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Heavy-metal removal from aqueous solution by fungus *Mucor rouxii*

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Abstract

Biosorption of lead, cadmium, nickel and zinc by live and dead *Mucor rouxii* biomass treated with NaOH was studied over a range of pH. In the case of dead biomass, low pH resulted in a decrease in the biosorption capacity. At pH 3.0 or less, the inhibition of biosorption of metal ions took place. At pH 4.0 or higher, the biosorption of metal ions increased sharply. Ho's pseudo-second-order model described the biosorption kinetics better than the Lagergren model. Live biomass had high biosorption capacity, i.e. 35.69, 11.09, 8.46 and 7.75 mg/g at pH 5.0 for Pb^{2+}, Ni^{2+}, Cd^{2+} and Zn^{2+}, respectively. The dead biomass adsorbed metal ions in the order of Pb^{2+}, Zn^{2+}, Cd^{2+} and Ni^{2+}, with the biosorption capability of 25.22, 16.62, 8.36 and 6.34 mg/g at pH 5.0, respectively. At pH 6.0, the capacity of the dead biomass increased to 53.75, 53.85, 20.31 and 20.49 mg/g, respectively. For bi- or multi-metal ion adsorption, biosorption capacity of individual metal ion was reduced in the presence of other metal ions, but the total biosorption capacity increased, indicating the capability of *M. rouxii* biomass in adsorbing multi-metal ions. In addition, *M. rouxii* biomasses cultured with different media exhibited the same level of capacity to bind metal ions. Metal ions adsorbed by the biomass could be eluted effectively with HNO_3, while distilled water demonstrated negligible metal elution capability. Regeneration of the biomass with NaOH regained or enhanced the biosorption capacity even after five cycles of adsorption–elution–regeneration.

Keywords: Fungal biomass; *Mucor rouxii*; Heavy-metal ions; Adsorption kinetics; Adsorption isotherms

1. Introduction

Heavy metals are present in nature and industrial wastewater. Due to their mobility in natural water ecosystems and their toxicity, the presence of heavy metals in surface water and groundwater poses a major inorganic contamination problem. Conventional techniques commonly applied to remove heavy metals from wastewater include chemical (precipitation/neutralization) or physical (ion exchange, membrane separation, electrodialysis and activated carbon adsorption) methods [1,2]. Generally, these processes are efficient in removing the bulk of metals from solution at high or moderate concentrations. However, chemical processes produce a large amount of metallic sludge, making metal recovery difficult [3]. The sludge also needs further disposal. In addition, effluent after such treatment usually has unacceptably high total dissolved solids. When applied to dilute metal waste or lower concentrations of metal ions, these processes are either ineffective or not cost-effective [1,4].

Biological methods of metal removal, defined as biosorption, have been recommended as cheaper and more effective techniques. In biosorption, either live or dead microorganisms or their derivatives are used, which complex metal ions through the action of ligands or functional groups located on the outer surface of the

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cell [5]. Microorganisms including bacteria, algae, fungi and yeast are found to be capable of efficiently accumulating heavy-metal ions [6–8]. Microbial biomass is produced in fermentation processes to synthesize valuable products such as antibiotics, enzymes, flavoring agents and organic acids. In such processes, a large amount of byproducts is generated, which can be used in biosorption of heavy metals [9]. In the case of fungal biomass, removal of metal ions from aqueous solutions has been studied with strains of *Penicillium* [10–11], *Rhizopus arrhizus* [12–14], *Rhizopus oryzae* and *Aspergillus oryzae* [4], and *Aspergillus niger* [15]. A limited information is available on the removal of some metal ions such as copper, silver and lanthanum by *Mucor rouxii*, a representative soil fungus [16,17], but information on its ability to remove lead, cadmium, nickel and zinc from aqueous solution is not available. Therefore, it was decided to study the removal of heavy metals using *M. rouxii*.

2. Materials and methods

2.1. Fungal biomass preparation

A laboratory strain of *M. rouxii* (ATCC# 24905) was routinely maintained on Bacto® potato dextrose agar (PDA). The *M. rouxii* culture streaked on PDA plates was stored at 4°C with routine transfers in a cycle of 2 months. The detailed procedure is described by Kapoor et al. [15]. The fungi was cultured in a filamentous form under aerobic conditions for 3 days using liquid media adopting the shake flask method. Three growth media were used separately: (1) YPG [18], i.e., yeast extract (3 g/l), peptone (10 g/l), and glucose (dextrose) (20 g/l); (2) YM broth, i.e. Bacto® yeast–malt broth (21 g/l) [16]; and (3) DP medium, used by Kapoor et al. [15], which contains dextrose (20 g/l), peptone (10 g/l), and other inorganic salts. Different culture media were used to examine their effects on biomass production and metal removal. The pH values of all the growth media were adjusted to 4.5 with 0.1 N H_2SO_4 and NaOH. Biomass was harvested by filtering the mixture of culture through a 150-μm sieve. The biomass collected was thoroughly washed with generous amounts of distilled–deionized water to remove the residual growth medium. The washed biomass (live biomass) was used immediately thereafter.

