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Journal of Hazardous Materials B109 (2004) 191–199
Journal of Hazardous Materials
www.elsevier.com/locate/jhazmat

Biosorption of Hg^{2+}, Cd^{2+}, and Zn^{2+} by Ca-alginate and immobilized wood-rotting fungus *Funalia trogii*

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Received 6 November 2003; received in revised form 28 March 2004; accepted 30 March 2004

Available online 5 May 2004

Abstract

Funalia trogii biomass was immobilized in Ca-alginate gel beads. The live and heat inactivated immobilized forms were used for the biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} ions by using plain Ca-alginate gel beads as a control system. The effect of pH was investigated and the maximum adsorption of metal ions on the Ca-alginate and both live and inactivated immobilized fungal preparations were observed at pH 6.0. The temperature change between 15 and 45 °C did not affect the biosorption capacity. The biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} ions on the Ca-alginate beads and on both immobilized forms was studied in aqueous solutions in the concentration range of 30–600 mg/L. The metal biosorption capacities of the heat inactivated immobilized *F. trogii* for Hg^{2+}, Cd^{2+} and Zn^{2+} were 403.2, 191.6, and 54.0 mg/g, respectively, while Hg^{2+}, Cd^{2+} and Zn^{2+} biosorption capacities of the immobilized live form were 333.0, 164.8 and 42.1 mg/g, respectively. The same affinity order on a molar basis was observed for single or multi-metal ions ($Hg^{2+} > Cd^{2+} > Zn^{2+}$). The Langmuir and the Freundlich type models were found to exhibit good fit to the experimental data. The experimental data were analyzed using the first-order (Langergren equations) and the second order (Ritchie equations). The experimental biosorption capacity with time is found to be best fit the second-order equations. The alginate-fungus system could be regenerated by washing with a solution of hydrochloride acid (10 mM). The percent desorption achieved was as high as 97. The biosorbents were reused in five biosorption–desorption cycles without significant loss of their initial biosorption capacity.

Keywords: Heavy metal ions; Biosorption; Ca-alginate; Immobilized biomass; *Funalia trogii*

1. Introduction

Biosorption is the non-specific term used to denote the complex process whereby biomass, usually microbial, is utilized to remove solutes or colloidal material during wastewater or other water treatment. Biosorption may involve one or more of several processes depending on physicochemical conditions and the origin and physiological state of the biomass. These include metal ion coordination complex, ion exchange and covalent linkage to biomass components and physiologically mediated intracellular uptake [1–5]. It has been shown that biosorption could be used to decontaminate metal-bearing wastewaters and to concentrate metals [6]. Live or inactivated microbial cells can be used to remove heavy metal ions, but maintaining the survivability of the microbial cells during biosorption process is difficult, because they require a continuous supply of nutrients and metal toxicity might take place for microbial cells [7–9]. Several researchers have also shown that non-living biomass is also able to bind heavy metals effectively. Therefore, the use of non-living microbial cells can eliminate these problems and can be regenerated and reused for many cycles [10–12].

In industrial or technical operations, the use of non-living microbial cells in the powdered form have several problems such as difficulty in separation of microbial cells after biosorption, mass loss during separation and low mechanical strength and small particle size, which make difficult to use in the batch and continuous systems [13,14]. These problems can be solved by immobilization of micro-

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doi:10.1016/j.jhazmat.2004.03.017

bial cells using natural or synthetic polymers. Natural polymers such as alginate, chitosan, chitin, and cellulose derivatives have been mostly used as the matrix for the immobilization of the microbial cells via entrapment [13–17]. These polymers are also known to bind metal ions strongly. Entrapment of microbial cells in these polymer supports could also enhance microbial cells performance and adsorptive capacity of the biosorbents systems for the heavy metal ions [18–21].

The purpose of this research was to study the enhancement of the adsorptive capacity of Ca-alginate beads for the removal of heavy metal ions from aqueous solution by combining them with a white-rot fungus *Funalia trogii* biomass. Information is available on the use of immobilized forms of other white-rot fungi species for removal heavy metals ions [8], but no study has been conducted on the use of free and immobilized biomass of *F. trogii* for this purpose. Therefore, there is a need to determine the heavy metal removal capacity of this immobilized fungus. *F. trogii* mycelia were immobilized on Ca-alginate, which is not toxic to fungal cells and is a biodegradable biopolymer. The immobilization method is also easy and can be performed under very mild conditions without damaging the living microbial cells. Both immobilized live and inactivated preparations were used for removal of Hg^{2+}, Cd^{2+} and Zn^{2+} ions from aqueous solutions. The effects of medium pH, temperature and the adsorption isotherm were investigated in a batch system. Finally, multi-metal ion biosorption and elution-reuse of Ca-alginate and immobilized fungal preparations were evaluated.

2. Materials and methods

2.1. Microorganism and media

Pure culture of *F. trogii* (*Trametes trogii*; MAFF 420251) was obtained from MAFF Genebank Culture Collection (Kannondai, Tsukuba, Ibaraki, Japan), and was maintained by subculturing on malt dextrose agar slants. The growth medium and growth conditions were previously described elsewhere [2].

