and Zn^{2+} ions from aqueous solution. CMC beads were prepared by cross-linking with trivalent ferric ions, which is considered to be a hard Lewis acid that has a strong affinity for oxygen-rich "*Lewis bases*" such as carboxylic, phosphate and sulphate [23]. Cross-linking should be a combination of metal co-ordination and ion exchange interaction between the carboxylic oxygen of CMC and trivalent ferric ions. These interactions cause CMC droplets to precipitate in the bead form in ferric chloride solution. The water content of CMC beads was 92%. These beads were very stable over the experimental pH range of 3.0–8.0.

The SEM micrographs of the plain CMC beads and fungus-immobilized bead are presented in Fig. 1A and B, respectively. The CMC beads are spherically shaped with approximately a 2 mm diameter. The SEM micrograph of fungus-immobilized CMC bead was completely different from the plain CMC bead. There was a uniform fungal growth on the bead surface indicating that immobilization of fungal mycelium is not localized. This uniform distribution of fungus is an important criterion for the proper biosorption of heavy metal ions on the whole surface area of the fungus-immobilized beads. Thus, immobilization

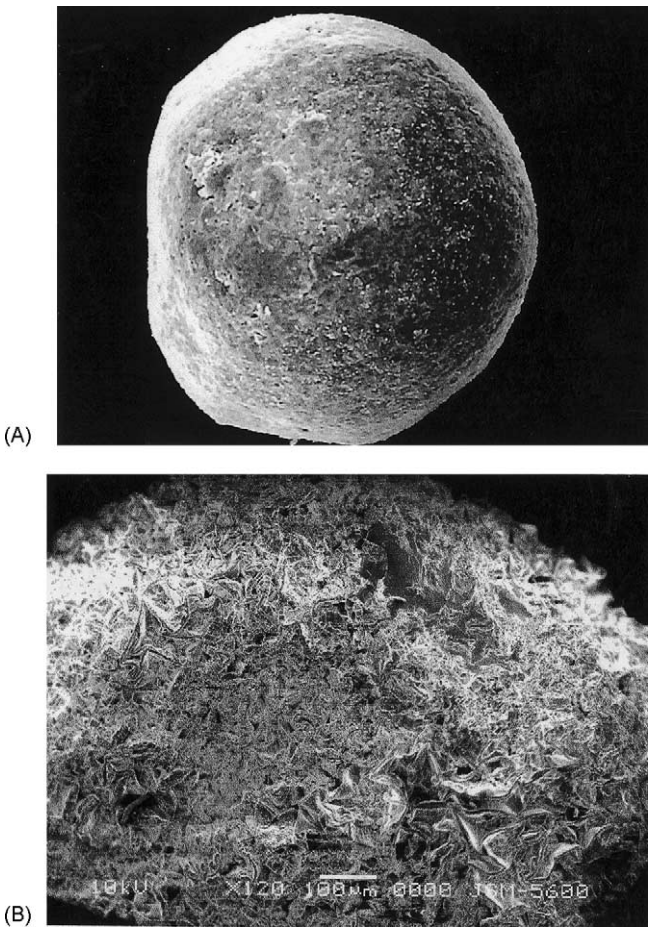

Fig. 1. SEM of plain and fungus-immobilized CMC bead. (A) Plain CMC bead and (B) fungus-immobilized CMC bead.

of fungal cells in the CMC beads could also provide an additional advantage over the freely suspended fungal cells; in batch culture fungal mycelia form individually distributed spherical clumps (Ø: 2–4 mm). This tight packing of the fungal cells could also lead to diffusional restrictions and fewer adsorptive sites for heavy metals than the CMC-fungal cells system [24].

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

The biosorption time of Cu^{+2}, Pb^{+2} and Zn^{2+} ions by both immobilized live and inactivated fungal biosorbents are important, for the assessment of the suitability of these fungal preparations to serve as biosorbents in a continuous flow system. Fig. 2 shows the biosorption time line of Cu^{2+} on CMC and on both immobilized live and inactivated preparations