2.2. Physical and chemical treatment of fungal biomass

The cells were soaked in 0.2 M NaOH solution for 30 min. Then, it was washed with generous amounts of deionized water until the pH of the wash attains the neutral range, i.e. 6.8–7.2. The biomass was then autoclaved for 30 min at 121°C and 124 kPa and dried for 24 h at 60°C under reduced pressure. Dried biomass was ground in a mortar and pestle and sieved through a sieve with 150-μm openings, and was ready for use in experiments.

2.3. Metal solutions

Separate metal solutions containing Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} with a concentration of approximately 10 mg/l were prepared using deionized water and nitric salts containing the metals. pH of the metal solutions was adjusted to the desired value using 0.1 M NaOH and 0.1 M HNO_3.

2.4. Apparatus and glassware

All the metal uptake experiments were carried out using 125 ml Erlenmeyer flasks. Metal ion concentrations were determined using Varian AA-10 atomic absorption spectrometer.

2.4.1. Effect of different culture media on biomass production

As mentioned earlier, three different culture media were used in the fungal biomass preparation to examine their effects on biomass production. Biomasses obtained after being autoclaved and dried for each medium were compared with respect to biomass production.

2.4.2. Batch kinetic study

A total of 0.05 g of NaOH-pretreated YPG biomass was added into 75 ml of metal ion solution with a concentration of approximately 10 mg/l. Initial pH values of 4.0, 5.0, or 6.0 were used. pH was not maintained constant in this study. The mixture was agitated at 125 rpm on a rotary shaker. Controls were run with a blank metal solution system. Samples were taken periodically from the mixture and filtered immediately through a 0.45-μm polycarbonate membrane filter. The filtrate was analyzed for the residual metal ion concentration. These experiments were conducted in duplicate and mean values were used in the analysis.

2.4.3. Batch isotherm study

A number of samples containing varying amounts (0.01–0.2 g) of live or NaOH-pretreated YPG biomass were shaken with approximately 10 mg/l metal solution at 125 rpm on a rotary shaker until the equilibrium time was reached, which was determined through kinetic study. Control containing no biomass was set up. Initial pH values of 4.0, 5.0, or 6.0 were used for pretreated biomass and 5.0 for live biomass (based on literature review). Then, samples were filtered and filtrate was measured for metal ion concentration. These experiments were conducted in duplicate and mean values were used in the analysis.

2.4.4. Metal removal efficiency of biomass grown in different culture media

A total of 0.05 g of live, autoclaved or NaOH-pretreated biomasses cultured with different media was added into 75 ml of metal solutions with an initial pH of 5.0. Samples were shaken at 125 rpm until equilibrium was reached. After measurement of metal concentrations in filtrate, metal removals by the biomasses cultured using different media were compared.

2.4.5. Interactive study

In bi- or multi-metal ion adsorption, biosorption capacity of biomass for each metal ion and for all the metal ions was examined in the presence of other metal ions. Concentration of each metal ion was maintained at approximately 5.0 mg/l. NaOH-pretreated YPG biomass was used in the study.

2.4.6. Elution, regeneration and reuse studies

Different chemicals were used to examine their elution efficiency in desorbing metal ions bound on the *M. rouxii* biomass (NaOH-pretreated YPG biomass). Biomass after the batch experiment was contacted with 25 ml of different elution solutions, such as deionized water, 0.05 M HNO_3, 0.2 M $CaCl_2$ or 0.2 M NaCl. The mixture was agitated at 125 rpm on a rotary shaker for 1 h. After elution, the mixture was filtered and filtrate was measured for metal ion concentration.

After elution, biomass was washed with generous amount of deionized water to remove residual H^+ from biomass till pH in wash water rose to 5.0 or higher. Then, the biomass was regenerated by two methods. The first method was to wash the biomass with deionized water till the pH of wash solution reached the range of 5.0–6.0. The second method was to soak and stir the biomass regenerated with first method in 0.2 M NaOH solution at a solid/liquid ratio (S/L) of 1 g/l for 30 min. Afterwards, a generous amount of deionized water was used to wash the regenerated biomass till pH of the wash solution was near the neutral range (7.0–8.0). Biomass regenerated was dried at 60°C for 12 h and then reused. Five cycles of biosorption–elution–regeneration experiments were conducted to examine the capability of the biomass to retain metal removal capability.

3. Results and discussion

3.1. Effect of different culture media on biomass production

The biomass production with YPG medium (4.84 g/l) was 13.3% higher than that with YM medium (4.27 g/l). However, the biomass production with DP medium was 2.54 g/l, only 52.5% and 59.5% of that with YPG and YM media, respectively. In addition, the pH values in the liquid cultures rose from an initial 4.5 to an average value of 6.41 for YPG medium and 6.26 for YM medium. The rise in pH could be due to loss of hydrogen ions by fungal biomass adsorption or interaction with hydroxyl ions released by the biomass. This is in agreement with the observation made by Bartnicki-Garcia and Nickerson in the early 1960s [18]. Nevertheless, the final pH was only 4.90 in the case of DP medium. The lower biomass production using DP medium may be ascribed to the influence of the composition of the culture medium, lower pH or inadequate aeration and insufficient intrusion of air into the medium. Dissolved oxygen was not measured in these experiments.