2.2. Immobilization of F. trogii

The immobilization of *F. trogii* mycelia via entrapment was carried out as follows: Na-alginate (2.0 g; from *Macrosytia pyrifera*, high viscosity, Sigma Chem. Co., USA) was dissolved in distilled water 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 $CaCl_2$ through a nozzle (2.0 cm length, 1.0 mm internal diameter) using a peristaltic pump, and the solution was stirred to prevent aggregation of the fungus mycelia immobilized Ca-alginate 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 Ca-alginate 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 Ca-alginate 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 $CaCl_2$ 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 at the immobilized preparations was determined by weighing (after drying in an oven at 50 °C overnight) the Ca-alginate beads before and after cell growth.

2.3. Biosorption studies

The biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} ions, on plain Ca-alginate beads and on the both immobilized live and heat inactivated *F. trogii* was investigated in batch biosorption equilibrium experiments. The stock solutions of metal ions (i.e., Hg^{2+}, Cd^{2+} and Zn^{2+}: 1.0 g/L) were prepared using nitrate salts in double distilled water.

The effect of pH on the biosorption rate 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 afterwards) at 25 °C. Solution containing 200 mg/L of Hg^{2+}, Cd^{2+} and Zn^{2+} ions and each Ca-alginate, live or heat inactivated fungus immobilized in beads were combined and the samples were stirred at 400 rpm.

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

The competitive biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} ions from their mixture was investigated in the same manner. The medium containing 0.2 mmol/L of each metal ion was incubated with the biosorbents in batch fashion.

2.4. Analytical procedure

Biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} ions from aqueous solutions were studied in batch systems. After the desired incubation period (up to 120 min) the aqueous phases were separated from the biosorbents and the concentration of Hg^{2+}, Cd^{2+} and Zn^{2+} ions in these phases were measured. A Shimadzu AA-6800 Flame Atomic Absorption Spectrophotometer was used for the determination of metal ions. Deuterium background correction was used and the spectral slit width was 0.5 nm. The working current/wavelength values for Cd^{2+} and Zn^{2+} were: 8.0 mA/228.8 nm and 8.0 mA/213.9 nm, respectively. For mercury measurement,

the instrument was equipped with a Mercury Vapor Unit (MVU-1A). Mercury determinations were realized by using Mercury Vapor Unit (MVU-1A). The working conditions were as follows:

- working current/wavelength: 6 mA/253.6 nm;
- concentration of $SnCl_2$: 1% (w/v);
- concentration of $KMnO_4$: 0.5% (w/v);
- concentration of H_2SO_4: 5% (v/v).

The instrument response was periodically checked with metal ion standard solutions. For each set of data reported, 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 Ca-alginate and both live and inactivated fungus immobilized preparations (mg metal ions/g dry beads) was obtained by using the following expression:

$$q_{\text{exp}} = \frac{(C_0 - C) \times V}{M} \quad (1)$$

where q_{exp} is the amount of metal ions adsorbed onto the unit mass of the adsorbent (mg/g), C_0 and C are the concentrations of the metal ions before and after biosorption (mg/L), V the volume of the aqueous phase (L), and M the amount of the adsorbent (g).

A known quantity of wet Ca-alginate or fungal immobilized preparations was used in the adsorption tests. After adsorption process, the adsorbents were dried in an oven at 50 °C overnight and the dry weight of the preparations was used in the calculations.

2.5. Desorption/reuse

In order to determine the reusability of the Ca-alginate and immobilized fungal preparations, consecutive adsorption–desorption cycles were repeated three times by using the same biosorbent. Desorption of Hg^{2+}, Cd^{2+} and Zn^{2+} ions were performed by 10 mM HCl solution. The Ca-alginate and immobilized fungal preparations loaded with metal ions were placed in the desorption medium and stirred at 400 rpm for 60 min at 25 °C. The final Hg^{2+}, Cd^{2+} and Zn^{2+}, ion concentrations in the aqueous phase were determined by using an AAS as described above. Desorption ratio was calculated from the amount of metal ions adsorbed on the immobilized preparations and the final metal ion concentration in the desorption medium. Desorption ratio was calculated from the following equation:

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

3. Results and discussion

3.1. Properties of the alginate based biosorbent systems

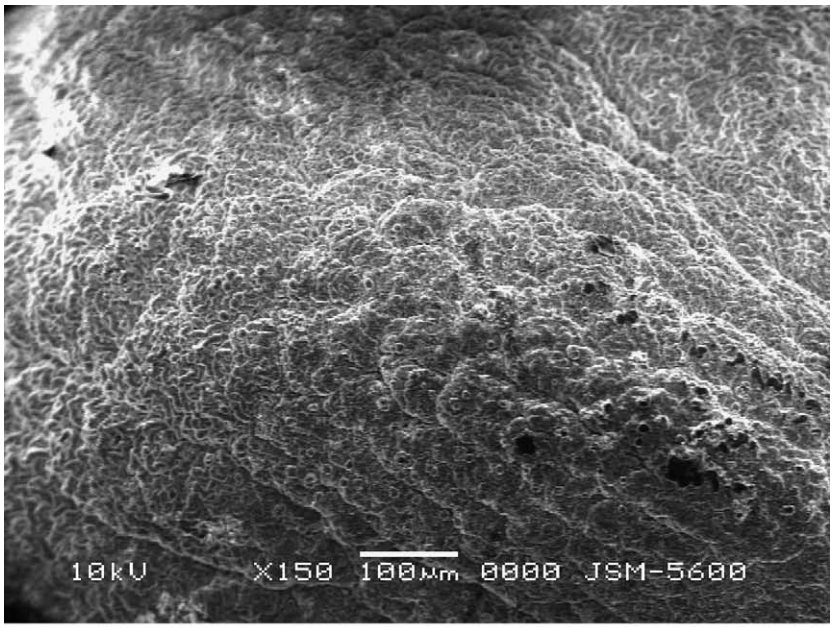
Alginic acid or alginate, the salt of alginic acid, is the common name given to the family of linear polysaccharides containing 1,4-linked β-D-mannuronic (M) and α-L-guluronic (G) acid residues arranged in a non-regular, block wise order along the chain [22–24]. The salts of alginic acid with monovalent ions are soluble in aqueous medium whereas those with divalent or polyvalent metal ions (except Mg^{2+}) are insoluble. Alginates were preferred over other materials because of their various advantages such as biodegradability, hydrophilic properties, presence of carboxylic groups, and natural origin. Other additional advantages are their low density and mechanical stability that make them highly suitable for many biotechnological applications.

In this research, Ca-alginate gel beads were used both as an adsorbent and a support material for the entrapment of a white-rot fungus *F. trogii*. Ca-alginate beads were prepared by cross-linking with divalent calcium ions. Cross-linking should be a combination of metal co-ordination and ion exchange interaction between the carboxylic groups of guluronic acid groups of alginate and divalent calcium ions [23,24]. These interactions cause alginate droplets to precipitate in the bead form in calcium chloride solution.

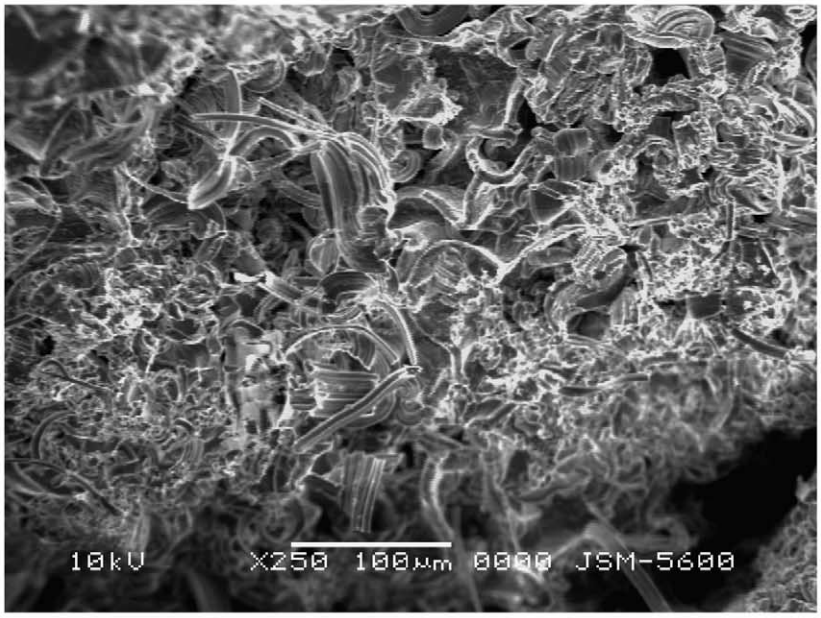
The water content of Ca-alginate beads was 245%. These beads were very stable over the experimental pH range of 3.0–7.0. The stability of a support under specified experimental conditions is a very important parameter in the cell immobilization. On the other hand, one of the most important disadvantages of the cells immobilization is the increase in the mass transfer resistance due to the polymeric matrix. The use of alginate instead of other synthetic support materials such as polyvinyl alcohol and acrylic polymers in the immobilization system eliminates such disadvantages. The structural properties of alginate enhance the heavy metal ions adsorption capacity of the immobilization system.

Cell walls of fungal biomass can be regarded as a mosaic of different functional groups where coordination complexes and/or ion exchange with metal ions can be formed. The functional groups for heavy metal ions binding on the fungal cell walls are carboxyl (–COOH), phosphate (PO_4^{3-}), amide ($–NH_2$), thiol (–SH), and hydroxide (–OH). In fungal cell walls, chitin and its associated proteins contain many carboxyl groups with pK_a values in the range of 4.0–5.0 [25]. Phosphate groups are present mainly in glycoproteins and are believed to play an important role in biosorption because they can exhibit a negative charge above pH 3.0 [26].