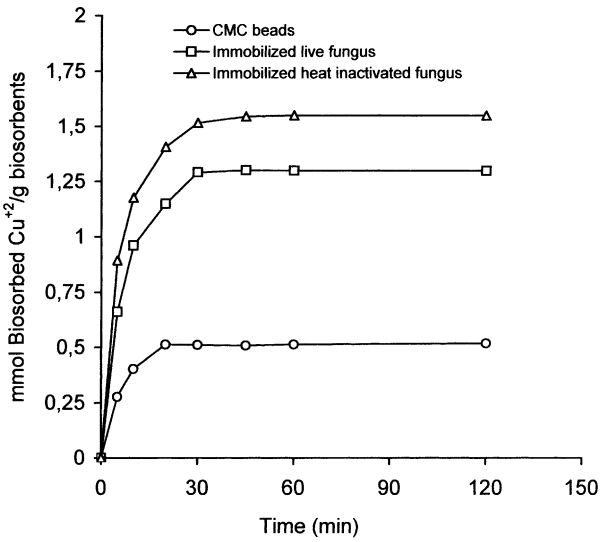

Fig. 2. Biosorption times of Cu^{2+} ions on CMC beads and both live and heat inactivated fungus immobilized on CMC beads from aqueous solutions at: pH, 6.0; temperature, 25 °C; and initial concentration of Cu^{2+} ions, 200 mg/l.

from solutions containing 200 mg/l of Cu^{2+} ions. As seen in this figure, most of the metal ions were biosorbed from aqueous solution within the first 30 min and there was almost no increase in the amount of biosorbed metal occurred after 60 min. The initial slope of the curve reflects the biosorption rate. It should be noted that there was no precipitation in these group of experiments. As seen in Fig. 2, high biosorption rates are observed at the beginning and plateau values (i.e. adsorption equilibrium) are gradually reached within 60 min.

Data on the adsorption rates of heavy metal ions by various biosorbents have shown a wide range of adsorption time. For example, polyvinyl alcohol–yeast and alginate–yeast biosorbent systems used for copper biosorption kinetic studies of 12 and 24 h equilibrium time have been reported, respectively [25]. The biosorption equilibrium time of Cr^{4+} on the dead and immobilized *R. arrhizus* was 2 h [26]. The biosorption equilibrium time of Hg^{2+} ions on the dead *Phanerochaete chrysosporium* was about 1 h [27]. The lead biosorption equilibrium time on *A. niger* biomass was 5 h [28]. The biosorption of Cd^{2+} onto pre-treated biomass of marine algae *Durvillaea potatorum* was studied and the biosorption process was very fast (up to 90% Cd^{2+} removed within 30 min) [29]. The biosorption equilibrium time of lead ions on the non-living biomass of marine algae was about 3 h [30]. Note that there are several parameters which determine the biosorption rate such as the stirring rate of the aqueous phase, structural properties both of the support and the biosorbent (e.g. protein and carbohydrate composition and surface charge density of the cell wall component of the immobilized fungal biomass), amount of sorbent, properties of the ion under study (e.g. ionic radius), initial concentration of ionic species and, of course, existence of other metal ions, which may compete with the ionic species of interest for the active biosorption sites. Therefore, it is very difficult to compare the biosorption rates reported.

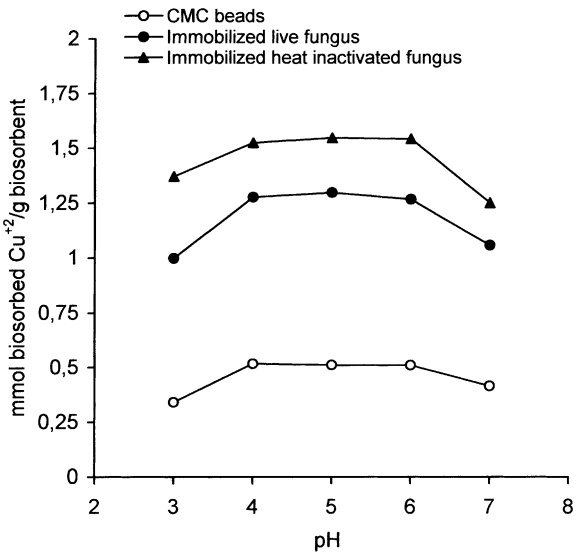

Fig. 3. Effect of pH on biosorption of Cu^{2+} ions on CMC beads and both live and heat inactivated fungus immobilized on CMC beads from aqueous solutions: temperature, 25 °C and initial concentration of Cu^{2+} ions, 200 mg/l.

