ELSEVIER

Journal of Hazardous Materials B131 (2006) 137–145

Journal of Hazardous Materials

www.elsevier.com/locate/jhazmat

Zn(II) biosorption properties of *Botrytis cinerea* biomass

Sibel Tunali *, Tamer Akar

Department of Chemistry, University of Eskisehir Osmangazi, Campus of Meşelik, 26480 Eskişehir, Turkey

Received 25 January 2005; received in revised form 6 September 2005; accepted 10 September 2005
Available online 18 October 2005

Abstract

The study was aimed of determining the Zn(II) sorption performance of *Botrytis cinerea* (*B. cinerea*) biomass as a new biosorbent. Heat inactivated biomass was used in the determination of optimum conditions. The rate and extent of accumulation were effected by pH, contact time and initial zinc ion concentrations. The uptake capacity of *B. cinerea* was increased by chemical and physical pretreatment of the cells when compared with the native biomass. The maximum removal of Zn(II) at pH 5.0–6.0 was found to be $12.98 \pm 0.9623\,mg\,g^{-1}$ at initial Zn(II) ion concentration of $100\,mg\,l^{-1}$ by heat inactivated biomass. Freundlich and Langmuir isotherm models were used to evaluate the data and regression constants were derived. The biosorbent was regenerated using 10 mM HCl solution, with up to 98% recovery and reused five times in biosorption–desorption cycles successivelly. Competitive biosorption experiments were performed with zinc in the presence of copper, cadmium and nickel ions simultaneously. The nature of the possible cell–metal ions interactions was also evaluated by chemical and instrumental analysis including infrared spectroscopy, scanning electron microscopy and X-ray energy dispersion analysis.

Keywords: *Botrytis cinerea*; Biosorption; Zinc (II); Freundlich isotherm; Langmuir isotherm; Desorption

1. Introduction

The high degree of industrialization and urbanization have resulted in environmental pollution [1–3]. The presence of heavy metals in the environment is of major concern because of their extreme toxicity and tendency for bioaccumulation in the food chain even in relatively low concentrations [4–6]. Heavy metals pollute the environment from various industries such as metal plating, electroplating, mining, ceramic, batteries, pigment manufacturing [7,8].

In 1978, the United States Environmental Protection Agency (USEPA) prepared a list of organic and inorganic pollutants which found in wastewater and constitute serious health hazards. The following 13 metals found in this list are antimony, arsenic, beryllium, cadmium, chromium, copper, lead, mercury, nickel, selenium, silver, thallium and zinc [9].

Zinc is one of the most important metals often found in effluents discharged from industries involved in acid mine drainage, galvanising plants, natural ores and munipical wastewater treatment plants and not biodegradable and travels through the food chain via bioaccumulation. Therefore there is significant interest regarding zinc removal from wastewaters [10] and its toxicity for humans at levels of 100–500 mg day^{-1} [11]. World Health Organization (WHO) recommended the maximum acceptable concentration of zinc in drinking water as $5.0\,mg\,l^{-1}$ [6].

Removal of heavy metals from aqueous solutions by biosorption plays an important role in water pollution control [12]. Biosorption can be defined as the ability of biological materials to accumulate heavy metals through metabolically mediated or physico-chemical pathways of uptake [10]. It has been showed that heavy metals can be removed by inexpensive biological materials such as fungi, bacteria and algae [13–16]. Biosorption is a rapid, reversible, economical and ecofriendly technology in contrast to traditional methods used for removal of heavy metals from aqueous streams such as chemical precipitation and reverse osmosis [17,18].

The present work investigates the potential use of pretreated and untreated *Botrytis cinerea* biomass as metal sorbent for zinc from aqueous solutions. *B. cinerea* was chosen as a biosorbent because of the relative lack of information about its sorption ability. Environmental parameters affecting the biosorption process such as pH, contact time and initial metal ion concentration. The equilibrium adsorption data evaluated by Freundlich and

* Corresponding author. Tel.: +90 222 2393750/2366; fax: +90 222 2393578.
E-mail address: stunali@ogu.edu.tr (S. Tunali).

0304-3894/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.jhazmat.2005.09.024

Langmuir isotherm models. Desorption studies and reusability of biosorbent were carried out in addition to competitive biosorption of Zn(II) in the presence of Cu(II), Cd(II) and Ni(II) simultaneously. The role played by the functional groups present in the biomass in sorption was also determined by FTIR, SEM and EDAX analysis.

2. Materials and methods

2.1. Microorganism and growth conditions

The strain used in this study was *B. cinerea* (AHU 9424), a pure culture, obtained from Faculty of Pharmacy of Anadolu University, Eskişehir, Turkey. The cultures were routinely maintained at 4 °C on agar-potato dextrose agar slants [19]. The pH of the liquid growth medium [20] was adjusted to 5.5 by the addition of 1N HNO_3 before autoclaving at 121 °C for at least 20 min. Erlenmeyer flasks containing the above media (100 ml) were innoculated with spore suspension (1 ml) obtained shaking sterile water (10 ml) with a mature slope of *B. cinerea* under sterile conditions. Growth was allowed to proceed for 7 days at 25 °C on a rotary shaker operating at 120 rpm. After the fungal growth, the biomass and the culture medium were separated by filtration and the resulting biomass was washed several times thoroughly with distilled water. The biomass obtained will be referred as native biomass in the text.

2.2. Metal solutions

A stock solution of Zn(II) used in this study was prepared by dissolving an accurate quantity of $ZnSO_4{\cdot}6H_2O$ in deionized water. Other concentrations prepared from stock solution by dilution varied between 5 and 300 mg l^{-1} and the pH of the working solutions was adjusted to desired values with 0.1 M HCl and 0.1 M NaOH. Fresh dilutions were used for each experiments. All the chemicals used were of analytical grade.

2.3. Pretreatment of biomass

An amount of live biomass (20 g wet weight) was subjected to pretreatment in an effort to study the effect of pretreatment on Zn(II) uptake capacity of fungal biomass. The live biosorbent was autoclaved for 15 min at 121 °C and 18 psi (referred as heat inactivated biomass), boiled for 15 min in 500 ml of 0.5N NaOH, commercial laundry detergent solution prepared by dissolving 2.5 g of the laundry detergent in water and 10% (v/v) AcOH solutions [21]. Following the desired pretreatment, biomass were collected by centrifugation and washed with deionized water until the pH of the washing solution was close to neutral range (pH 6.9–7.1). All the pretreated biomass were then spread on petri dishes and dried in an oven at 60 °C for 15 h. They were powdered using a mortar and pestle and sieved to select particles 150 μm for use as a biosorbent. For the untreated control sample, living mycelium of *B. cinerea* was directly used in the experiments.

