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ORIGINAL PAPER

Isotherm and kinetic models and cell surface analysis for determination of the mechanism of metal sorption by *Aspergillus versicolor*

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Abstract The isolate *Aspergillus versicolor* was obtained from an estuary, which is exposed to metal contamination. It was found to have a good metal tolerance and sorption capacity. Further studies revealed that the rate of metal removal from solution is very rapid in the first 5–10 min, and is favoured by a pH of 6.0. The biosorption data obtained was explained by the Freundlich adsorption isotherm model and followed a pseudo-second order kinetics reaction. The fungus showed a higher accumulation of fatty acids when grown in presence of metals as compared to the mycelium grown in absence of the metal; there was also an increase in the saturation index of fatty acids in presence of Cu^{2+} which serves as a protective mechanism for the fungus. Fourier Transform Infrared, scanning electron microscopy and EDAX analysis indicated that metal removal from solution by *A. versicolor* occurred by a passive adsorption to the fungal cell surface, involving an ion exchange mechanism.

Keywords *Aspergillus versicolor* · Lead · Copper · Freundlich isotherm · Kinetics · Fatty acid · Ion exchange

Introduction

Environmental contamination by improper disposal of industrial, mining, agricultural, municipal, and other residues is known worldwide. Lead is highly hazardous (Volesky 1994), while copper, though not potentially toxic in low concentrations, can pose serious health problems due to extensive and prolonged use and a consequent increase in levels in the environment (Bueno et al. 2008). Thus, release and accumulation of lead and copper in the aquatic environment could result in toxicity to both human and aquatic life.

The use of living and dead biomass of bacteria, algae, fungi, and plants as biosorbents to sequester metal ions in trace level from contaminated effluents forms the foundation of *biosorption technology* that offers a promising and economical alternative to treat wide variety discharges of metal-containing industrial effluents (Akar et al. 2007; Alluri et al. 2007; Singh 2006). Biomass characteristics, physicochemical properties of the target metals, and factors, such as pH, temperature, initial concentrations of biomass and metal ions significantly affect their biosorption capacities (Al-Garni et al. 2009). The biosorbent, unlike mono functional ion exchange resins, contains a variety of functional sites including carboxyl, imidazole, sulphydryl, amino, phosphate, sulfate, thioether, phenol, carbonyl, amide and hydroxyl moieties.

Biosorbents are cheaper, more effective for the removal of metallic elements, especially heavy metals from aqueous solution (Akhtar et al. 1996; Bairagia et al. 2011). Because of the high surface to volume ratio of microorganisms and their ability to detoxify metals they are considered as potential alternative to synthetic resins for remediation of dilute solutions of metals and solid wastes (Magyarosy et al. 2002).

Fungi are present in aquatic sediments, terrestrial habitats and water surfaces, and play a significant part in natural remediation of metal. Furthermore, fungal hyphae can penetrate contaminated soil and have advantages over bacteria in natural environments (Leitão 2009). The use of non-living fungal biomass in batch treatment of metal waste is of advantage since it does not depend on

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requirements for growth. The problem of toxicity of metals does not affect this type of biomass, which is seen as one of the major advantages of biosorption.

In this investigation, a composite study has been made on removal of Pb^{2+} and Cu^{2+} from solution by *A. versicolor*, with respect to the factors affecting sorption capacity and rate, together with the isotherm models and kinetics, the mechanism of sorption as determined by FTIR, SEM and EDAX analysis, and the response of the fungus to heavy metals through changes in its fatty acid composition.

Materials and methods

Culture and growth conditions

The isolate *A. versicolor* EM2wt41, obtained from the top water of Mandovi estuary, Goa, India, showed a high resistance to heavy metals such as lead and copper, as well as a good capacity for removal from aqueous solution. The culture was maintained on Czapek Dox Agar containing 2 % salt (S-CzA).

Spore suspension (10^6 spores) of a freshly grown agar slant culture was inoculated in 100 mL of Czapek Dox Broth containing 2 % salt (S-CzB) and incubated for 3 days at 30 °C, 150 rpm.