3.3. *Effect of pH and the temperature on the biosorption capacity of the biosorbents*

It is well known that metal ion adsorption on both non-specific and specific sorbents is pH dependent [1,31]. The medium pH affects the solubility of metal ions and the ionization state of the functional groups (i.e. carboxylate, phosphate, and amino groups) on the fungal cell wall [1]. The effect of pH on biosorption of Cu^{2+}, Pb^{2+} and Zn^{2+} ions on the biosorbents were evaluated at different pH values for Cu^{2+} biosorption (Fig. 3). The maximum Cu^{2+}, Pb^{2+} and Zn^{2+} ions biosorption occurred between pH 4.0 and 6.0. A reduction in metal ions removal with increasing pH beyond 6.0 has been attributed to reduced solubility and precipitation [32,33]. The amount of adsorbed Cu^{2+}, Pb^{2+} and Zn^{2+} ions (from 200 mg/l Cu^{2+}, Pb^{2+} and Zn^{2+} solution) on the dry bases of the immobilized live and heat inactivated fungal preparation at pH 6.0 were found to be 1.30 and 1.55 mmol Cu^{2+}, 0.78 and 1.00 mmol Pb^{2+} and 1.16 and 1.47 mmol Zn^{2+} per g of biosorbents, respectively, whereas the amount of adsorbed metal ions on the plain CMC beads was 0.52 mmol Cu^{2+}, 0.32 mmol Pb^{2+} and 0.39 mmol Zn^{2+} per g of dry beads, respectively.

The temperature of the adsorption medium could be important for energy dependent mechanisms in metal biosorption by microorganisms. Energy-independent mechanisms are less likely to be affected by temperature since the processes responsible for biosorption are largely physicochemical in nature. The biosorption of Cu^{2+}, Pb^{2+} and Zn^{2+} by CMC and both immobilized live and inactivated preparations appears to be temperature independent over the temperature range tested (15–45 °C). Similar results have been reported by other researchers [34].

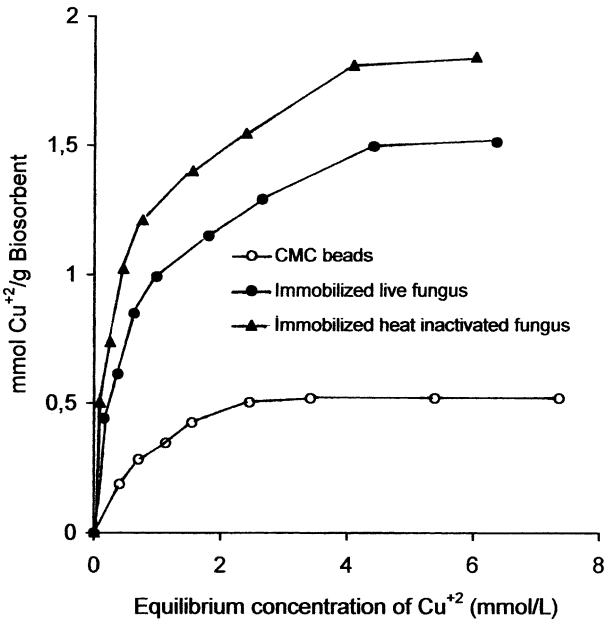

Fig. 4. Equilibrium biosorption values of Cu^{2+} ions on CMC beads and both live and heat inactivated fungus immobilized on CMC beads from aqueous solutions: pH, 6.0 and temperature, 25 °C.