2.4. Batch biosorption studies

All batch experiments were carried out with biosorbent samples (0.1 g) at 25 °C in erlenmeyer flasks (250 ml) on an orbital shaker operating at 120 rpm to elucidate the optimum conditions (pH, contact time and initial metal ion concentration) using heat inactivated biomass.

The effect of pH on biosorption capacity of *B. cinerea* was determined by equilibrating the biosorption mixture containing heat inactivated biomass (0.1 g) and metal solutions (50 ml of 100 mg l^{-1} solution) at different pH values (1–7). The period of contact time was varied up to 135 min determined by using the same sorption mixture described above. For the assessment of effect of initial metal ion concentration on biosorption at optimum pH and contact time, the concentration of Zn(II) ranging from 5 to 300 mg l^{-1} were prepared and used. Their contents were centrifuged at 4500 rpm for 5 min and the supernatant was analysed for residual metal ion concentration.

Competitive biosorption of Zn(II) in the presence of Cu(II), Cd(II) and Ni(II) was also investigated using heat inactivated biomass. The medium containing 100 mg l^{-1} of each metal ion was incubated with 0.1 g of biosorbent at pH 5.0–6.0 for 30 min.

2.5. Desorption and reuse studies

Different mineral acids revealed a metal elution efficiency close to 100%. In this work heat inactivated biomass were subjected to 10 mM HCl solution in order to determine the reusability of heat inactivated *B. cinerea* biomass. Consecutive biosorption–desorption cycles were repeated five times using the same biosorbent. For this purpose 0.1 g of biosorbent was contacted with 50 ml Zn(II) solution (100 mg l^{-1}). After the adsorption process, the bound zinc ions were eluted. Each biosorption and desorption cycle were allowed 30 min of contact time in solutions containing biosorbent-zinc ions or biosorbent-desorbent agent for achieving sorption or desorption equilibrium. The final Zn(II) ion concentrations of the solutions were determined by using the procedure described below. The eluted biosorbent was washed repeatedly with bidistilled deionized water to remove any residual desorbing solution and placed into metal solution for the next biosorption cycle. Desorption efficiency was calculated by using following equation:

$$\text{Desorption efficiency} = \frac{\text{Amount of Zn(II) desorbed}}{\text{Amount of Zn(II) adsorbed}} \times 100$$

2.6. Metal analysis

The concentrations of unadsorbed Zn(II) in solutions were determined using flame atomic absorption spectrophotometer (Model 180-70, Hitachi, Japan) after the separation of biosorbent by centrifugation at 4500 rpm for 5 min. Zinc hallow cathode lamp was used. The spectral slit width and specific current/wavelengths were 1.3 nm and 10 mA/213.8 nm, respectively. The instrument response was periodically checked by standart metal solutions. The amount of adsorbed metal ions (q_e) per gram of biomass was calculated using the general defi-

nition:

$$q_e = [(C_i - C_f)]V/M \quad (1)$$

where q_e is the amount of metal ions adsorbed on the biomass at equilibrium (mg g^{-1}), C_i the initial metal ion concentration in solution (mg l^{-1}), C_f the final metal ion concentration in solution (mg l^{-1}), V the volume of the medium (l) and M is the amount of the biomass used in the reaction mixture (g).

2.7. Statistical analysis

All the experiments were carried out in triplicate and data presented are the mean values from these independent experiments. Standard deviation and error bars are indicated wherever necessary. All statistical analysis was done using SPSS 9.05 for Windows where it is possible to evaluate whether the effect and the interaction among the investigated factors are significant with respect to the experimental error.

2.8. FTIR, SEM and EDAX studies

The chemical characteristics of heat inactivated *B. cinerea* biomass before and after Zn(II) sorption were analysed and interpreted by FTIR spectroscopy. The spectra were recorded in a Bruker Tensor 27 Fourier transform infrared spectrometer with the samples prepared as KBr discs.

The surface structure of biosorbent was analysed by scanning electron microscopy (SEM) coupled with energy dispersive X-ray analysis (EDAX) using JEOL 560 LV SEM at 20 keV with background substraction and a summation of 60 scans. Unloaded and zinc-loaded heat inactivated *B. cinerea* biomass samples were mounted on a stainless steel stab with a double-stick tape followed by coating with a thin layer of gold under vacuum to increase the electron conduction and to improve the quality of the micrographs.

3. Results and discussion

3.1. Effect of pH on the biosorption capacity

Biosorption of heavy metal ions onto the surface of the microorganism is affected by initial pH of the biosorption medium [22]. Metal ion uptake is generally suppressed by increasing H^+ concentration, although exceptions exist [23]. The pH dependence of metal uptake is due to solubility of metals and the ionization state of the various functional groups (carboxylate, phosphate and amino groups) on fungal cell walls. This functional groups carry negative charges that allow the functional cell wall components to be potential binding sites for cations [24]. Since high proton concentration at lower pH, heavy metal uptake was decreased because of the positive charge density on metal binding sites. Namely hydrogen ions effectively compete with metal ions to bind the sites. The negative charge density on the cell surface increases with increasing in pH due to deprotonation of the metal binding sites. The metal ions then become more competitive against to bind the sites which increases the biosorption [25].

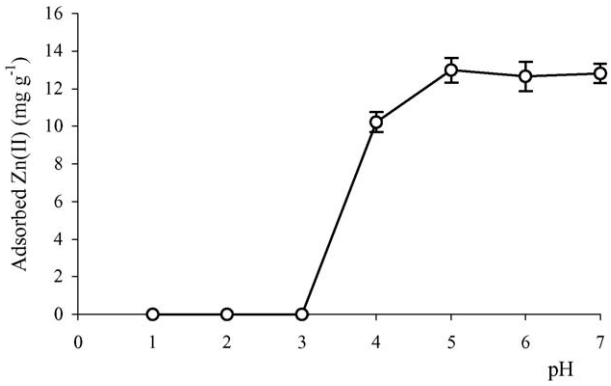

Fig. 1. Effect of pH on Zn(II) ion removal by heat inactivated biomass of *B. cinerea*. Initial Zn(II) ion concentration = 100 mg l^{-1} and the biosorbent concentration = 2 g l^{-1}. The bars represent the standard error of the mean.