The SEM micrographs of the plain Ca-alginate bead and fungus immobilized bead are presented in Fig. 1A and B, respectively. The Ca-alginate bead is spherically shaped with approximately a 2 mm diameter. The SEM micrograph of fungus immobilized Ca-alginate bead was completely different from the plain Ca-alginate bead. There was a uniform fungal growth on the bead surface indicating that immobi-



(A)



(B)

Fig. 1. SEM of plain and fungus immobilized Ca-alginate bead: (A) plain Ca-alginate bead; (B) fungus immobilized Ca-alginate bead.

lization of fungal mycelium is not localized. The uniform distribution of fungal mycelia is an important criterion for the proper biosorption of heavy metal ions on the whole surface area of the fungus immobilized Ca-alginate beads.

3.2. Biosorption rate

The biosorption rates of heavy metal ion species on the Ca-alginate and both immobilized live and inactivated fungal mycelia were obtained by following the decrease in the concentration of Hg^{2+}, Cd^{2+} and Zn^{2+} ions within the adsorption medium with time. The biosorption rate of cadmium is exemplified in Fig. 2. The metal ions adsorption rate is high at the beginning of adsorption process and saturation levels are completely reached at about 60 min for all metal ions. After this equilibrium period, the amount of adsorbed metal ions on the biosorbents does not significantly change with time. This trend in binding of metal ions suggests that the binding may be through interactions with functional groups located on the surface of the biosorbents. Data on the adsorption rates of heavy metal ions by various biosorbents have shown a wide range of adsorption time. The Hg^{2+} biosorp-

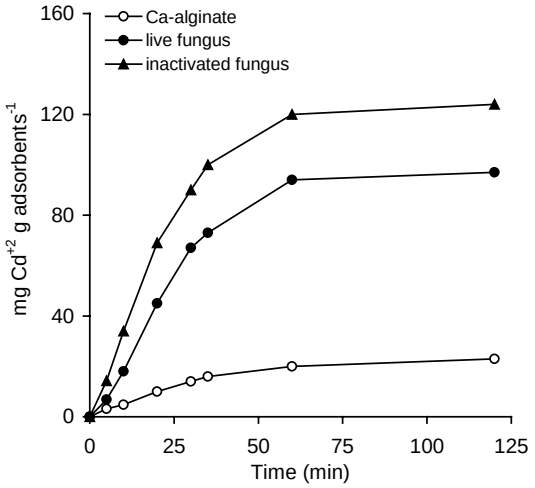


Fig. 2. Biosorption rate of Cd^{2+} ions by plain Ca-alginate and both immobilized live and inactivated *F. trogii*. Adsorption conditions: initial concentration of metal ions: 200 mg/L, pH 6.0, temperature: 20 °C.

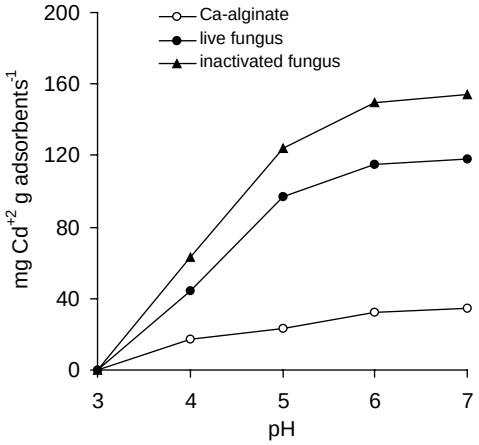


Fig. 3. Effect of pH on the biosorption capacity of Cd^{2+} on the Ca-alginate and both immobilized live and inactivated *F. trogii*. Biosorption conditions: initial concentration of metal ions: 200 mg/L; volume of biosorption medium: 25 mL; temperature: 20 °C; biosorption time: 60 min.

tion rate of *Phanerochaete chrysosporim* is fast and reached saturation value within 1 h [26]. The biosorption of Cd^{2+} onto pre-treated biomass of marine alga *Durvillaea potatorum* has been studied and the biosorption process was very fast, 90% uptake taking place within 30 min [27]. Pagnanelli et al. [28] have studied Cu^{2+} and Cd^{2+} biosorption in single and multi-metal systems on *Arthrobacter* sp. *biomass* and the biosorption equilibrium was established within 30 min. There are several parameters that determine the biosorption rate such as stirring rate, structural properties of the support and the biosorbent (e.g., protein and carbohydrate composition and surface charge density, surface topography and area), amount of biosorbent, properties of the ions under study, initial concentration of ionic species and the presence of other metal ions that may compete with the ionic species of interest for the active binding sites. Therefore, it is difficult to compare the biosorption rates reported here.