3.4. Effect of initial metal ion concentration

Copper, lead and zinc ions biosorption capacities of the CMC and both immobilized live and inactivated fungal preparations are shown in Figs. 4–6 as a function of the equilibrium concentration of Cu^{2+}, Pb^{2+} and Zn^{2+} ions within the aqueous biosorption medium. These figures were prepared by using the plateau values of the biosorption rate curves (example is given in Fig. 2). The amount of Cu^{2+}, Pb^{2+} and Zn^{2+} ions adsorbed per unit mass of the biosorbent (i.e. biosorption capacity) increased, as expected, with the initial concentration of metal ions. In order to reach the plateau values, which represent saturation of the active sites (which are available for specific interaction with metal ions) on the biosorbent, in other terms to obtain the maximum biosorption capacity of the CMC and both immobilized live and inactivated fungal preparations for the Cu^{2+}, Pb^{2+} and Zn^{2+} ions, the initial concentration of these ions was increased up to 400 mg/l. As seen from these figures, the amount of Cu^{2+}, Pb^{2+} and Zn^{2+} ions adsorbed at equilibrium on the plain CMC beads was 0.52, 0.36 and 0.42 mmol (or 32.9 ± 1.3, 73.6 ± 2.9 and 27.5 ± 1.8 mg) per g of dry CMC beads, respectively. The maximum biosorption capacities for immobilized live and heat inactivated preparations (*T. versicolor)* were 1.51 and 1.84 mmol (or 96.3 ± 2.7 and 117.2 ± 3.2 mg) for Cu^{2+}, 0.85 and 1.11 mmol (or 176.4 ± 2.4 and 227.8 ± 2.8 mg) for Pb^{2+} and 1.33 and 1.67 mmol (or 87.4 ± 1.8 and 109.3 ± 2.1 mg) for Zn^{2+} per g of dry biosorbents, respectively. The biosorption capacity of the fungus-immobilized CMC beads was higher (up to 4.0 times) than that of the CMC beads. After heat treatment, the

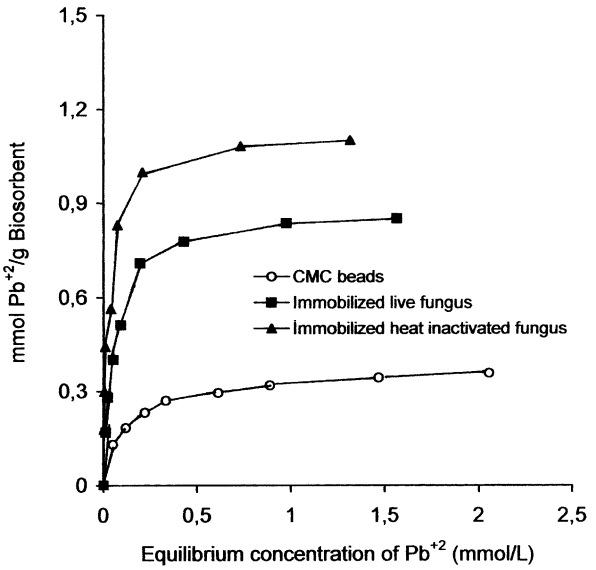

Fig. 5. Equilibrium biosorption values of Pb^{2+} ions on CMC beads and both live and heat inactivated fungus immobilized on CMC beads from aqueous solutions: pH, 6.0 and temperature, 25 °C.

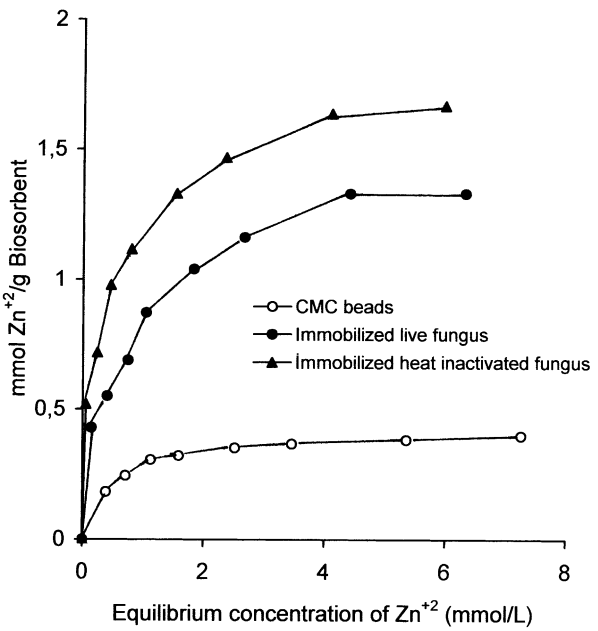

Fig. 6. Equilibrium biosorption values of Zn^{2+} ions on CMC beads and both live and heat inactivated fungus immobilized on CMC beads from aqueous solutions: pH, 6.0 and temperature, 25 °C.

adsorption capacity of the fungus-immobilized biosorbent increased approximately 18% for Cu^{2+}, 22% for Pb^{2+} and 20% for Zn^{2+} metal ions.