Batch equilibrium studies were carried out with different initial pH values ranging from 1.0 to 7.0 in order to investigate the effect of pH on the biosorption capacity of *B. cinerea* for Zn(II). The results presented in Fig. 1 showed that the maximum uptake of Zn(II) was observed at pH 5.0–6.0. Under highly acidic conditions (pH 1–3) no biosorption was occured. The difference in biosorption capacity was found to be statistically significant at pH values between 1.0 and 5.0 comparing with pH 5.0 ($p < 0.05$). At pH 5.0–7.0 biosorption capacity did not differ significantly from pH 5.0 ($p > 0.05$). Maximum biosorption capacity of 12.98 ± 0.9623 mg g^{-1} for Zn(II) was reached for pH 5.0–6.0. Similar results have been reported by other researchers (Table 1).

3.2. Effect of biosorption time

Fig. 2 shows the explained biosorption time of Zn(II) by *B. cinerea* from solutions containing 100 mg l^{-1} of Zn(II) ions. As seen in this figure the biosorption equilibrium was established in 30 min for Zn(II). This suggested that the biosorption process is quite fast. After this equilibrium period the amount of biosorbed metal ions did not change significantly with contact

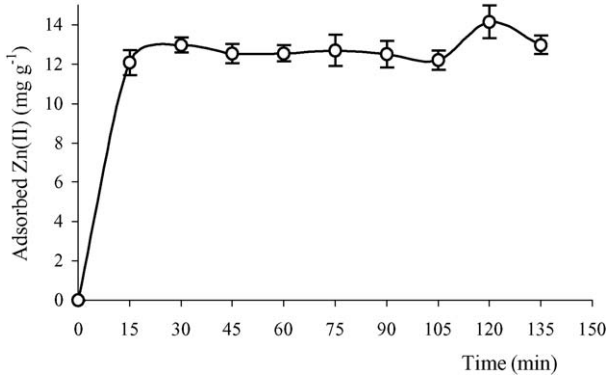

Fig. 2. Biosorption capacity of Zn(II) on heat inactivated biomass of *B. cinerea*. Initial Zn(II) ion concentration = 100 mg l^{-1}, the biosorbent concentration = 2 g l^{-1} and pH 5.0–6.0. The bars represent the standard error of the mean.

Table 1
Biosorption results of zinc ions from the literature by various biosorbents and operating conditions

Biosorbent material	Operating conditions					
	Biosorption capacity (mg g^{-1})	pH	T (°C)	Initial concentration range (mg l^{-1})	Biomass (g l^{-1})	References
Activated carbon	31.11	4.5	25	1–1000	4.0	[6]
Streptoverticillium cinnamoneum	21.3	5.5	28	50–1000	2.0	[8]
Fontinalis antipyretica	12 ± 1[a]	5.0	5	100	2.0	[12]
Aspergillus niger 405	4.70	5.0	25	10	10.0	[18]
Penicillium digitatum	9.7	5.5	25	25	7.0	[23]
Streptomyces noursei	1.6	5.8	30	0.6–65	3.5	[28]
Mucor rouxii (live)	4.89	5.0	n.a.	10	n.a.	[31]
Mucor rouxii (NaOH pretreated)	5.63	5.0	n.a.	10	n.a.	[31]
Mucor rouxii (Na_2CO_3 pretreated)	3.26	5.0	n.a.	10	n.a.	[31]
Mucor rouxii ($NaHCO_3$ pretreated)	6.28	5.0	n.a.	10	n.a.	[31]
Pseudomonas syringae	8.0	n.a.	22	0–13	0.28	[35]
Rhizopus arrhizus	13.5	6–7	n.a.	10–600	3.0	[36]
Phanerochaete chrysosporium[b]	39.0	7.0	25	30–600	n.a.	[37]
Azolla filiculoides	45.2	6.0	18	1000	4.0	[38]
Citrobacter strain MCMB-181	23.62	6.5	25	6.58–115	2.0	[39]
Sargassum sp.	24.35	4.5	30	98	4.0	[40]
Bark of *Pinus sylvetris*	43.5	6.1	n.a.	1045	10.0	[41]
Peat	11.2	4.7	25	587	50.0	[42]
Animal bones	11.55	5.0	20	15–79	4.0	[43]
Botrytis cinerea (heat inactivated)	12.98 ± 0.9623[a]	5.0–6.0	25	100	2.0	This study

n.a. = not available.
[a] Standard deviation of the mean.
[b] Ca-alginate immobilized.

time ($p > 0.05$) and it is thus fixed as the optimum contact time. This trend for metal ions in binding suggests that the binding may take place through interactions with functional groups on the surface of the biosorbent [26].

3.3. Effect of initial Zn(II) ion concentration

The biosorption experiments with heat inactivated *B. cinerea* biomass were conducted using metal ion solutions ranging from 5 to 300 mg l^{-1} at pH 5.0–6.0. The biomass exhibited quite fast metal loading capacity for Zn(II) in the first 30 min, which was found as 12.98 ± 0.9623 mg Zn(II) g^{-1} biomass at the initial concentration of 100 mg l^{-1}. Then the biosorption capacity reached a saturation value and did not change further with initial metal ion concentration ($p > 0.05$) (Fig. 3). The observed enhancement of metal uptake could be due to an increase in electrostatic interactions (relative to covalent interactions) which involve sites of progressively lower affinity for metal ions [27]. The results obtained from this study were found to be comparable with many of the reported literature values (Table 1).

3.4. Analysis of adsorption isotherms

Analysis of the obtained equilibrium data is essential to develop an equation which precisely represents the results and can be used for design purposes. There are two widely accepted and easily linearized adsorption isotherm models, Freundlich and Langmuir models, commonly used in the literature. These two isotherm equations tested in the present study.