3.3. Effect of pH

It is well known that biosorption of heavy metal ions by biosorbents depends on the pH of the solution. The pH affects the availability of metal ions in solution and the metal binding sites on biosorbent surface. Fig. 3 shows the effect of pH on the biosorption of cadmium. As can be seen from the figure, the maximum biosorption of Cd^{2+} ions on the alginate and both live and inactivated immobilized fungal mycelia were observed around pH 6.0. Similar results were observed for all the other tested metal ions (data not shown). There was an increase in the adsorbed amount of heavy metal ions per unit weight of fungal biomass with increasing pH from 3.0 to 6.0. The observed increase in the biosorption levels with increasing pH can be explained by the strong relation of biosorption to the number of surface negative charges, which depends on the dissociation of functional groups. The low biosorption capacity at pH values below 4.0 was attributed to hydrogen ions that compete with metal ions on the sorption sites [29,30].

3.4. Effect of initial metal ion concentration

Metal ions biosorption capacities of the Ca-alginate and both immobilized live and inactivated *F. trogii* biomass are presented as a function of the initial concentration of metal ions (in the range of 30–600 mg/L) within the aqueous biosorption medium in Fig. 4a–c. The amount of Hg^{2+}, Cd^{2+} and Zn^{2+} ions adsorbed per unit mass of the biosorbent (i.e., biosorption capacity) increased with increasing initial concentration of metal ions in the adsorption medium, as expected. From the figures, the maximum of Hg^{2+}, Cd^{2+}, and Zn^{2+} ions adsorbed on the plain alginate beads were 34.89 ± 1.84, 30.90 ± 1.52 and 31.05 ± 1.03 mg/g dry alginate beads, respectively. Maximum biosorption capacities for immobilized live and inactivated mycelia of *F. trogii* were found as 333.0 ± 5.88 and 403.2 ± 5.32 mg/g for Hg^{2+} 164.8 ± 3.04 and 191.6 ± 6.70 mg/g for Cd^{2+} and 42.1 ± 1.21 and 54.0 ± 2.15 mg/g for Zn^{2+}, respectively. In a previous study, the biosorption capacities of the immobilized live and inactivated white-rot fungus *L. sajur-caju* were 104.8 and 123.5 mg/g for Cd^{2+} ions, respectively [8]. The heat treatment of both immobilized white-rot fungi resulted an increase in the biosorption capacities for metal ions. Similar observations were reported for other biomasses, including fungi and yeast [31,32] and can be attributed to a variety of resistance mechanisms. These mechanisms include: (i) extracellular complexation with metal binding proteins such as metallothionin and phytochelatins which are proteins that contain large amounts cysteine and bind heavy metal ions [33] (ii) efficient pumping out metal ions from the living cell [1,34].

The electronegative values of Hg^{2+}, Cd^{2+}, and Zn^{2+} metal ions are 2.00, 1.69 and 1.65, respectively. The more

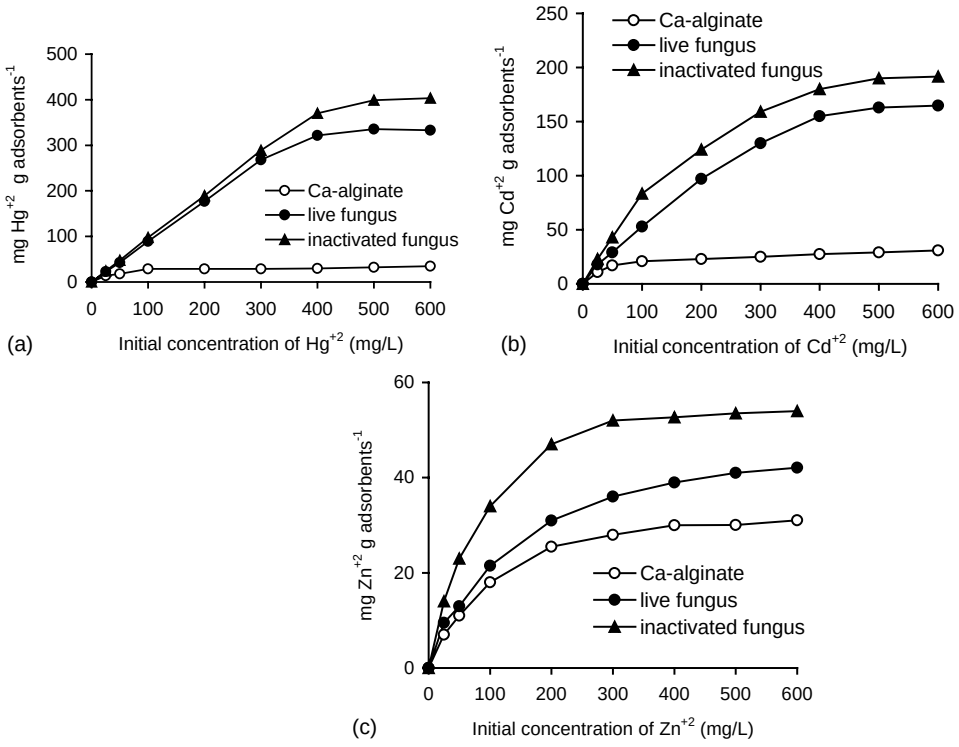


Fig. 4. (a) Biosorption capacities of plain Ca-alginate and both immobilized live and inactivated *F. trogii* for Hg^{2+} ions. Biosorption conditions: volume of the biosorption medium: 25 mL; pH 6.0; temperature: 20 °C; biosorption time: 60 min. (b) Biosorption capacities of plain Ca-alginate and both immobilized live and inactivated *F. trogii* for Cd^{2+} ions. Biosorption conditions: volume of the biosorption medium: 25 mL; pH 6.0; temperature: 20 °C; biosorption time: 60 min. (c) Biosorption capacities of plain Ca-alginate and both immobilized live and inactivated *F. trogii* for Zn^{2+} ions. Biosorption conditions: volume of the biosorption medium: 25 mL; pH 6.0; temperature: 20 °C; biosorption time: 60 min.