3.2. Effect of pH on biosorption

Properties of adsorbent, pH, concentration of adsorbate, and the presence of co-ions in solution affect the biosorption of metal ions from aqueous solutions [19,20]. NaOH-pretreated *M. rouxii* bioamss (0.05 g) was contacted with Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} ions in separate solutions (75 ml) at a metal ion concentration of approximately 10 mg/l for 14 h, with an initial pH value of the solutions ranging from 2.0 to 6.0. Figs. 1(a)–(d) show the effect of pH on biosorption of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} ions. At an initial pH 4.0 or lower, little biosorption occurred. Especially at pH 2.0, almost no biosorption was observed (except for nickel). A sharp increase in biosorption capacity took place in the pH range of 4.0–5.0. Above pH 5.0, biosorption of lead was found to be relatively constant; biosorption of cadmium and nickel still increased but to a lesser extent. Thus, different metals have different pH optima, due to the different solution chemistry of the metals [21]. The low biosorption capacity at pH values below 4.0 was attributed to hydrogen ions that compete with metal ions on the sorption sites [22,23]. In other words, at lower pH, due to protonation of the binding sites resulting from a high concentration of protons, negative charge intensity on the sites was reduced, resulting in the reduction or inhibition of the binding of metal ions [15]. In fact, most microbial surfaces are negatively charged because of the ionization of functional groups, thus contributing to the metal binding [24,25]. Generally, fungal surfaces have a negative charge in the pH range of 2–6 examined in this study. At low pH, some functional groups will be positively charged and may not interact with metal ions [9,14]. For example, removal of copper by *P. spinulosum* decreased at lower pH [26]. Galun et al. [6] found that biosorption of Pb, Cd, Ni and Zn by *Penicillium digitatum* was severely inhibited when pH was below 3. *Rhizopus nigricans* also had a significantly low sorption of lead at pH values below 3 [20]. Zinc biosorption on *Saccharomyces cerevisiae* occurred above pH 4 [27]. Therefore, biosorption of

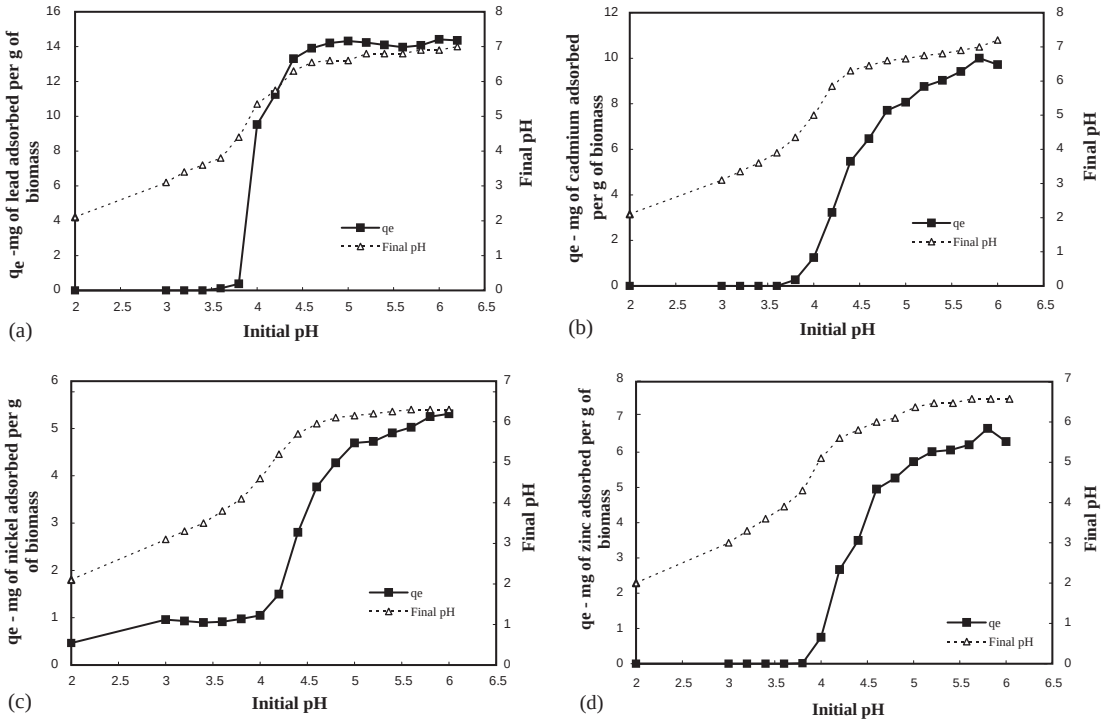

Fig. 1. Effect of pH on biosorption of (a) lead (b) cadmium (c) nickel and (d) zinc on pretreated *M. rouxii* biomass.

metal ions is dependent to a larger extent on pH of metal ion solution. The pH plays an important role mainly by its influence on metal or cell wall chemistry [3]. The increase observed in final pH values of reaction mixtures could be either from the adsorption of hydrogen ions from aqueous solutions by fungal biomass or neutralization of H^+ with OH^- released from the biomass. Rao et al. [28] and Kapoor et al. [15] also observed such an increase in pH for copper biosorption on alkali-treated *A. niger*.