Biosorption experiments

Stock solutions of metals containing 1,000 mg L^{-1} of Pb^{2+} as $Pb(NO_3)_2$ and of Cu^{2+} as $CuSO_4.5H_2O$ were prepared. The stock solutions were appropriately diluted to get the desired concentrations of metal as per the experiment.

The culture was grown as described above; the biomass was harvested by filtering through double layered muslin cloth, washed with deionized water and then treated by boiling in 5 % KOH for 15 min followed by washing with deionized water until the pH of the solution was neutral. The treated biomass was dried by lyophilization and used as biosorbent.

All batch experiments were carried out by incubating 0.1 g of the biosorbent in 20 mL of metal solution under the desired conditions of pH and metal concentration as per the experiment, incubated at 150 rpm, 30 °C. The biosorbent was then removed and the metal remaining in solution was estimated by atomic absorption spectrophotometry (AAS) using Varian AA2402. Metal controls containing only Pb^{2+} and Cu^{2+} solution without biosorbent were maintained. Standards of lead and copper solutions (Reagecon) for AAS were prepared in 0.1 N HNO_3 in the range of 0–10 ppm Pb^{2+} or 0–2 ppm Cu^{2+} for obtaining the standard curve. The samples were diluted so as to obtain metal concentrations within this range and read against the standards. Each experiment was conducted in duplicates and plotted with the standard error bars.

The amount of heavy metal ions removed from solution by the biosorbent was calculated using the equation q_e (mg g^{-1}) = $(C_i - C_f)$ V/M where q_e is the specific metal biosorption (mg metal g^{-1} biosorbent), V is the volume of metal solution (L), C_i and C_e are the initial and equilibrium concentration of metal (mg metal L^{-1}) respectively, and M is the dry weight of the biosorbent (g), (Volesky and Holan 1995; Yen and Viraraghavan 2003).

Effect of pH on metal biosorption was studied at pH 4, 5, 6 and 7, for a contact time of 1 h; the pH of the metal solution (20 mL) containing 0.1 g % of Pb^{2+} or of Cu^{2+} ions, was adjusted with 0.1N HCl.

Effect of contact time on metal biosorption was determined by incubating the biosorbent in different flasks containing 20 mL of 0.1 g % metal solution, pH 6.0; pH was maintained constant throughout. Flasks in duplicate were removed at specified time intervals of up to 180 min and treated as above to determine the residual metal.

Effect of initial metal ion concentration on biosorption was assessed by incubating the biosorbent with metal ion concentrations ranging from 50 to 300 mg L^{-1}, at pH 6.0 for 6 h.

Equilibrium adsorption isotherms

Freundlich and Langmuir isotherms were employed to analyze the equilibrium distribution between metal ions adsorbed and ions in solution.

The Freundlich method is described by the equation:

$log\ q_e = log\ K_F + 1/n\ log\ C_e$, where q_e is the amount of metal ions adsorbed onto the biosorbent at equilibrium (mg g^{-1}), C_e is the heavy metal ions concentration in the solution (ppm) at equilibrium and K_F (L g^{-1}) and n are the Freundlich adsorption isotherm constants.

Langmuir isotherm equation is given by the following equation:

$C_e/q_e = 1/q_{max}\ K_L + C_e/q_{max}$ where q_e and q_{max} are the amount of heavy metal ions removed at equilibrium and maximum uptake capacity (mg g^{-1}), respectively, C_e is the equilibrium concentration (ppm), and K_L is the Langmuir constant.

Kinetic models

The pseudo-first order and pseudo-second order kinetic models were employed to determine the mechanism of biosorption of Pb^{2+} and Cu^{2+} (Ho and McKay 1999).

The pseudo-first order rate equation is expressed as $log\ (q_e - q_t) = log\ q_e - k_1/2.303t$, where q_e and q_t are the amounts of lead ions (mg g^{-1}) adsorbed at equilibrium and

at time t respectively, and k_1 is the first-order rate constant (min^{-1}). The value of k_1 was calculated from the slope of the plot of $log\ (q_e - q_t)$ versus t.