The Freundlich model is based on the relationship between the metal uptake capacity "q_e" (mg g^{-1}) of biomass and the residual (equilibrium) metal ion concentration "C_e" (mg l^{-1}). The general Freundlich equation is as follow:

$$q_e = kC_e^{1/n} \quad (2)$$

and linearized form of this model is

$$\ln q_e = \ln k + 1/n \ln C_e \quad (3)$$

where intercept $\ln k$ ($\ln K_f$) is a measure of adsorption capacity and the slope $1/n$ is the intensity of adsorption.The general

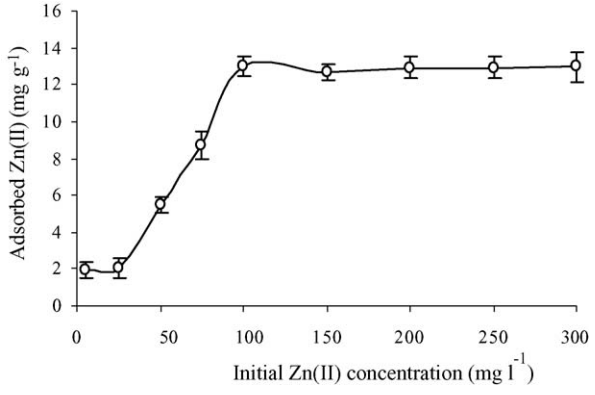

Fig. 3. Effect of initial metal ion concentration on Zn(II) removal by heat inactivated biomass of *B. cinerea*. The biosorbent concentration = 2 g l^{-1}, pH 5.0–6.0 and contact time = 30 min. The bars represent the standard error of the mean.

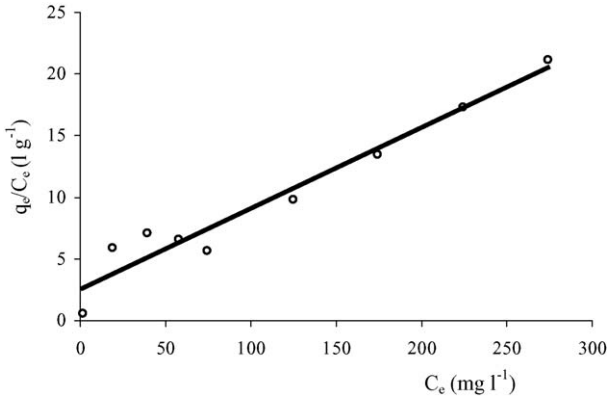

Fig. 4. Langmuir adsorption isotherm of Zn(II) ions on the heat inactivated biomass of *B. cinerea*.

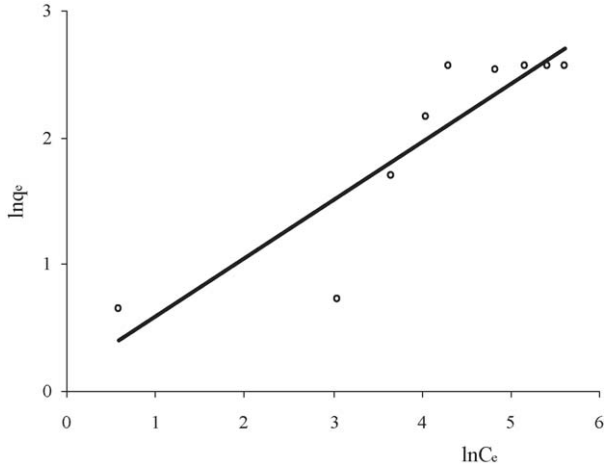

Fig. 5. Freundlich adsorption isotherm of Zn(II) ions on the heat inactivated biomass of *B. cinerea*.

Langmuir equation is commonly presented as:

$$q_e = \frac{Q_0 b C_e}{1 + bC_e} \quad (4)$$

and the equation may be linearized as follow:

$$\frac{C_e}{q_e} = \frac{1}{Q_0 b} + \frac{C_e}{Q_0} \quad (5)$$

where q_e is the amount of metal ion removed (mg g^{-1}), C_e the equilibrium concentration (mg l^{-1}), Q_0 and b are the Langmuir constants related to adsorption capacity and adsorption energy, respectively. The Langmuir and Freundlich constants, along with the regression coefficients have been calculated from the corresponding plots (Figs. 4 and 5) for biosorption of Zn(II) on the biosorbent and the results are presented in Table 2. Langmuir model seemed to define the experimental data obtained from this study well with the regression coefficient (R^2) value of 0.9488. Small b value (0.1682) indicated that Zn(II) ions were binded strongly to heat inactivated fungal biomass. Therefore biosorption process in this study may be interpreted as monolayer adsorption. According to Langmuir model, sorption occurs uniformly on the active sites of the sorbent, and once a sorbate occupies a site, no further sorption can take place at this site [28].

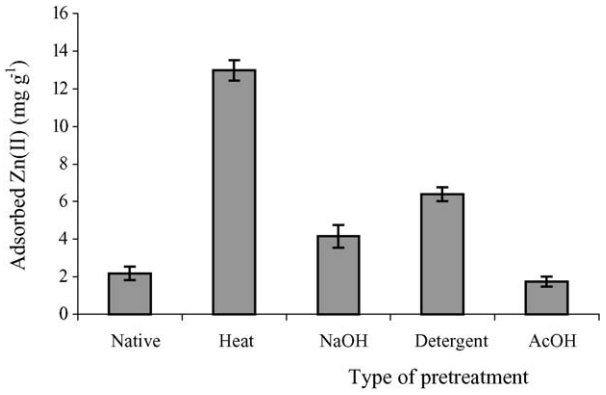

Fig. 6. Effect of pretreatments on the Zn(II) uptake of *B. cinerea* (1: live biomass; 2: heat inactivated biomass; 3: detergent pretreated biomass; 4: sodium hydroxide pretreated biomass; 5: acetic acid pretreated biomass). Initial Zn(II) ion concentration = 100 mg l^{-1}, the biosorbent concentration = 2 g l^{-1}, pH 5.0–6.0 and contact time = 30 min. The bars represent the standard error of the mean.

3.5. Effect of biomass pretreatments on Zn(II) sorption

The metal adsorbing capacity of dead cells may be greater, equivalent to, or less than that of living cells. This depends on various factors which include the fungus under consideration, pretreatment method used and type of metal ions being studied. The use of dead or pretreated cells offers some advantages over the corresponding live cells such as no limitations for toxicity, no requirement for growth media and nutritients, easily desorption of biosorbed metal ions and reusability of biomass as well as usability of dead biomass in traditional adsorption models [29,30].