3.3. Adsorption kinetics

Figs. 2(a)–(d) show the lead, cadmium, nickel and zinc concentration profiles versus agitation time using pretreated *M. rouxii* at initial pH values of 4, 5 and 6. It can be seen that biosorption consisted of two phases: a primary rapid phase and a second slow phase, which is more apparent at pH 5.0 and 6.0. The rapid phase accounted for the major part in the total metal biosorption, while the second phase contributed to a relatively small part. The first phase lasted approximately 45 min at pH 6.0 and was observed to increase with a decrease in pH. Biosorption reached equilibrium in approximately 5 h for lead, 6 h for cadmium, 7 h for nickel and 7 h for zinc at pH 6.0. With a decrease in pH, equilibrium time increased. At pH 5.0, the equilibrium time was 7, 13, 10 and 12 h for lead, cadmium, nickel and zinc, respectively. Biosorption of metal ions was lower at pH 4, especially for cadmium and zinc. Zhang et al. [20] observed that at pH of 4 lead biosorption on non-living *R. nigricans* reached equilibrium in 2 h and the biosorption rate was fast in the first 20 min. Cadmium biosorption on *A. oryzae* reached equilibrium in 1 h with 90% biosorption taking place in the initial 10 min at pH 6.0 [29]. However, biosorption of metal ions i.e. lead, cadmium, copper and nickel by *A. niger* took a longer time to reach equilibrium at a pH of 4–6 [15].

Two reaction rate equations were used to analyze the adsorption kinetics. Ho et al. [30] used a pseudo-second-order reaction rate equation (Eq. (1)) to describe the kinetics of adsorption of heavy metals on peat:

$$\frac{t}{q_t} = \frac{1}{2K'q_e^2} + \frac{t}{q_e}, \tag{1}$$

where q_t is the amount of metal ions adsorbed (mg/g) at any given time t (h), q_e is the amount of metal ion adsorbed (mg/g) at equilibrium and K' is the second-order reaction rate constant for adsorption (g/mg h).

The model developed by Lagergren [31] has been used by Lee et al. [32] and others in a study of kinetics of heavy-metal adsorption. The model has the following form:

$$\log(q_e - q_t) = \log(q_e) - \frac{K_L}{2.3}t, \tag{2}$$

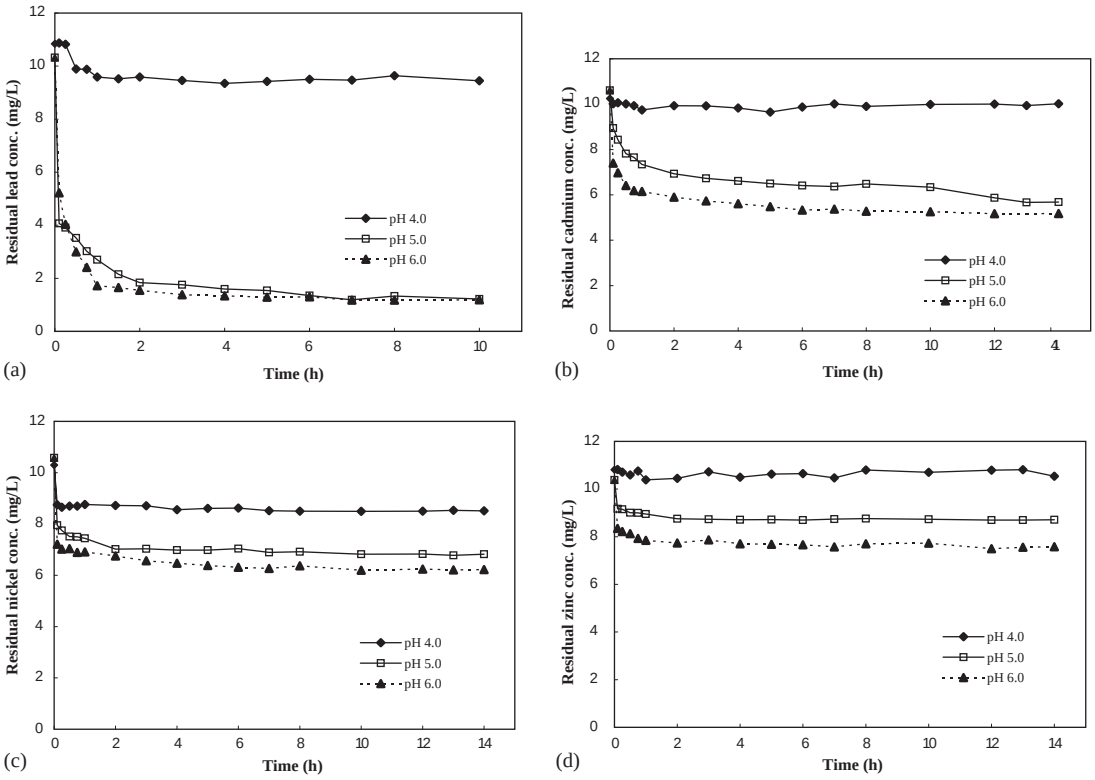

Fig. 2. Plots of (a) lead (b) cadmium (c) nickel and (d) zinc concentration with time at pH 4.0, 5.0 and 6.0.