The pseudo-second-order kinetic model has the linear form of $t/q_t = 1/k_2\ q_2^2 + 1/q_2 t$), where q_2 is the maximum adsorption capacity (mg g^{-1}) for the pseudo-second order adsorption and k_2 is the equilibrium rate constant for the pseudo-second order adsorption (gm $g^{-1}min^{-1}$). The values of k_2 and q_2 were calculated from the plot of t/q_t versus t.

FTIR

The functional chemical groups present on the cell walls of the fungal biomass which are involved in heavy metal biosorption was analyzed by Fourier Transform Infrared (FTIR) spectroscopy. The biosorbent before and after exposure to heavy metals, was dried at 60 °C for 12 h; sample disks of finely ground biomass encapsulated with KBr (1:10, w/w) was prepared and analyzed using IR (Prestige-21 FTIR- Shimadzu).

SEM, EDX analysis

The cell-metal interactions were also evaluated for alterations in the cell-surface morphology and confirmation of the presence of the metal ion bound to the cell-surface by scanning electron microscopy (JEOL Model: 15800, LV, Japan) equipped with energy-dispersive X-ray analyzer (SEM-EDX). The samples of biosorbent before and after exposure to heavy metals, were dehydrated by immersion of mycelial strands in acetone of increasing concentrations from 10 to 100 % for 30 min. Samples were then coated with a thin layer of gold–palladium prior to analysis.

Lipid extraction and fatty acids analysis

Spore suspension (10^6 spores) of a freshly grown agar culture was inoculated in 100 mL of S-CzB containing 2 mM Pb^{2+} or 1 mM of Cu^{2+} ions incubated for 3 days at 30 °C, 150 rpm; the control of cultures grown in the absence of the heavy metal was also maintained. The biomass was then harvested, washed and dried as detailed above, then treated using a modification of the method of Bligh and Dyer (1959) as described by Volkman et al. (1989) for fatty acid methyl esters (FAME) analysis. The lyophilized biomass (0.25 g) was saponified in 10 % KOH in methanol: water (80:20) at 80 °C for 2 h in a screw-capped Pyrex tube and cooled. The samples were then extracted thrice with 15 mL of hexane-diethyl ether (9:1). The aqueous layer was acidified to pH 2 by addition of concentrated HCl and extracted thrice with 15 mL of hexane: diethyl ether (9:1). The pooled extracts were dried over anhydrous sodium sulphate, purified by passage through a silica gel column (60–120 mesh) and elution with hexane:ethyl acetate (4:1). The eluate was concentrated under vacuum. FAMEs were analyzed by using a Varian CP-3800 gas chromatograph interfaced with GC/MS Saturn 2200 mass spectrometer. Fungal fatty acids were identified by comparison with the retention times of the authentic standards (Sigma, Supelco) and expressed as μg g^{-1} dry weight biomass.

Results and discussion

Effect of pH on biosorption of lead and copper ions

The sorption capacity of the biosorbent for both metal ions was markedly influenced by the levels of initial pH in the solution, being enhanced substantially with increase in pH from 4.0 to 6.0 and then decreasing at pH 7.0. The biosorption capacity at pH 6.0 was 17.35 and 12.0 mg g^{-1} for lead and copper respectively (Fig. 1). Similar results for pH effect on lead or copper biosorption were also reported by earlier investigators (Gabr et al. 2008; Hefne et al. 2008; Hussain et al. 2009; Joo et al. 2011; Mukhopadhyay et al. 2007; Oh et al. 2009; Ozsoy 2010; Say et al. 2001).

At low pH, cell wall ligands would be closely associated with the hydronium ions H_3O^+, that restrict the binding of metal cations as a result of repulsive forces (Dursun 2006). The increase in the biosorption capacity with increase in pH can be attributed to a change in the charge density of the biosorbent. The pH results in a deprotonation of the metal binding sites of the cell wall, such as carboxyl, phosphate and amino groups, thus increasing the negative charge density of these functional groups. Consequently there is a corresponding increase in attraction of metal cations and biosorption on to the cell wall (Akar et al. 2007; Bairagia et al. 2011; Kapoor et al. 1999; Karaca et al. 2010). This indicated the involvement of ionic attractions