In order to investigate the influence of different pretreatments on Zn(II) uptake of *B. cinerea* cells, the cells were treated with heat, sodium hydroxide, detergent and acetic acid. Data obtained from pretreatment experiments in this study (see Fig. 6) indicated that all pretreatment methods altered the adsorption capacity of *B. cinerea* biomass which ranged from 1.75 ± 0.4590 to 12.98 ± 0.9623 mg g^{-1} biomass at 100 mg l^{-1} initial concentration of Zn(II) when compared with the native biomass.

Table 2
Isotherm model constants and regression coefficients for biosorption of Zn(II) ions by *B. cinerea* from aqueous solution

Experimental, q_e (mg g^{-1})	Langmuir constant			Freundlich constant		
	Q_0 (mg g^{-1})	b (l mg^{-1})	R^2	K_f	n	R^2
12.98 ± 0.9623[a]	15.26	0.1682	0.9488	1.13	2.17	0.7999

[a] Standard deviation of the mean.

The highest Zn(II) loading value ($12.98 \pm 0.9623\,mg\,g^{-1}$) was obtained by heat inactivated biomass. This was followed by detergent and sodium hydroxide treatments which increased the uptake of Zn(II) from 2.18 ± 0.6245 to 6.38 ± 0.6520 and $4.14 \pm 1.0678\,mg\,g^{-1}$, respectively. In a study by Galun et al. [23] *Penicillium* biomass pretreated at 100 °C for 5 min increased the biosorption Zn(II), Ni(II), Cu(II), Cd(II) and Pb(II) and the increase was attributed to the activation of the latent binding sites after pretreatment.

A small increase observed by alkali pretreatment on biosorption could be explained by the fact that autolytic enzymes could be destroyed. This may not only cause putrefaction of biomass but also remove lipids and proteins that mask binding sites, causing the release of certain biopolymers from the cell wall that have a high affinity towards heavy metal ions [31]. Luef et al. [32] explained the increase observed in zinc uptake by *Aspergillus niger* after sodium hydroxide treatment by suggesting that the removal of certain polysaccharides from the cell wall after alkali pretreatment generates more accessible spaces within the β-glucan chitin skeleton, allowing more zinc ions to be sequestered by this structure. An increase observed by detergent pretreatment on biosorption could be explained in the similar ways as described above since commercial detergents contain alkalies which may cause similar effects as the alkali pretreatment.

The effect of acid pretreatments on the biosorption ability of organisms varies from high to none depending on the type of microorganisms used and the type of heavy metal ions studied. Kapoor and Viraraghavan [21] reported that acid pretreatment decreased the biosorption capacity of *A. niger* for cadmium and nickel whereas Huang and Huang [33] indicated that acid pretreatment increased the biosorption capacity of *A. oryzae*. They also reported that acid pretreatment caused no change in the biosorption capacity of *Rhizopus oryzae*. A similar observation was made in the studies on the biosorption of nickel and copper ions by *Pseudomans aeruginosa* and *Penicillium* biomass suspended in acidic solutions [23,34].

In this study acetic acid pretreatment reduced the Zn(II) loading capacity of the biomass from 2.18 ± 0.6245 to $1.75 \pm 0.4590\,mg\,g^{-1}$. This is because metal ions compete with protons in acidic solutions to bind the binding sites of the biomass. The difference in results after a specific pretreatment could be due to the different strains of fungi, type of metal ion and the form of biomass, such as live and dead, when it is used in biosorption process.

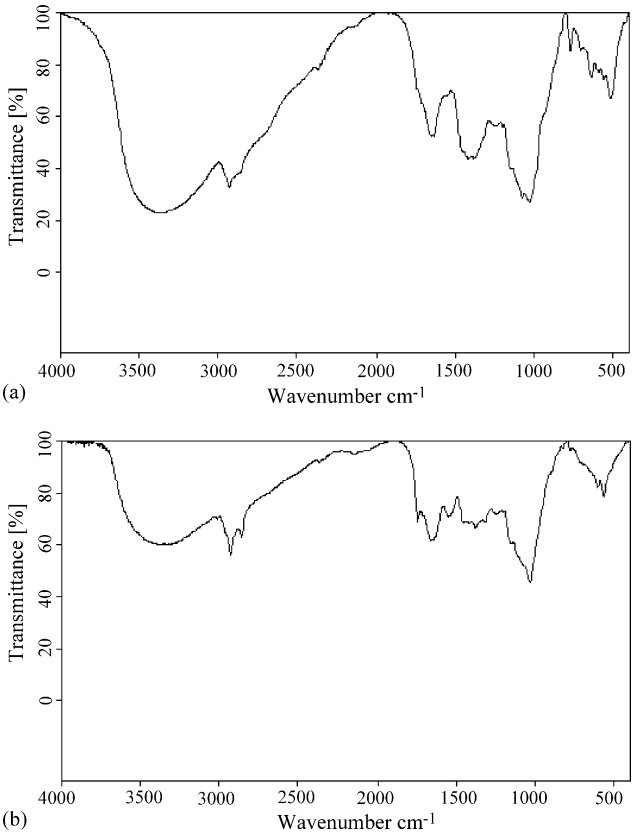

Fig. 8. Infrared spectra of heat inactivated *B. cinerea* biomass: (a) unloaded and (b) Zn(II) loaded biomass.

3.6. Desorption and reuse

The regeneration of the biosorbent is one of the key factors in assessing their potential for commercial application. The

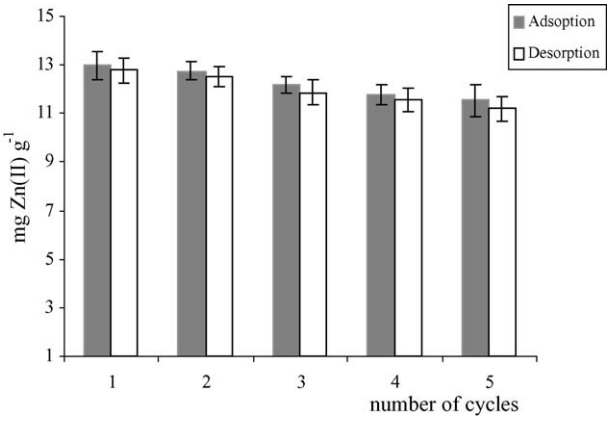

Fig. 7. Adsorption–desorption cycles for heat inactivated *B. cinerea* biomass. The bars represent the standard error of the mean.