The inactivated form of the immobilized white-rot fungus *"T. versicolor"* had a higher adsorption capacity for all the tested metal ions than the living immobilized form. This might be due to an increase in the surface area for adsorption of Cu^{2+}, Pb^{2+} and Zn^{2+} ions because of cell rupture upon dead. Similar observations have been reported for other biomasses, including fungus and yeast [35], and can be devoted a variety of resistance mechanisms. These mechanisms include extra-cellular complexation with metal binding proteins such as metallothioneins and phytochelatings (which are proteins that contain large amounts of cysteine and bind heavy metal ions) [36] and efficient pumping out if metal ions enter the cell.

3.5. Adsorption isotherm modeling

The adsorption process is a mass transfer operation that can be described mathematically by an equilibrium process and a rate process. The equilibrium is established between the concentration of the metal ions dissolved in aqueous phase and that bound to the biosorbent.

The modeling of the adsorption processes on CMC and both immobilized live and heat inactivated preparations was performed by using different adsorption isotherms. Among the several isotherm equations available, two isotherms (Langmuir and Freundlich adsorption isotherms) were investigated. These isotherms are widely used to analyze data for water and wastewater treatment process.

The model is described by the following equilibrium:

$$\mathrm{M} + \mathrm{L} \underset{k_2}{\overset{k1}{\rightleftarrows}} \mathrm{ML} \tag{3}$$

Metal ions and ligands on the biosorbents are donated by M and L, respectively. k_1 and k_2 are the forward and reverse interaction rates, respectively, which include the metal ion movement from the bulk phase to the biosorbent surface layer.

Eq. (3) can be expressed in the form of a rate equation with a second-order forward and a first-order reverse kinetics, namely

$$\frac{d_\mathrm{q}}{d_\mathrm{t}} = k_1 C_\mathrm{eq}(q_\mathrm{m} - q_\mathrm{eq}) - k_2 q_\mathrm{eq} \tag{4}$$

Eq. (4) leads to

$$q_\mathrm{eq} = q_\mathrm{m} \frac{C_\mathrm{eq}}{(k_\mathrm{d} + C_\mathrm{eq})} \tag{5}$$

where C_eq and q_eq also show the residual metal concentration and the amount of metal adsorbed on the adsorbent at equilibrium, respectively, $k_\mathrm{d} = k_2/k_1$ is the Langmuir constant of the system.

The semi-reciprocal plot of $C_\mathrm{eq}/q_\mathrm{eq}$ versus C_eq was employed to generate the intercept of $K_\mathrm{d}/q_\mathrm{m}$ and the slope of $1/q_\mathrm{m}$. The maximum capacity q_m determined from the Langmuir isotherm defines the total capacity of the biosorbents for Cu^{2+}, Pb^{2+} and Zn^{2+} ions (Fig. 7).

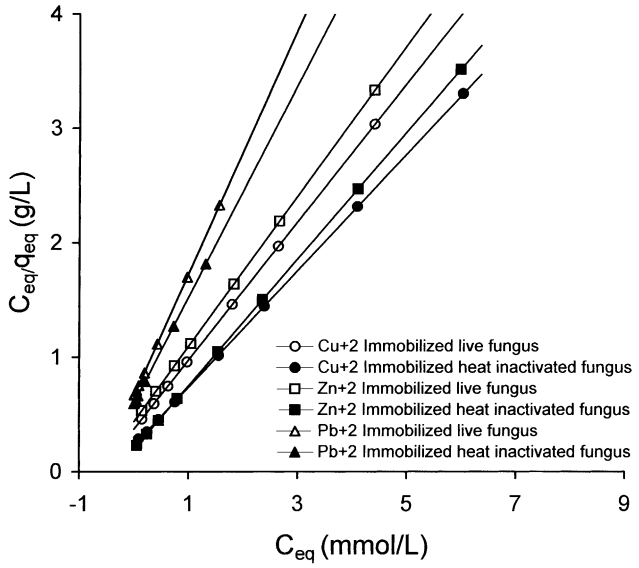

Fig. 7. The Langmuir plots of Cu^{2+}, Pb^{2+} and Zn^{2+} ions on both live and heat inactivated fungus immobilized on CMC beads.

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

$$q_{eq} = K_F(C_{eq})^{1/n} \tag{6}$$

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 (Fig. 8).

The Langmuir and Freundlich constants, along with correlation coefficients (R^2) have been calculated from the plots for biosorption of Cu^{2+}, Pb^{2+} and Zn^{2+} on the biosorbents and the results are presented in Table 1.