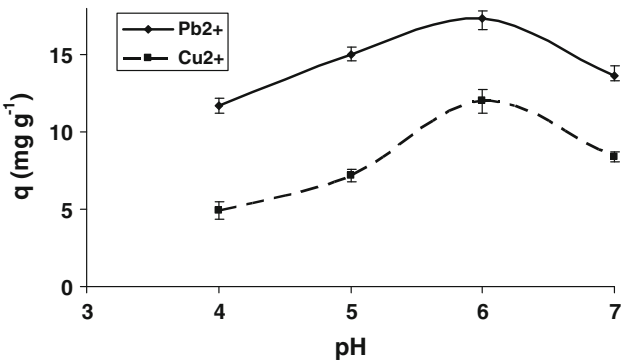

Fig. 1 Effect of pH on biosorption of Pb^{2+} and Cu^{2+} onto *A. versicolor*

between cell wall ligands and the metal ions as a mechanism in metal biosorption by the fungi. At pH greater than 6, metal ions precipitate as hydroxides or hydrated oxides, resulting in decreased amounts of Pb^{2+} and Cu^{2+} in the solution, thus limiting the biosorption process at higher pH solutions (Oh et al. 2009).

Effect of contact time on lead and copper ions biosorption

The Pb^{2+} and Cu^{2+} removal capacity by *A. versicolor* biosorbent as a function of time is presented in Fig. 2. It can be seen that sorption occurred rapidly in the first 5–10 min, followed by a slower phase until equilibrium was attained within 30 min, with no further increase in the sorption after 60 min. This could be due to the mechanism of ionic attraction between negatively charged cell wall ligands and the metal cations; at the initial phase, the functional groups are abundantly available for binding by the metal ions, and as the ligands get saturated, the sorption rate decreases. The high initial concentration of heavy metal ions enhances the contact proximity and consequently, the rate of adsorption to the cell wall. This corroborates earlier reports on sorption by *A. parasiticus, A. niveus* and other species (Akar et al. 2007; Dursun 2006; Karaca et al. 2010; Oh et al. 2009). The rate of metal sorption was seen to be faster than that obtained by *A. versicolor* MTCC 280 which reached equilibrium after 3 h (Bairagia et al. 2011) and by *Rhizopus oligosporus*, which required 6 h for maximum sorption (Ozsoy 2010); however, it was slower than that obtained by commercial natural bentonite for Pb^{2+} sorption (Hefne et al. 2008). The natural econiche from where the isolate *A. versicolor* $EM2w_t41$ was obtained, and which is exposed to metal contamination, could have caused the isolate to develop a resistance to metals (Ezzouhri et al. 2009) and a mechanism for a speedy passive metal removal as a means of survival.

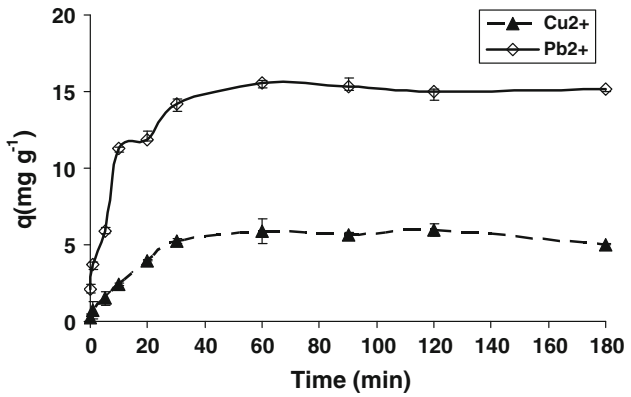

Fig. 2 Effect of contact time on the biosorption of Pb^{2+} and Cu^{2+} onto *A. versicolor*