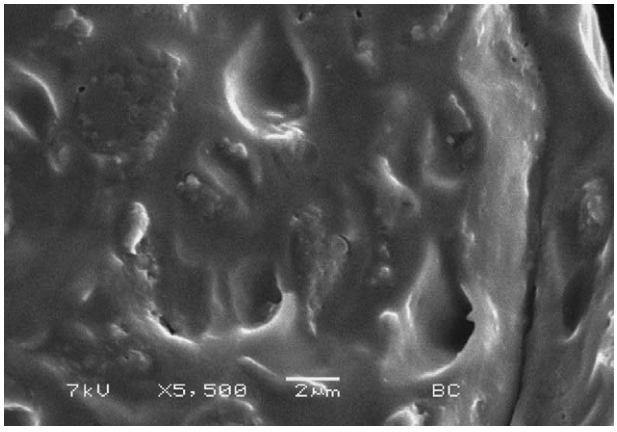

Fig. 9. Typical SEM micrograph of heat inactivated *B. cinerea*.

desorption of adsorbed Zn(II) ions was studied with the form having the highest biosorption capacity, i.e. heat inactivated *B. cinerea*. HCl (10 mM) solution was used as a desorption agent. Higher than 97% of the adsorbed lead ions were desorbed from the biosorbent. The reusability of the biosorbent was tested in five consecutive adsorption–desorption cycles using the same preparation (see Fig. 7). The adsorption efficiency of biomass for Zn(II) did not change significantly and only a maximum 11% decrease was observed after five cycles. These results indicated that the heat inactivated *B. cinerea* biomass offers potential to be used repeatedly in zinc adsorption studies without any detectable loss in the total adsorption capacity.

3.7. Competitive biosorption

Competitive biosorption of Zn(II) in the presence of Cu(II), Cd(II) and Ni(II) ions on heat inactivated *B. cinerea* biomass was conducted in the solution containing these four metal ions simultaneously. The results indicated that the biosorption capacity of the biomass for Zn(II) decreased with the presence of the competing metal ions when compared with that of noncompetitive conditions. The highest Zn(II) loading capacity was found to be $8.16 \pm 0.6482\,mg\,g^{-1}$ at $100\,mg\,l^{-1}$ initial concentration under competitive conditions.

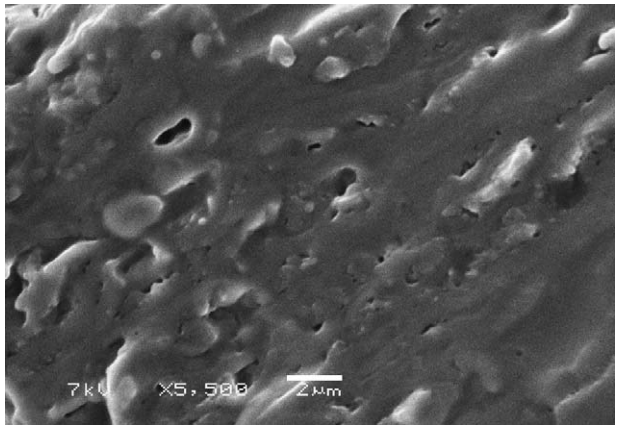

Fig. 10. Typical SEM micrograph of heat inactivated *B. cinerea* (Zn(II) loaded).

3.8. FTIR spectral analysis

The FTIR spectra of dried and zinc loaded *B. cinerea* biomass in the range of 400–4000 cm^{-1} were taken to obtain information on the nature of the possible cell–metal ions interactions and presented in Fig. 8. The FTIR spectroscopic analysis of zinc loaded biosorbent of *B. cinerea* indicated intensity decrease and shifted strong asymetrical stretching bands at 3369 cm^{-1} (indicative of

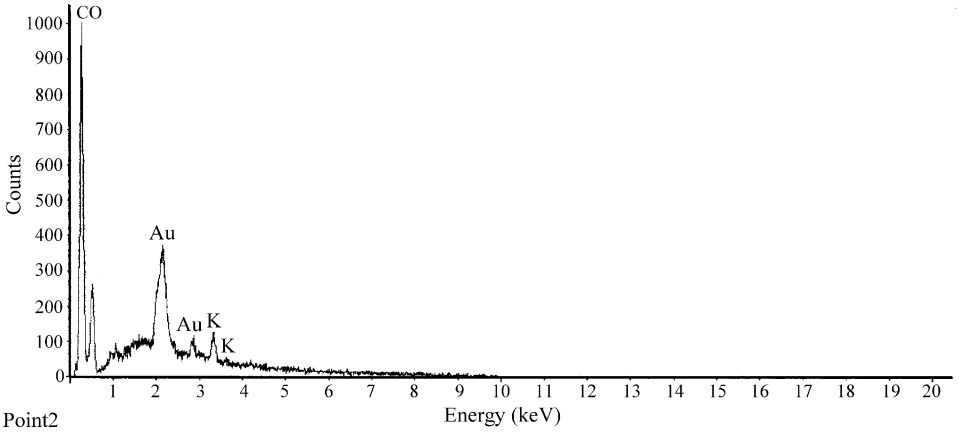

Fig. 11. EDAX spectra of heat inactivated *B. cinerea*.

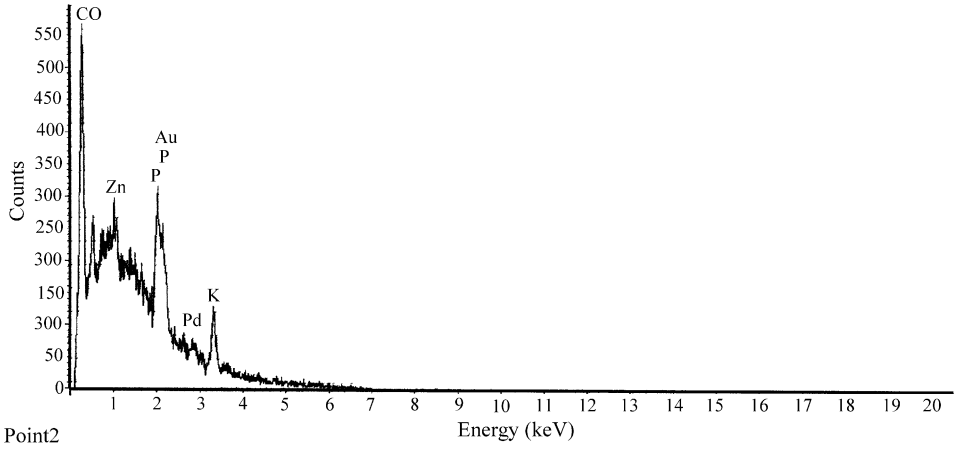

Fig. 12. EDAX spectra of heat inactivated *B. cinerea* (Zn(II) loaded).