The correlation regression coefficients show that the adsorption process could be well described by Langmuir equation. The Langmuir constant (q_m) values were fit the experimental values, and this could be evidence that biosorption stops at one monolayer, consistent with specific and strong adsorption onto specific sites. Because the exchange reaction between surface sites and previously adsorpbed ions is of the only a monolayer or less, there is an accumulation of matter at the solid–solution interface, without the creation of a three-dimensional structure. On the other hand, the magnitudes of K_F and n (Freundlich constants) show easy separation of metal ions from aqueous medium and indicate favorable

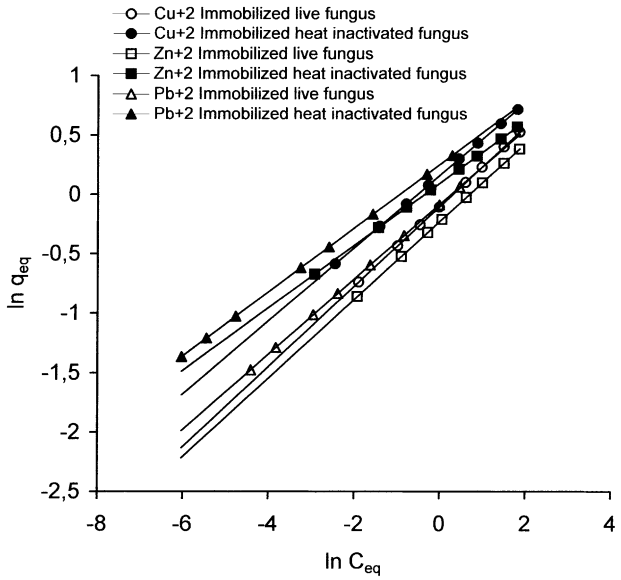

Fig. 8. The Freundlich plots of Cu^{2+}, Pb^{2+} and Zn^{2+} ions on both live and heat inactivated fungus immobilized on CMC beads.

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. As seen from Table 1 for all experimentally tested biosorbents, n values were found high enough for separation.

Table 1
Isotherm model constants and correlation coefficients for biosorption of Cu^{2+}, Pb^{2+} and Zn^{2+} ions from aqueous solution

Biosorbent	Experimental q_{eq} (mmol/g)	Langmuir constant			Freundlich constant		
		q_m (mmol/g)	k_d ($\times 10^4$ M)	R^2	K_F	N	R^2
Plain CMC beads Cu^{2+}	0.52	0.59	6.32	0.997	0.31	2.92	0.926
Live immobilized *Trametes versicolor* Cu^{2+}	1.51	1.65	6.11	0.908	0.71	2.98	0.984
Inactivated immobilized *Trametes versicolor* Cu^{2+}	1.84	1.96	4.98	0.998	1.18	3.26	0.984
Plain CMC beads Pb^{2+}	0.36	0.38	1.33	0.999	0.32	3.76	0.974
Live immobilized *Trametes versicolor* Pb^{2+}	0.85	0.94	6.13	0.980	0.92	3.16	0.945
Inactivated immobilized *Trametes versicolor* Pb^{2+}	1.11	1.08	6.40	0.950	1.29	3.72	0.946
Plain CMC beads Zn^{2+}	0.42	0.42	4.98	0.999	0.26	4.05	0.947
Live immobilized *Trametes versicolor* Zn^{2+}	1.33	1.52	6.60	0.996	0.80	3.03	0.988
Inactivated immobilized *Trametes versicolor* Zn^{2+}	1.67	1.80	3.50	0.999	1.10	3.80	0.981

3.6. Competitive biosorption

The surface of fungal cells can act as an ion exchange resin. The major cell wall component of fungi is chitin units, which are long chain polymers of *N*-acetyl D-glucoseamine linked by β1–4 glycosidic bonds. Other major cell wall components include polyuronic acid, polyphosphates, proteins and lipids. The functional groups on the fungal cell wall components, such as amino, carboxylic, sulfydryl, phosphate and thiol groups, differ in their affinity and specificity for metal binding. The amount of a metal ion bound on the fungal cell surface would be determined by the relative affinities of the sites for the metal ions and the other cations present, and the residual concentrations of these metal ions not uptaken in the solution [37,38].