electronegative metal ions will be more strongly attracted to the fungal cells surfaces. Hg^{2+} has the highest affinity for both immobilized live and inactivated fungal preparations and it has a greater electronegativity than both Cd^{2+} and Zn^{2+}. The sorption capacity of both biosorbents for Cd^{2+} ions is also greater than Zn^{2+} ions and the same trend was observed by their respective electronegativities.

3.5. Equilibrium studies

In order to optimize the design of a biosorption system to remove metal ions it is important to establish the most appropriate correlations for the equilibrium curves. Two isotherm equations have been tested in the present study, namely Langmuir and Freundlich.

The most widely used equation for modeling equilibrium data is the Langmuir equation, which for dilute solutions may be represented as

$$q_e = \frac{K_L C_e}{1 + a_L C_e} \tag{3}$$

where q_e is the amount of adsorbed metal ions at time t (mg/g) and C_e the equilibrium concentration (mg/L).

The constants K_L (L/g) and a_L (L/mg) are the parameters of the Langmuir equation and can be determined from a linearized form of the above equation

$$\frac{C_e}{q_e} = \left[\left(\frac{1}{K_L}\right) + \left(\frac{a_L}{K_L}\right) C_e\right] \tag{4}$$

Therefore, a plot of C_e/q_e versus C_e gives a straight line of slope a_L/K_L and intercept $1/K_L$ (Fig. 5). The constant K_L is the Langmuir equilibrium constant and the ratio K_L/a_L gives the theoretical monolayer saturation capacity.

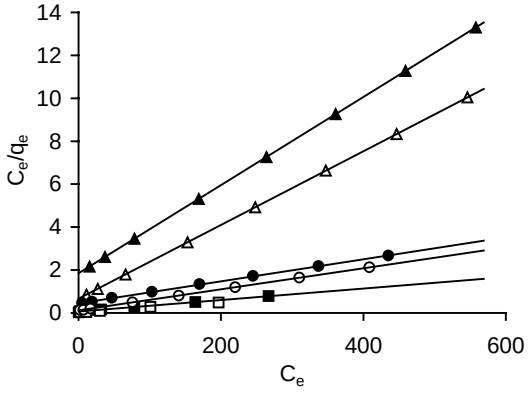


Fig. 5. The Langmuir plots of Hg^{2+}, Cd^{2+} and Zn^{2+} ions on both immobilized live and inactivated *F. trogii*: (□) live fungus Hg^{2+}; (■) inactivated fungus Hg^{2+}; (△) live fungus Zn^{2+}; (▲) inactivated fungus Zn^{2+}; (○) live fungus Cd^{2+}; (●) inactivated fungus Cd^{2+}.

Table 1
Isotherm models constants and correlation coefficients for biosorption of Hg^{2+}, Cd^{2+}, and Zn^{2+} ions from aqueous solution

Immobilized fungal mycelia	q_{exp} (mg/mL)	Langmuir			Freundlich		
		K_L/a_L (mg/mL)	$1/K_L$ ($\times 10^{-3}$ mol/L)	R^2	a	b	R^2
Live *F. trogii*, Hg^{2+}	333.0	374.5	0.34	0.989	18.2	0.59	0.970
Inactivated *F. trogii*, Hg^{2+}	403.2	425.2	0.12	0.998	50.7	0.49	0.975
Live *F. trogii*, Cd^{2+}	164.8	199.9	3.92	0.989	5.6	0.59	0.991
Inactivated *F. trogii*, Cd^{2+}	191.6	203.3	1.04	0.998	20.5	0.40	0.981
Live *F. trogii*, Zn^{2+}	42.1	48.7	28.20	0.998	2.9	0.44	0.983
Inactivated *F. trogii*, Zn^{2+}	54.0	58.2	10.09	0.999	7.2	0.38	0.988

The Freundlich expression is an empirical equation based on adsorption on a heterogeneous surface. The Freundlich equation is commonly presented as

$$q_e = aC_e b \tag{5}$$

where q_e is the amount of adsorbed metal ions at time t (mg/g), C_e the equilibrium concentration (mg/L). a and b are the equilibrium constants indicative of adsorption capacity and adsorption intensity, respectively, and Eq. (5) may be linearized by taking logarithms:

$$\ln q_e = b \ln C_e + \ln a \tag{6}$$

A plot of $\ln q_e$ versus $\ln C_e$ enables the constant a and exponent b to be determined.