–OH and –NH groups) when compared with that of unloaded biomass which showed the same absorption at 3359 cm^{-1}. The similar band shift was observed for the double bands of the carboxylate ion (from 1660 and 1416 to 1659 and 1382 cm^{-1}) with a difference of 1 and 34 cm^{-1}, respectively in addition to intensity decrease of 1416 cm^{-1}. These observations could indicate the involvement of these functional groups in the biosorption process. There was also clear disappearence of the band at 771 cm^{-1} possibly belonging to mono substituted aromatic protons of the biosorbent showing possibly involvement of aromatic amino acids in biosorption. Finally, it should be noted that peaks in the region of lower wavenumbers (under 700 cm^{-1}) appeared as a broad singlet after zinc biosorption in comparison with that of unloaded dried biomass which contained multiply absorption peaks. This could be attributed to an interaction between Zn(II) ions and N-containing bioligands.

3.9. SEM and EDAX analysis

SEM micrographs and EDAX spectra obtained before and after Zn(II) biosorption onto heat inactivated *B. cinerea* biomass are presented in Figs. 9–12, respectively. These micrographs indicated the presence of new particles over the surface of zinc-loaded heat inactivated *B. cinerea* cells. This observation was confirmed by EDAX analysis which revealed Zn(II) signals together with the presence of gold peaks in all spectra.

4. Conclusion

The present work evidenced the possibility of using *B. cinerea* for removal of zinc from aqueous solutions. The biosorption process has been shown to be affected from experimental conditions such as pH, initial metal ion concentration and contact time. The experimental data were shown to be described appropriately by the Langmuir isotherm model. Physical and chemical pretreatments enhanced the biosorption capacity of *B. cinerea* except acid pretreatment when compared with the native biomass. Competitive biosorption studies showed that adsorption yield of Zn(II) ions was reduced by the presence of the other competing ions in reaction mixture. Desorption and reusability studies indicated that the biosorbent could be regenerated using 10 mM HCl solution with up to 98% recovery and reused five times in biosorption–desorption cycles successively. The interactions between Zn(II) ions and functional groups on the cell wall surface of the fungal cells were confirmed by FTIR, SEM and EDAX analysis. It could be concluded based on these results that heat inactivated biomass of *B. cinerea* may be used as an inexpensive, effective and easily cultivable biosorbent for the removal of Zn(II) ions from aqueous solutions.

Acknowledgements

We would like to thank Dr. Fatih Demirci from Anadolu University for providing *Botrytis cinerea* and Dr. Ismail Kiran and Prof. Cengiz Baycu from Osmangazi University, Eskişehir, Turkey for laboratory facilities, SEM/EDAX studies, respectively.

References

[1] S.P. Mishra, D. Tiwari, R.S. Dubey, M. Mishra, Biosorptive behaviour of casein for Zn^{2+}, Hg^{2+} and Cr^{3+}: effects of physico-chemical treatments, Bioresour. Technol. 63 (1998) 1–5.
[2] K. Selvaraj, S. Manonmani, S. Pattabhi, Removal of hexavalent chromium using distillery sludge, Bioresour. Technol. 89 (2003) 207–211.
[3] S. Karabulut, A. Karabakan, A. Denizli, Y. Yürüm, Batch removal of copper(II) and zinc(II) from aqueous solutions with low-rank Turkish coals, Sep. Purif. Technol. 18 (2000) 177–184.
[4] P. Malakul, K.R. Srinivasan, H.Y. Wang, Metal adsorption and desorption characteristics of surfactant–modified clay complexes, Ind. Eng. Chem. Res. 37 (1998) 4296–4301.
[5] T. Viraraghavan, G.A.K. Rao, Adsorption of cadmium and chromium from wastewater by flyash, J. Environ. Sci. Health A 26 (1991) 721–753.
[6] D. Mohan, K.P. Singh, Single-and multi-component adsorption of cadmium and zinc using activated carbon derived from bagasse-an agricultural waste, Water Res. 36 (2002) 2304–2318.
[7] M. Iqbal, R.G.J. Edyvean, Biosorption of lead, copper and zinc ions on loofa sponge immobilized biomass of *Phanerochaete chrysosporium*, Miner. Eng. 17 (2004) 217–223.
[8] P.R. Puranik, K.M. Paknikar, Biosorption of lead and zinc from solutions using *Streptoverticillium cinnamoneum* waste biomass, J. Biotechnol. 55 (1997) 113–124.
[9] R.L. Ramos, L.A.B. Jacome, J.M. Barron, L.F. Rubio, R.M.G. Coronado, Adsorption of zinc(II) from an aqueous solution onto activated carbon, J. Hazard. Mater. B 90 (2002) 27–38.
[10] L. Norton, K. Baskaran, McKenzie S T., Biosorption of zinc from aqueous solutions using biosolids, Adv. Environ. Res. 8 (2004) 629–635.
[11] K.H. Chong, B. Volesky, Description of 2-metal biosorption equilibria by Langmuir-type models, Biotechnol. Bioeng. 47 (1995) 451–460.
[12] R.J.E. Martins, R. Pardo, R.A.R. Boaventura, Cadmium(II) and zinc(II) adsorption by the aquatic moss *Fontinalis antipyretica*: effect of temperature, pH and water hardness, Water Res. 38 (2004) 693–699.
[13] G. Yan, T. Viraraghavan, Heavy metal removal in a biosorption column by immobilized *M. rouxii* biomass, Bioresour. Technol. 78 (2001) 243–249.
[14] C.L. Brierley, Bioremediation of metal contaminated surface and groundwater, Geomicrobiol. J. 8 (1990) 201–223.
[15] R.H. Crist, J.R. Martin, D. Carr, J.R. Watson, H.J. Clarke, D.R. Crist, Interaction of metals and protons with algae. 4. Ion exchange versus adsorption models and a reassessment of Scatchard plots: ion-exchange rates and equilibria compared with calcium alginate, Environ. Sci. Technol. 28 (1994) 1859–1866.
[16] E. Fourest, C. Canal, J.C. Roux, Improvement of heavy metal biosorption by mycelial dead biomasses (*Rhizopus arrhizus*, *Mucor miehei* and *Penicillium chrysogenum*): pH control and cationic activation, FEMS Microbiol. 14 (1994) 325–332.
[17] P. Ahuja, R. Gupta, R.K. Saxena, Zn^{2+} biosorption by *Oscillatoria anguistissima*, Process Biochem. 34 (1999) 77–85.
[18] Z. Filipovič-Kovačević, L. Sipos, F. Briški, Biosorption of chromium, copper, nickel and zinc ions onto fungal pellets of *Aspergillus niger* 405 from aqueous solutions, Food Technol. Biotechnol. 38 (2000) 211–216.
[19] I. Kiran, H.N. Yildirim, J.R. Hanson, P.B. Hitchcock, The antifungal activity and biotransformation of diisophorone by the fungus *Aspergillus niger*, J. Chem. Technol. Biotechnol. 79 (2004) 1366–1370.
[20] S. Tunali, I. Kiran, T. Akar, Chromium(VI) biosorption characteristics of *Neurospora crassa*, Min. Eng. 18 (2005) 681–689.
[21] A. Kapoor, T. Viraraghavan, Biosorption of heavy metals on *Aspergillus niger*: effect of pretreatment, Bioresour. Technol. 63 (1998) 109–113.
[22] A. Özer, D. Özer, H.I. Ekiz, Application of Freundlich and Langmuir models to multistage purification process to remove heavy metal ions by using *Schizomeris leibleinii*, Process Biochem. 34 (1999) 919–927.