Effect of initial metal concentration on biosorption

The effect of initial metal concentration on biosorption of Pb^{2+} and Cu^{2+} is shown in Fig. 3. As the initial metal ion concentration (C_i) was increased, the biosorption capacity also increased. Similar results were observed in studies on *A. niger* and *A. niveus* (Goyal et al. 2003; Karaca et al. 2010; Mukhopadhyay et al. 2007). The effect of initial metal ion concentration on sorption is related to the number of available active sites on the cell surface for binding (Bhatti et al. 2008; Joo et al. 2010). The increase in concentration of the metal ions in the vicinity of the binding sites would also enhance the sorption rate, till a point where the active sites are fully bound to the metal ions. Yalcin et al. (2010) state that the initial metal concentrations provide an important driving force to overcome all mass transfer resistances between the metal solution and the fungal cell wall. Furthermore, the number of collisions between metal ions and the adsorbent are reported to enhance the biosorption process (Wang et al. 2010). The percentage sorption was enhanced a little with increase in initial metal concentrations up to 250 mg L^{-1}, and then showed a slight decrease at 300 mg L^{-1}. This could be due to a competition of increasing metal ions for ligands on the cell surface, coupled with a decrease in number of available sites with increasing metal concentrations (Khambhaty et al. 2009; Ozsoy 2010).

The sorption capacity q_e of the isolate was 51.978 mg Pb^{2+} and 43.66 mg Cu^{2+} g^{-1} biosorbent at C_i of 300 mg L^{-1}, higher than results obtained with *A. niger* (Dursan 2006) but less than that obtained by *Fusarium solani* (Bhatti et al. 2008). It was also observed that q_e values were in the order of $Pb^{2+} > Cu^{2+}$ for a given initial metal concentration. This preferential type of adsorption may be ascribed to the difference in their ionic radii (Gabr et al. 2008). The ionic radius of Pb^{2+} is 1.20 Å while that of

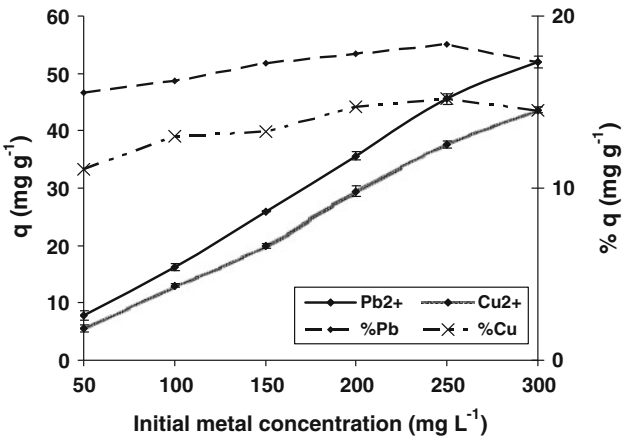

Fig. 3 Effect of initial metal ion concentration on biosorption of Pb^{2+} and Cu^{2+} onto *A. versicolor*

Cu^{2+} is 0.73 Å. The smaller the ionic radius, the greater its tendency to be hydrolyzed, leading to reduced biosorption, for this reason the fungal biomass has greater affinity for lead rather than copper. These results were in accord with earlier studies by Akar and Tunali (2006) and Iskandar et al. (2011) on *Aspergillus* species, and by (Bueno et al. 2008; Oh et al. 2009) on bacteria *Rhodococcus opacus* and *Pseudomonas stutzeri*.

Biosorption isotherms

The non-linearized adsorption isotherms of each metal are given in Fig. 4. The biosorption isotherm curve represents the equilibrium distribution of metal ions between the aqueous and solid phase. The isotherms indicate that the biosorption rate increases with an increase in equilibrium concentration of the metal.

The Langmuir and Freundlich isotherms are given in Figs. 5 and 6 respectively. The Langmuir isotherm model