The Langmuir and Freundlich constants along with the correlation coefficients (R^2) have been calculated from the corresponding plots for biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} ions on the biosorbents and the results are presented in Table 1. The Langmuir model was able to describe the experimental equilibrium data for biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} ions on both immobilized preparations under given experimental conditions (Table 1). The magnitudes of a and b (Freundlich constants) show easy separation of metal ions from aqueous medium and indicate favorable adsorption. The correlation regression coefficients also show that the adsorption process can be well defined by both Langmuir and Freundlich equations.

3.6. Biosorption kinetics modeling

In order to examine the controlling mechanism of biosorption process such as mass transfer and chemical reaction, kinetic models were used to test the experimental data. The large number and different chemical groups on the cell wall of the fungal mycelia (e.g., –COOH, $–NH_2$, =NH, –SH, –OH) imply that there are many types of fungal mycelia–metal ions interactions. The kinetic models (the first-order and second-order equations) can be used in this case assuming that measured concentrations are equal to cell surface concentrations.

The first-order rate equation of Langergren is one of the most widely used for the sorption of solute from a liquid solution [35,36]. It may be represented as follows:

$$\frac{dq_t}{dt} = k_1(q_{eq} - q_t) \tag{7}$$

where k_1 is the rate constant of first-order biosorption (min^{-1}) and q_{eq} and q_t denote the amounts of biosorption at equilibrium and at time t (mg/g), respectively. After integration by applying boundary conditions, $q_t = 0$ at $t = 0$ and $q_t = q_t$ at $t = t$, gives

$$\log\left(\frac{q_{eq}}{q_{eq} - q_t}\right) = \frac{k_1 t}{2.303} \tag{8}$$

Eq. (8) can be rearranged to obtain a linear form

$$\log(q_{eq} - q_t) = \log q_{eq} - \frac{k_1 t}{2.303} \tag{9}$$

A plot of $\log(q_{eq} - q_t)$ against t should give a straight line to confirm the applicability of the kinetic model. In a true first-order process $\log q_{eq}$ should be equal to the intercept of a plot of $\log(q_{eq} - q_t)$ against t.

Ritchie proposed a second-order rate equation for the kinetic adsorption of gases on solids [37]. The author assumed that θ was the fraction of surface available site for adsorption of solute; n is the number of surface sites occupied by each molecule of adsorbed gas and k the rate constant, then

$$\frac{d\theta}{dt} = k(1 - \theta)^n \tag{10}$$

when $t = t$, $\theta = q_t/q_{eq}$ and $t = 0$, θ=0. The integration form of Eq. (10) becomes second order for $n = 2$:

$$\frac{q_{eq}}{q_{eq} - q_t} = kt + 1 \tag{11}$$

The linear form of equation is

$$\frac{1}{q_t} = \frac{1}{kq_{eq}t} + \frac{1}{q_{eq}} \tag{12}$$

From Eq. (12), a plot of $1/q_t$ versus $1/t$ should give a straight line and the sorption capacity q_{eq} and the rate constant k can be calculated from the intercept and the slope of the linear second-order equation, respectively (Fig. 6).

Table 2
The first-order and second-order kinetic constants for biosorption of Hg^{2+}, Cd^{2+} and Zn^{2+} ions on the immobilized live and inactivated fungus *F. trogii*

Immobilized fungal mycelia	Experimental	First-order kinetic constants			Second-order kinetic constants		
	q_{eq} (mg/g)	k_1 ($\times 10^2$ min^{-1})	q_{eq} (mg/g)	R^2	k_2 ($\times 10^2$ g mg^{-1} min^{-1})	q_{eq} (mg/g)	R^2
Live *F. trogii*, Hg^{2+}	333.0	4.21	242.9	0.962	10.20	353.2	0.997
Inactivated *F. trogii*, Hg^{2+}	403.2	4.81	290.8	0.964	11.82	428.8	0.996
Live *F. trogii*, Cd^{2+}	164.8	4.95	163.7	0.967	7.60	169.8	0.992
Inactivated *F. trogii*, Cd^{2+}	191.6	5.16	195.6	0.972	8.20	200.1	0.990
Live *F. trogii*, Zn^{2+}	42.1	4.15	33.9	0.993	9.15	44.3	0.998
Inactivated *F. trogii*, Zn^{2+}	54.0	5.83	49.9	0.979	9.12	59.3	0.995

The essential assumption of the Ritchie second-order model was that on adsorbate was adsorbed onto two surface sites. Therefore, the chemical equation becomes

$$2M_{(solid)} + Cd_{(aqueous)}{}^{2+} \rightarrow M_2Cd_{(adsorbed\ phase)}{}^{2+} \quad (13)$$

The sorption rate equation can be established based on the adsorption mechanism as shown in Eq. (13)

$$\frac{d\theta}{dt} = k(1-\theta)^2 \quad (14)$$

In order to analyze the biosorption kinetics of Hg^{2+}, Cd^{2+} and Zn^{2+} ions, the Langergren first-order and the Ritchie second-order kinetics models were applied to the experimental data [36,38]. The second-order equation fitted well with the experimental data. The comparison of experimental biosorption capacities and the theoretical values estimated from the above two equations and are presented in Table 2. The theoretical q_{eq} values estimated from the first-order kinetic model gave significantly different values compared to experimental values, and the correlation coefficients were also found to be slightly lower. These results showed that the biosorption systems were not well described by the first-order kinetic model.