Table 1
Parameter values calculated using Ho's and Lagergren models for the biosorption of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} on pretreated *M. rouxii* biomass

Metal ion	Initial pH	Ho's model		R^2	Lagergren model		R^2
		q_e	K'		q_e	K_L	
Pb^{2+}	4.0				1.76	0.91	0.82
	5.0	12.89	0.64	0.95	4.72	0.51	0.86
	6.0	13.75	0.38	0.99	3.70	0.64	0.80
Cd^{2+}	5.0	6.62	0.26	0.96	3.83	0.19	0.84
	6.0	7.78	0.73	0.97	2.85	0.34	0.89
Ni^{2+}	4.0	2.54	18.38	0.97	0.61	0.35	0.67
	5.0	5.38	1.83	0.97	1.46	0.32	0.70
	6.0	6.16	2.60	0.96	1.78	0.35	0.80
Zn^{2+}	5.0	2.43	3.98	0.96	0.90	0.88	0.88
	6.0	4.03	2.77	0.97	1.01	0.20	0.54

R = correlation coefficient. Initial concentration of metal ion is approximately 10 mg/l.

where q_t is the amount of metal ions adsorbed (mg/g) at any time t, q_e is the amount of metal ion adsorbed (mg/g) at equilibrium and K_L is the Lagergren rate constant for adsorption (h^{-1}).

Batch kinetic data was fitted to the models by non-linear regression analysis using software Statistica (Release 5.0) and results showed that the kinetics of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} adsorption on *M. rouxii*

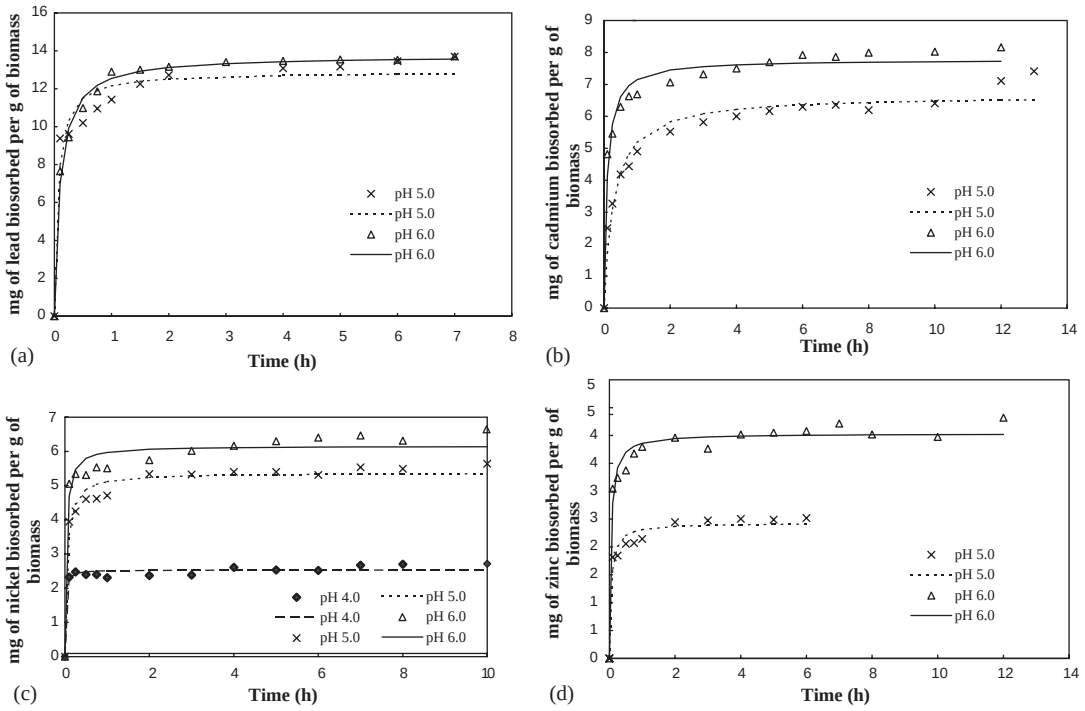

Fig. 3. Experimental data points and prediction curves for the Ho's model applied to biosorption of (a) lead (b) cadmium (c) nickel and (d) zinc on pretreated *M. rouxii* biomass.

followed the models. Table 1 shows the parameters calculated for the adsorption of lead, cadmium, nickel and zinc on pretreated *M. rouxii* biomass. Figs. 3(a)–(d) present the comparison between the experimental data and predicted values by Ho's model.

In the case of lead, cadmium and zinc, the kinetic data obtained at pH 4.0 could not be described by Ho's model. Likewise, the kinetic data obtained at pH 4.0 for cadmium and zinc could not be described by the Lagergren model either. Ho's model fits better the adsorption kinetics of heavy-metal ions on *M. rouxii* biomass than the Lagergren model. Effect of pH is evident and q_e values calculated with Ho's model decreased with pH.