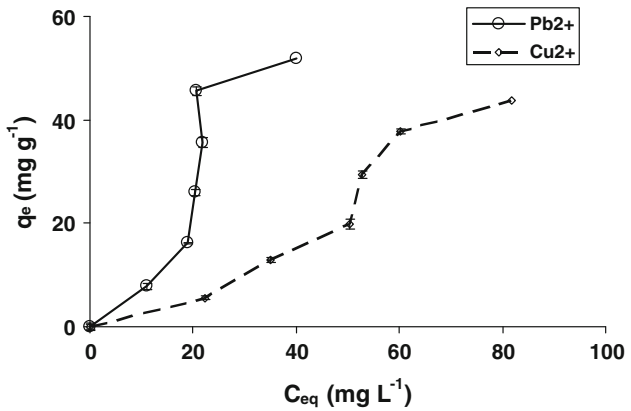

Fig. 4 Adsorption isotherm of Pb^{2+} and Cu^{2+} onto *A. versicolor*

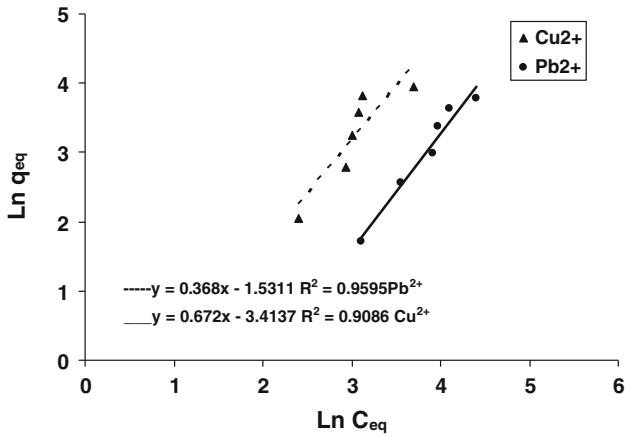

Fig. 5 The linear form of Freundlich adsorption isotherms of Pb^{2+} and Cu^{2+} onto *A. versicolor*

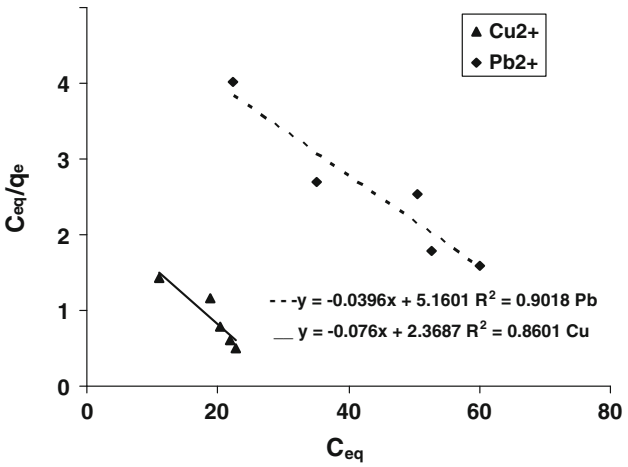

Fig. 6 The linear form of Langmuir adsorption isotherms of Pb^{2+} and Cu^{2+} onto *A. versicolor*

is based on monolayer sorption onto a surface containing a given number of identical sorption sites which are homogeneously distributed over the sorbent surface; the Freundlich isotherm equation describes the sorption based on a heterogeneous surface. The constants of Langmuir and Freundlich isotherm models for Pb^{2+} and Cu^{2+} biosorption onto lyophilized cells of *A. versicolor* are presented in Table 1. The equilibrium data fitted the Freundlich adsorption isotherm better than the Langmuir model for Pb^{2+} and Cu^{2+} biosorption at various initial concentrations, as seen from the correlation coefficient r^2.

As shown in Table 1, the magnitude of the K_f intercept in the Freundlich equation showed a high Pb^{2+} adsorptive capacity of *A. versicolor*, which was higher than reported values for *A. versicolor* (Bairagia et al. 2011), while that for Cu^{2+} was lower. The intensity of sorption, denoted by 'n' (Table 1), which is related to the distribution of bound ions on the sorbent surface, was greater than unity, indicating that there is a favourable sorption of lead and copper ions by the biomass of *A. versicolor.*

The maximum adsorption capacities for Pb^{2+} and Cu^{2+} biosorption by *A. versicolor* calculated from Langmuir adsorption isotherm were 25.25 and 13.15 mg g^{-1}, respectively (Table 1) that for lead being higher than that for copper. The biosorption capacity of *A. versicolor* was seen to be better than that of other aspergilli such as

Table 1 The Freundlich and Langmuir parameters for the biosorption of $Pb^{2+}$and Cu^{2+} onto *A. versicolor*

Metal ions	Freundlich			Langmuir		
	K_f	n	r^2	b (l mg^{-1})	q_{max} (mg g^{-1})	r^2
Pb^{2+}	30.413	2.717	0.959	0.007	25.25	0.901
Cu^{2+}	4.623	1.48	0.908	0.032	13.15	0.860