[23] M. Galun, E. Galun, B.Z. Siegel, P. Keller, H. Lehr, S.M. Siegel, Removal of metal ions from aqueous solutions by *Penicillium* biomass: kinetic and uptake parameters, Water Air Soil Pollut. 33 (1987) 359–371.
[24] P. Yin, Q. Yu, B. Jin, Z. Ling, Biosorption removal of cadmium from aqueous solution by using pretreated fungal biomass cultured from starch wastewater, Water Res. 33 (1999) 1960–1963.
[25] A. Kapoor, T. Viraraghavan, D.R. Cullimore, Removal of heavy metals using the fungus *Aspergillus niger*, Bioresour. Technol. 70 (1999) 95–104.
[26] M.Y. Arıca, G. Bayramoğlu, M. Yılmaz, S. Bektaş, Ö. Genç, Biosorption of Hg^{2+}, Cd^{2+}, and Zn^{2+} by Ca-alginate and immobilized wood-rotting fungus *Funalia trogii*, J. Hazard. Mater. B 109 (2004) 191–199.
[27] S. Al-Asheh, Z. Duvnjak, Adsorption of copper and chromium by *Aspergillus carbonarius*, Biotechnol. Progr. 11 (1995) 638–642.
[28] B. Mattuschka, G. Straube, Biosorption of metals by a waste biomass, J. Chem. Technol. Biotechnol. 58 (1993) 57–63.
[29] G. Yan, T. Viraraghavan, Heavy-metal removal from aqueous solution by fungus *Mucor rouxii*, Water Res. 37 (2003) 4486–4496.
[30] G.M. Gadd, C. White, Removal of thorium from simulated acid process steams by fungal biomass, Biotechnol. Bioeng. 33 (1989) 592–597.
[31] G. Yan, T. Viraraghavan, Effect of pretreatment on the bioadsorption of heavy metals on *Mucor rouxii*, Water SA 26 (2000) 119–123.
[32] E. Luef, T. Prey, C.P. Kubicek, Biosorption of zinc by fungal micelial wastes, Appl. Microbiol. Biotechnol. 34 (1991) 688–692.
[33] C. Huang, C.P. Huang, Application of *Aspergillus oryzae* and *Rhizopus oryzae* for Cu(II) removal, Water Res. 30 (1996) 1985–1990.
[34] P. Sar, S.K. Kazy, R.K. Asthana, S.P. Singh, Metal adsorption and desorption by lyophilized *Pseudomans aeruginosa*, Int. Biodeter. Biodegr. 44 (1999) 101–110.
[35] J.P.S. Cabral, Selective binding of metal ions to *Pseudomonas syringae* cells, Microbios 71 (1992) 47–53.
[36] E. Fourest, J. Roux, Heavy metal biosorption by fungal mycelium byproducts: mechanisms and influence of pH, Appl. Microbiol. Biotechnol. 37 (1992) 399–403.
[37] M.Y. Arica, C. Arpa, A. Ergene, G. Bayramoglu, O. Genc, Ca-alginate as a support for Pb(II) and Zn(II) biosorption with immobilized *Phanerochaete chrysosporium*, Carbohyd. Polym. 52 (2003) 167–174.
[38] M. Zhao, J.R. Duncan, R.P. Van Hille, Removal and recovery of zinc from solute on and electroplating effluent using *Azolla filiculoides*, Water Res. 33 (1999) 1516–1522.
[39] P.R. Puranik, K.M. Paknikar, Biosorption of lead, cadmium and zinc by *Citrobacter* strain MCMB-181: characterization studies, Biotechnol. Progr. 15 (1999) 228–237.
[40] A.J.P. Esteves, E. Valdman, S.G.F. Leite, Repeated removal of cadmium and zinc from an industrial effluent by waste biomass *Sargassum* sp., Biotechnol. Lett. 22 (2000) 499–502.
[41] I. Gaballah, G. Kiberus, Elimination of As, Hg and Zn from synthetic solutions and industrial effluents using modified bark, in: M. Misra (Ed.), Separation Process: Heavy Metals, Ions and Materials, The Mineral, Metals and Materials Society, 1994, pp. 15–26.
[42] T. Gosset, J.-L. Trancart, D.R. Thevenot, Batch metal removal by peat: kinetics and thermodynamics, Water Res. 20 (1986) 21–26.
[43] F. Banat, S. Al-Asheh, F. Mohai, Batch zinc removal from aqueous solution using dried animal bones, Sep. Purif. Technol. 21 (2000) 155–164.