3.4. Effect of different culture media

Metal removals by live, autoclaved or NaOH-pretreated *M. rouxii* biomasses cultured with different media are shown in Table 2. For live biomasses cultured with YPG and YM media, there is no large difference with respect to their metal removals for Pb, Ni and Zn, but the biomass cultured with DP medium exhibited lower biosorption capacities. Cd adsorption by live biomass was higher for the YM medium compared to YPG or DP media. The low capacities exhibited by the biomass cultured with DP medium could be attributed to the influence of low pH at equilibrium, which was between 4.8 and 5.2, rather than between 5.7 and 6.0 for YPG cultured biomass and between 5.3 and 7.0 for YM cultured biomass. For autoclaved biomass, biomass cultured with DP medium still showed lower metal biosorption capacities than those cultured with YPG and YM media. Lower pH (5.1–5.4) should also be the factor affecting the capacities. However, in the case of NaOH-treated biomasses, there is little difference among the biomasses cultured with three media in terms of metal biosorption capacities due to higher final pH values of 6.0–6.5 at equilibrium.

3.5. Adsorption isotherms

Both the Langmuir and Freundlich models were used to describe adsorption isotherm. The Langmuir equation has the form

$$q = \frac{Q^0 b C_e}{1 + b C_e}, \tag{3}$$

where q is the amount adsorbed at time t (mg/g), C_e is the equilibrium concentration (mg/l), b is a constant related to the energy or net enthalpy of adsorption (l/mg), and Q^0 is the mass of adsorbed solute completely required to saturate a unit mass of adsorbent (mg/g).

The Freundlich model is as follows:

$$q = K C_e^{1/n}, \tag{4}$$

Table 2
Metal removal of *M. rouxii* biomass under different culture media

Pretreatment	Medium type	Metal removal (mg/g)			
		Pb	Cd	Ni	Zn
Live biomass	YPG	17.13	6.94	5.24	4.89
	YM	16.96	9.40	5.82	5.58
	DP	4.78	5.61	2.57	4.74
Autoclaved	YPG	10.02	5.04	1.67	2.56
	YM	8.79	5.79	2.35	3.18
	DP	4.04	2.34	0.50	1.65
NaOH treated	YPG	13.43	8.81	5.09	5.80
	YM	13.76	7.47	3.92	5.30
	DP	12.65	9.17	5.18	5.67

Table 3
Adsorption parameters calculated using the Langmuir and the Freundlich isotherms

Metal ion	Biomass type and initial pH	Langmuir model		R^2	Freundlich model		R^2
		Q^0	b		K'	n	
Pb^{2+}	*Live biomass*						
	5.0	35.69	0.80	0.95[a]	14.31	2.10	0.88[a]
	Pretreated biomass						
	5.0	25.22	0.87	0.86[a]	10.73	2.50	0.80[a]
	6.0	53.75	0.27	0.99[a]	10.88	1.45	0.97[a]
Cd^{2+}	*Live biomass*						
	5.0	8.46	5.93	0.87[a]	6.34	5.56	0.69[a]
	Pretreated biomass						
	5.0	8.36	2.67	0.72[b]	6.81	12.94	0.57[b]
	6.0	20.31	0.28	0.56[b]	5.22	2.05	0.64[a]
Ni^{2+}	*Live biomass*						
	5.0	11.09	0.46	0.83[a]	3.74	2.25	0.93[a]
	Pretreated biomass						
	5.0	6.34	0.53	0.47[b]	3.05	4.00	0.52[b]
	6.0	20.49	0.07	0.67[b]	1.88	1.50	0.70[a]
Zn^{2+}	*Live biomass*						
	5.0	7.75	0.80	0.97[a]	3.42	2.53	0.96[a]
	Pretreated biomass						
	5.0	16.62	0.10	0.71[b]	2.07	1.65	0.75[b]
	6.0	53.85	0.03	0.71[b]	1.94	1.12	0.65[a]

R = correlation coefficient. Initial concentration of metal ion is approximately 10 mg/l.
[a] Model parameters are statistically significant (t-test) at 95% confidence level.
[b] Model parameters are not statistically significant (t-test) at 95% confidence level.

where q is the amount adsorbed at time t (mg/g), C_e is the equilibrium concentration (mg/l). K and n are equilibrium constants indicative of adsorption capacity and adsorption intensity, respectively.

The non-linear regression analysis was carried out with the use of software Statistica (Release 5.0). Table 3 presents the adsorption parameters calculated for biosorption of Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+} on live and pretreated *M. rouxii* biomass. Figs. 4(a)–(d) show the experimental data points for biosorption of lead, cadmium, nickel and zinc. Lead, cadmium and nickel removal capacities of live biomass were higher than those of pretreated biomass obtained at pH 4.0 and 5.0, but lower at pH 6.0. For zinc, removal capacity of live biomass was higher than that of pretreated biomass at pH 4.0, but lower than those obtained at pH 5.0 and