A. flavus (Akar and Tunali 2006) and *A. niger* (Dursun et al. 2003), and of clays such as bentonite (Donat et al. 2005) and kaolinite (Gupta and Bhattacharyya 2005), as well as *A. nidulans* (Gazem and Nazareth 2012) but less than that obtained by *A. niveus* (Karaca et al. 2010), *Fusarium solani* (Bhatti et al. 2008), *Rhizopus arrhizus* (Fourest and Roux 1992), *Pseudomonas stutzeri* (Oh et al. 2009), *Rhodococcus opacus* (Bueno et al. 2008), *Spirogyra neglecta* (Hussain et al. 2009) and a bentonite sample as shown by Hefne et al. (2008).

The Langmuir constant, 'b', values obtained for Pb^{2+} and Cu^{2+} were found to be 0.070 and 0.035, respectively, which indicate that lyophilized cells of *A. versicolor* possess a high adsorption affinity for Cu^{2+} as compared to Pb^{2+}.

Sorption of lead and copper ions by *A. flavus* and *A. niger* was also found to follow the Freundlich model (Akar and Tunali 2006; Kapoor et al. 1999; Parvathi et al. 2007) a similar observation was made using the basidiomycetes *Phanerochaete chrysosporium, Trametes versicolor, Pleurotus eryngii* and compost (Say et al. 2001; Bayramoglu et al. 2003; Joo et al. 2011; Seelsaen et al. 2007).

Biosorption kinetics

The linear form of the pseudo-first order model and the pseudo-second order model for the adsorption of Pb^{2+} and Cu^{2+} is given in Figs. 7 and 8. The biosorption kinetics of heavy metals provides the mechanism of the sorption reaction, describing the metal uptake, which in turn controls the time during which the metal remains at the solid-solution interface.

The correlation coefficients (r^2) for the linear plots using the pseudo-first order model was 0.4010 for Pb^{2+} and 0.839 for Cu^{2+} sorption, while the correlation coefficients for the linear plots from the pseudo-second order model was 0.996 for Pb^{2+} and 0.999 for Cu^{2+} sorption (Table 2). The adsorption capacities calculated ($q_{e\ calc}$) by the pseudo-second-order

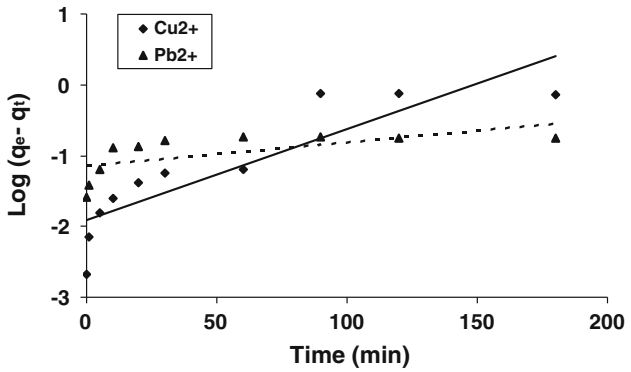

Fig. 7 Pseudo-first order kinetics plot for sorption of Pb^{2+} and Cu^{2+} ions onto *A. versicolor*

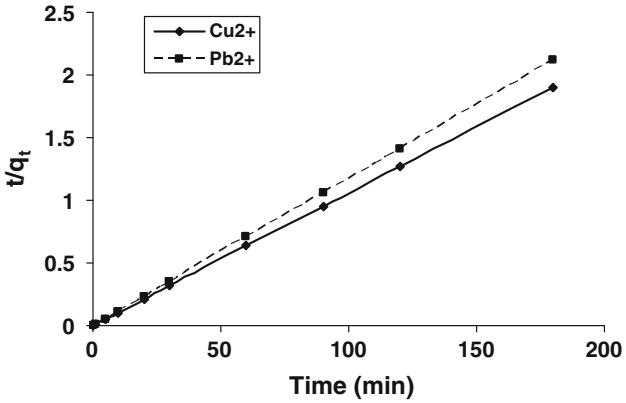

Fig. 8 Pseudo-second order kinetics plot for sorption of Pb^{2+} and Cu^{2+} ions onto *A. versicolor*