The competitive biosorption capacities of the immobilized live and inactivated fungal preparations were 0.61 and 0.75 mmol for Cu^{2+}, 0.36 and 0.43 mmol for Pb^{2+}, and 0.48 and 0.59 mmol for Zn^{2+} per g of dry biosorbents, respectively. The biosorption order under competitive conditions was $Cu^{2+} > Zn^{2+} > Pb^{2+}$ in mmol basis for all the tested biosorbents. The competitive biosorption capacities of the immobilized fungal preparations for all metal ions were lower than non-competitive conditions. The immobilized *T. versicolor* exhibits the highest biosorption ability for Cu^{2+} ions. The selectivity factor could be due to the different charge/mass ratio, which favors Cu^{2+} biosorption. Similar results are reported in the literature in which Cu^{2+} is preferentially biosorbed with respect to other metal ions [28,39]. Many of the functional groups present on the fungal cell wall and different cations compete for the binding sites. The differences in the biosorption affinities could also be contributed to differences in the electrode potentials of various ions [39]. In competitive biosorption, the complex interactions of several factors such as ionic charge, ionic radii and electrode potential would affect the biosorption of metal ions on the immobilized fungal preparation.

3.7. Desorption and reuse

The desorption of the adsorbed Cu^{2+}, Pb^{2+} and/or Zn^{2+} ions from the biosorbents was studied in a batch system. The Cu^{2+}, Pb^{2+} and Zn^{2+} ions adsorbed onto biosorbents were eluted with 10 mM HCl. More than 95% of the adsorbed Cu^{2+}, Pb^{2+} and Zn^{2+} ions were desorbed from the biosorbents. In order to show the reusability of the biosorbents, adsorption–desorption cycle of Cu^{2+}, Pb^{2+} and Zn^{2+} ions was repeated five times by using the same preparations. The adsorption capacities for all the biosorbents did not noticeably change (a maximum 3% change was observed with the tested biosorbent during the repeated adsorption–desorption operations). These results showed that CMC beads and both immobilized live and inactivated preparations could be repeatedly used in heavy metal adsorption studies without detectable losses in their initial adsorption capacities.

4. Conclusion

Plain CMC beads and both immobilized live and heat inactivated *T. versicolor* have been successfully used as the biosorbing agent for removal of Cu^{2+}, Pb^{2+} and Zn^{2+} ions from

an aqueous medium. The biosorption of Cu^{2+}, Pb^{2+} and Zn^{2+} ions on the biosorbents depend on the experimental conditions particularly medium pH and the concentration of metals ion in the medium. The biosorption medium temperature had no significant effect on the biosorption capacity between 15 and 45 °C. The equilibrium was well described by Langmuir and Freundlich adsorption isotherms.

The CMC beads with immobilized live and heat inactivated fungus have shown improved performance in the batch system, especially the heat inactivated immobilized preparation of *T. versicolor* could be used as an efficient biosorbent system for the treatment of heavy metals containing wastewater streams. These biosorbents were not only able to remove Cu^{2+}, Pb^{2+} and Zn^{2+} ions from single metal ions solution, but were also able to adsorb these metal ions from multi metal solution. The competitive biosorption capacities of the immobilized fungal preparations for all metal ions were lower than non-competitive conditions. The immobilized *T. versicolor* exhibits the highest biosorption ability for Cu^{2+} ions. The biosorbents can be regenerated by acid treatment and reused. The biosorbents were reused in five biosorption and desorption cycles with negligible decrease (up to 97% recovery) in the biosorption capacities. The immobilized heat inactivated fungus with a higher metal adsorption capacity than the free counterpart that can be used with existing treatment technologies. The non-living immobilized fungal preparation eliminates the problem of metal toxicity and possible adverse operating conditions in packed bed and continuous flow systems.