The correlation coefficients for the linear plots of $1/q_t$ against $1/t$ for the second-order equation are greater than 0.990 for all the biosorbents for contact times of 120 min (Fig. 6). The theoretical q_{eq} values for all the tested biosorbent systems were very close to the experimental q_{eq} values in the case of second-order kinetics. The second-order kinetics best described the data.

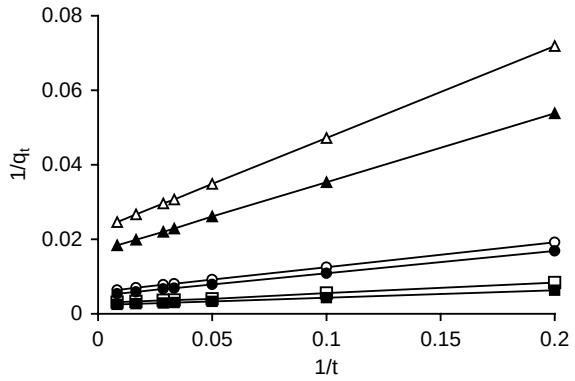


Fig. 6. The second-order plots of Hg^{2+}, Cd^{2+} and Zn^{2+} ions on both immobilized live and inactivated *F. trogii*: (□) live fungus Hg^{2+}; (■) inactivated fungus Hg^{2+}; (△) live fungus Zn^{2+}; (▲) inactivated fungus Zn^{2+}; (○) live fungus Cd^{2+}; (●) inactivated fungus Cd^{2+}.

3.7. Multi-metal ions biosorption studies

The amount of metal ions bound on the fungal cell walls 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 in the solution [39,40]. The biosorption capacities of the immobilized live and inactivated fungal preparations in the multi-metal ions system were 0.67 and 0.87 mmol for Hg^{2+}, 0.53 and 0.61 mmol for Cd^{2+}, and 0.32 and 0.41 mmol for Zn^{2+}/g of dry biosorbents, respectively. The biosorption order under multi-metal ions conditions was $Hg^{2+} > Cd^{2+} > Zn^{2+}$ in mmol basis for all the tested biosorbents. This affinity order is the same as in the single metal biosorption studies. The total biosorption capacities of the immobilized fungal preparations in the multi-metal system were lower than the single metal system. The presence of other heavy metal ions slightly decreased the total biosorption capacity of the immobilized fungal preparations under given experimental conditions. The immobilized *F. trogii* exhibits the highest biosorption ability for Hg^{2+} ions. Many of the functional groups present on the fungal cell walls 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 [41]. 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.

3.8. Desorption and reuse

The desorption of the adsorbed Hg^{2+}, Cd^{2+} and Zn^{2+} ions from the tested biosorbents were studied in a batch system. The metal ions adsorbed onto biosorbents were eluted with 10 mM HCl. More than 95% of the adsorbed metal ions were desorbed from the biosorbents.

In order to show the reusability of the biosorbents, adsorption–desorption cycle of metal ions was repeated three times by using the same preparations. The adsorption

capacities did not noticeably change (only a maximum 3% change was observed with the tested biosorbent) during the repeated adsorption–desorption operations. These results showed that alginate beads and both immobilized live and inactivated fungal preparations could be repeatedly used in heavy metal adsorption studies without detectable losses in their initial adsorption capacities.

4. Conclusion

The plain Ca-alginate and both immobilized live and inactivated *F. trogii* have been successfully used as biosorbents for removal of Hg^{2+}, Cd^{2+} and Zn^{2+} ions from aqueous solutions. The kinetics of Hg^{2+}, Cd^{2+} and Zn^{2+} ions biosorption on the biosorbents depend on the experimental conditions particularly medium pH and metals ion concentration. The biosorption capacity of the immobilized fungus was enhanced greatly when biosorption took place following heat inactivation. As the pH increased, the metal biosorption capacity increased significantly up to pH 6.0. The distribution of Hg^{2+}, Cd^{2+} and Zn^{2+} ions between liquid phase and solid phase was analyzed by the Langmuir and the Freundlich isotherm models. The characteristic biosorption parameters for each isotherm were determined. Comparing the equilibrium capacities (q_{eq}) of the kinetic models "namely first and second order" with the experimental equilibrium capacities of the biosorbents, the calculated maximum capacities from second-order equation seems to describe best the experimental data.

Acknowledgements

The authors wish to thank on behalf of the National Institute of Agrobiological Sciences to Dr. Hiroshi Shinbo (Director of Genebank) for supplying the white-rot fungus *F. trogii* (MAFF 420251).

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