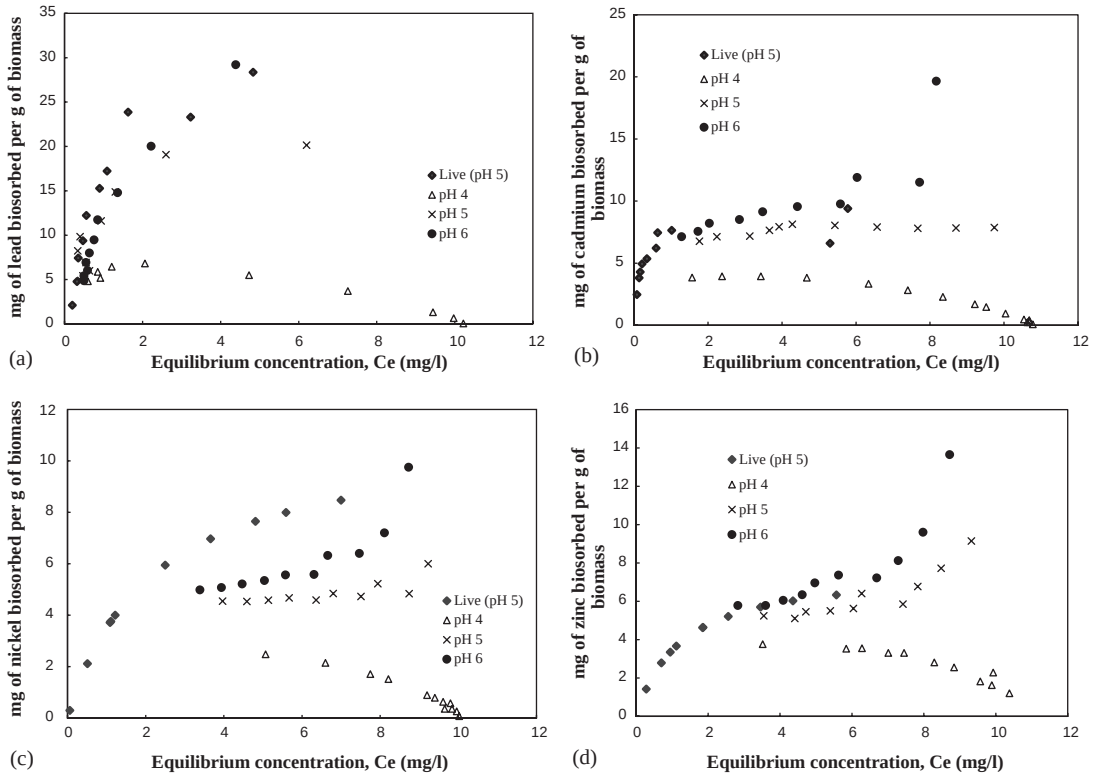

Fig. 4. Biosorption of (a) lead (b) cadmium (c) nickel and (d) zinc on *M. rouxii* biomass at various pH values.

6.0. The higher adsorption of the live biomass within some pH range might be explained by the fact that biosorption of metal ions on live biomass is due to surface binding followed by intracellular uptake. The intracellular uptake could account for a substantial part in total uptake for some fungal stains of live biomass [33], while the biosorption of metal ions on dead biomass is achieved via only surface and wall binding, which is non-metabolic [34]. This surface binding involves specific chemical sites or functional groups on the cell wall [35], performance of which is affected by pH and other ions. This may explain why biosorption capacity of the dead NaOH-pretreated biomass exceeded that of live biomass for certain pH values. Biosorption data for all the four metal ions at pH 4.0 were not described by both models as biosorption capacity at pH 4.0 increased with a decrease in final equilibrium concentration, which is contrary to the condition for favorable adsorption. The Langmuir model was able to describe the experimental data for biosorption of all metal ions at pH 5.0 by live biomass, and only Pb ion by pretreated biomass at pH 5.0 and 6.0. The Freundlich model explained the data better for biosorption of Cd, Ni and Zn by pretreated biomass at pH 6.0.

It was also observed that the final pH of the reaction mixture using live biomass was higher than the initial pH values. This is due to the growth property of *M. rouxii* which, under aerobic condition, tends to neutralize the medium with pH less than 7.0 [18]. Even though no substrate was added to the reaction mixture in the experiment, the biomass was still active to some extent, possibly due to endogenous respiration.

3.6. Interactive study

Table 4 shows the adsorption capacities of pretreated *M. rouxii* in the presence of single, two or multi-metal ions for a sample containing 0.1 g biomass in a reaction volume of 75 ml at pH 5.0. Presence of other metal ions affected the adsorption of a metal ion on *M. rouxii*. From the data shown in Table 4, it can be seen that compared with that of single ion system, biosorption capacity of individual metal ion was reduced in the presence of other metal ions, indicating the existence of competitive binding with cell surfaces. The single ion sorption for lead did not follow the trend and it was lower than with two ions and sometimes with three ions. However, in comparison with single-ion system, total biosorption capacity increased, with higher values in

Table 4
Biosorption of lead, cadmium, nickel and zinc when present in one-, two- or three-metal system

Metal ion	mg of each metal ion biosorbed per g of biomass (total metal ions biosorbed (μmol) per g of biomass)										
	Only	Pb + Cd	Pb + Ni	Pb + Zn	Cd + Ni	Cd + Zn	Ni + Zn	Pb + Cd + Ni	Pb + Ni + Zn	Pb + Cd + Zn	Ni + Cd + Zn
Pb^{2+}	3.47	3.62	3.84	3.66				3.18	3.55	3.56	
	(16.75)	(45.23)	(62.48)	(58.81)				(71.30)	(74.62)	(65.16)	
Cd^{2+}	3.26	3.12			2.29	2.61		2.23		1.92	2.15
	(29.00)	(45.23)			(61.59)	(64.37)		(71.30)		(65.16)	(81.40)
Ni^{2+}	2.80		2.58		2.42		1.95	2.12	1.92		2.12
	(47.69)		(62.48)		(61.59)		(63.35)	(71.30)	(74.62)		(81.40)
Zn^{2+}	2.89			2.69		2.69	1.97		1.62	2.02	1.71
	(44.21)			(58.81)		(64.37)	(63.35)		(74.62)	(65.16)	(81.40)

Initial concentration of each ion is approximately 5.0 mg/l.