References

[1] P. Baldrian, Enzyme Microb. Technol. 32 (2003) 78.
[2] K.C. Sekhar, C.T. Kamala, N.S. Chary, Y. Anjaneyulu, Int. J. Miner. Process. 68 (2003) 37.
[3] R.S. Bai, T.E. Abraham, Water Res. 36 (2002) 1224.
[4] E. Valdman, L. Erijman, F.L.P. Pessoa, S.G.F. Leite, Process. Biochem. 36 (2001) 869.
[5] B. Koumanova, P. Peeva, S.J. Allen, K.A. Gallagher, M.G. Healy, J. Chem. Technol. Biotechnol. 77 (2002) 539.
[6] F. Pagnanelli, A. Esposito, L. Toro, V. Veglio, Water Res. 37 (2003) 627.
[7] G.M. Gadd, C. White, Biotechnol. Bioeng. 33 (1989) 592.
[8] J.I. Zhou, Appl. Microbiol. Biotechnol. 51 (1999) 686.
[9] A. Kapoor, T. Viraraghavan, Biores. Technol. 53 (1995) 195.
[10] A. Jarosz-Wilkolazka, E. Malarczyk, J. Pirszel, T. Skowronski, A. Leonowicz, Cell Biol. Int. 26 (2002) 603.
[11] G. Bayramoğlu, A. Denizli, S. Bektaş, M.Y. Arıca, Microchem. J. 72 (2002) 63.
[12] R.A.A. Muzzarelli, F. Tanfani, G. Scarpini, Biotechnol. Bioeng. 22 (1980) 885.
[13] A. Sağlam, Y. Yalçinkaya, A. Denizli, M.Y. Arıca, Ö. Genç, S. Bektaş, Microchem. J. 71 (2002) 73.
[14] M. Tsezos, D.M. Keller, Biotechnol. Bioeng. 25 (1983) 201.
[15] A. Groboilot, D.K. Boadi, D. Poncelet, R.J. Neufeld, Crit. Rev. Biotechnol. 14 (1994) 75.
[16] W. Jianlong, N. Horan, E. Stentiford, Q. Yi, Process. Biochem. 35 (2000) 465.
[17] K.H. Chu, J. Hazard. Mater. 90 (2002) 77.
[18] A.K. Pandey, S.D. Pandey, V. Misra, S. Devi, J. Hazard. Mater. 98 (2003) 177.
[19] S. Babel, T.A. Kurniawan, J. Hazard. Mater. 97 (2003) 219.
[20] M.Y. Arıca, C. Arpa, B. Kaya, S. Bektas, A. Denizli, O. Genç, Biores. Technol. 89 (2003) 145.
[21] Y. Yalçinkaya, L. Soysal, A. Denizli, M.Y. Arıca, S. Bektas, Ö. Genç, Hydrometallurgy 63 (2002) 31.
[22] M.Y. Arıca, Polym. Int. 49 (2000) 775.
[23] G. Bayramoğlu, B. Kaya, M.Y. Arıca, Chem. Eng. Sci. 57 (2002) 2323.
[24] M.Y. Arıca, C. Arpa, A. Ergene, G. Bayramoglu, O. Genc, Carbohydr. Polym. 52 (2003) 167.
[25] Y-P. Ting, G. Sun G, J. Chem. Technol. Biotechnol. 75 (2000) 541.
[26] R.S. Prakasham, J.S. Merrie, R. Sheela, N. Saswathi, S.V. Ramakrishna, Environ. Pollut. 104 (1999) 421.

[27] N. Saglam, R. Say, A. Denizli, S. Patir, M.Y. Arıca, Process. Biochem. 71 (1999) 73.
[28] A. Kapoor, T. Viraraghavan, D.R. Cullimore, Biores. Technol. 70 (1999) 95.
[29] J.T. Matheickal, Q. Yu, G.M. Woodburn, Water Res. 33 (1999) 335.
[30] R. Jalali, H. Ghafourian, Y. Asef, S.J. Davarpanah, S. Sepehr, J. Hazard. Mater. 92 (2002) 253.
[31] A. Delgado, A.M. Anselmo, J.M. Novais, Water Environ. Res. 70 (1998) 370.
[32] C-P. Huang, D. Westman, C. Huang, A.L. Morehart, Water Sci. Technol. 20 (1988) 369.
[33] G.N. Manju, K.A. Krishnan, V.P. Vinod, T.S. Anirudhan, J. Hazard. Mater. 91 (2002) 221.
[34] J.M. Tobin, C. White, G.M. Gadd, J. Ind. Microbiol. 13 (1994) 126.
[35] G.M. Gadd, New Phytol. 124 (1993) 25.
[36] W.Y. Baik, J.H. Bae, K.M. Cho, W. Hartmeier, Biores. Technol. 81 (2002) 167.
[37] K.H. Chu, J. Hazard. Mater. 90 (2002) 77.
[38] J.M. Tobin, D.G. Cooper, R.J. Neufeld, Appl. Environ. Microbiol. 47 (1984) 821.
[39] S.A. White, P.R. Farina, I. Fulton, Appl. Environ. Microbiol. 8 (1979) 323.