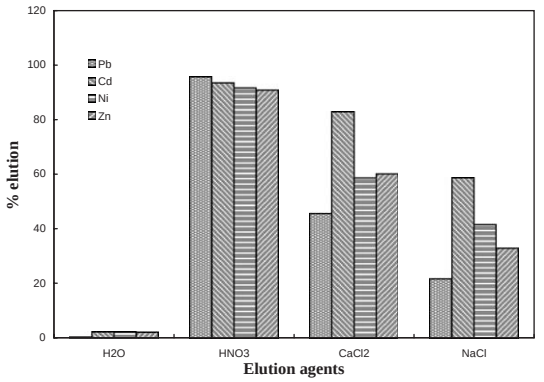

Fig. 5. Effectiveness of metal in desorption from *M. rouxii* biomass using various elutants.

three-metal ion systems than those in bi-metal ion systems, indicating the capability of *M. rouxii* biomass in adsorbing multi-metal ions.

3.7. Elution, regeneration and reuse studies

Various elutants were used to desorb the metal ions loaded on pretreated biomass. Fig. 5 shows the results of desorption tests. The effectiveness of an elutant was expressed as a percentage of ion desorbed from biomass to that biosorbed on biomass. HNO_3 proved to be a more effective elutant than $CaCl_2$ and NaCl, with more than 90% elution for Pb^{2+}, Cd^{2+}, Ni^{2+} and Zn^{2+}, while deionized water exhibited negligible desorption capability. Zhang et al. [20] reported that more than 80% of lead could be desorbed from non-living *R. nigricans* with the use of mineral acids i.e. HCl and HNO_3. The mineral acids are proton-exchange agents, which dislodge high valence metal ions from biomass. Tobin and Roux [36] showed the effectiveness of H_2SO_4 in eluting biosorbed chromium from *M. meihi* biomass.

Biosorption capability of fungal biomass and its reuse will decide its potential as a biosorbent in an

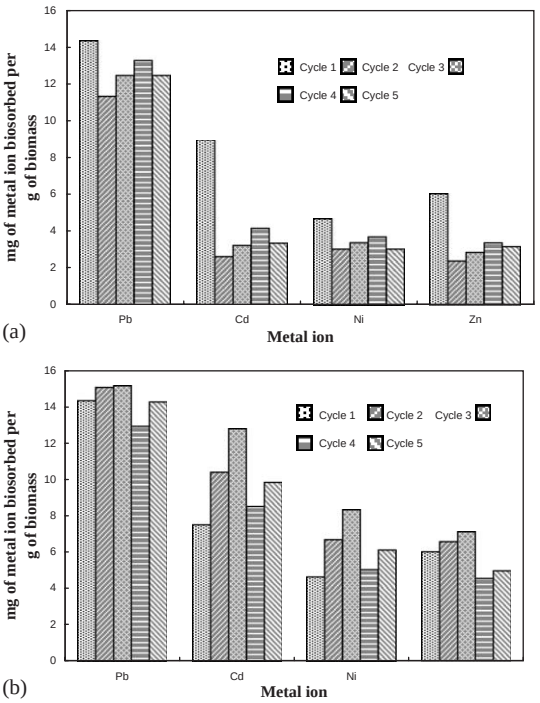

Fig. 6. Reuse of *M. rouxii* biomass regenerated with (a) deionized water and (b) NaOH solution.

application. After biosorption of metal ion, biomass was desorbed using HNO_3 and then regenerated by washing with deionized water or followed by NaOH regeneration. Biomass was used for 5 or 6 cycles of biosorption–elution–regeneration. Fig. 6a shows that biomass regenerated by washing with only deionized water lost some of its metal removal capability in comparison with that of raw NaOH-pretreated biomass. However, biomass regenerated with NaOH regained its initial metal removal capacity. The values in the cycle 2–5 were even higher than that obtained in cycle 1 using raw NaOH-pretreated biomass in many cases (Fig. 6b). This

indicated that biomass could be repeatedly subjected to alkaline treatment without losing its adsorption properties [10,11]. The caustic regeneration decreases protonation and substitutes sodium ions on functional groups. These ions can be readily displaced by heavy-metal ions [37]. Hunt [38] and Fourest et al. [9] showed that enhancement of hydroxide concentration could activate binding sites, thus regaining the biosorption capacity.

4. Conclusions

The NaOH-pretreated *M. rouxii* biomass showed a high adsorption capability for the removal of lead, cadmium, nickel and zinc from aqueous solution. It exhibited good biosorption capacity in bi- or multi-metal ion systems in terms of total adsorption capability. pH was found to be critical in biosorption, with an optimum pH being 6.0 or higher. High recovery of biosorbed metal ions could be achieved with acid elution. Caustic regeneration of eluted biomass rehabilitated the metal ion biosortion capacity of the biomass even after five cycles of reuse.

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