model are also close to those determined by experiments ($q_{e\ exp}$). These results indicated that the pseudo-first order model is less suitable to describe the biosorption process. It was therefore concluded that the pseudo-second order adsorption model is more applicable to describe the adsorption kinetics of Pb^{2+} and Cu^{2+} by lyophilized culture of *A. versicolor*. This was also observed in the biosorption of Pb^{2+} and Cu^{2+} by *Fusarium solani* (Bhatti et al. 2008), *Pseudomonas stutzeri* (Oh et al. 2009), *Rhodococcus opacus* (Bueno et al. 2008), *Spirogyra neglecta* (Hussain et al. 2009), as well as by bentonite (Hefne et al. 2008).

FTIR

The IR spectrum of the KOH-treated, lyophilized biomass in comparison to the untreated native biomass (Fig. 9), indicated a broadening of the –NH and –OH bands and a shift or an increase in sharpness and intensity of peaks, that could be assigned to functional groups of carbonyl, carboxyl and amide groups of biomolecules of the mycelium, thus indicating that K^+ must have adsorbed to the mycelial mass. The IR spectra of the KOH treated biosorbent after metal sorption showed a further broadening of the –NH and –OH band at 3,500–3,200 cm^{-1}, intensified in presence of Cu^{2+}, and an increase in the intensity of the peaks corresponding to the –CH stretching vibrations of CH_2 and CH_3 groups at 3,000–2,800 cm^{-1}, a shift in peaks of the carbonyl –C=O of amide or carboxyl groups at 1,670–1,650 cm^{-1} and 1,550–1,540 cm^{-1}, the amide or sulfamide at 1,381 cm^{-1}, the C–O or C–N stretching vibrations of proteins at 1,153 cm^{-1} and the P–O–C linkage of the organo-phosphorous groups about 1,026 cm^{-1}. This would indicate an ion exchange mechanism, including a replacement of the K^+ ions adsorbed on the biosorbent with the Pb^{2+} and Cu^{2+} ions. This ion-exchange mechanism also supports the observation that at low pH, H^+ ions compete with metal ions for the binding sites on the cell

Table 2 Kinetic parameters for the adsorption of $Pb^{2+}$and Cu^{2+} onto *A. versicolor*

Metal ions	$q_{e\ exp}$(mg g^{-1})	Pseudo-first order			Pseudo-second order		
		k_1	$q_{e\ calc}$ (mg g^{-1})	r^2	k_2	$q_{e\ calc}$ (mg g^{-1})	r^2
Pb^{2+}	15.13	0.0076	13.826	0.401	1.413	16.05	0.999
Cu^{2+}	5.02	0.0377	50.35	0.778	0.445	4.71	0.996

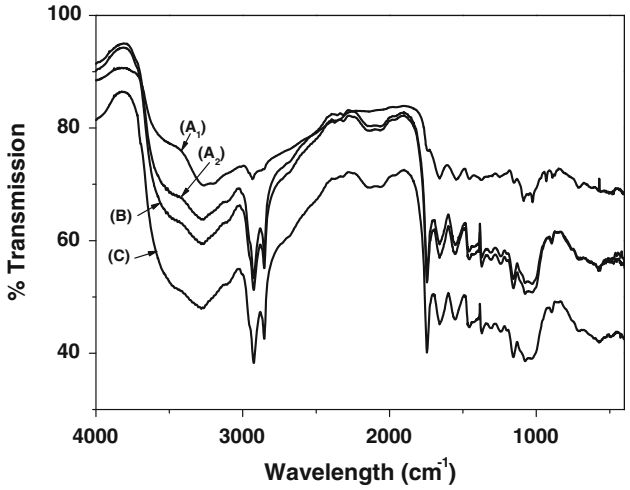

Fig. 9 IR spectra of *A. versicolor*: mycelial biomass control-untreated (A_1) control-KOH treated (A_2) mycelia after sorption with lead (*B*) and with copper (*C*)

wall, causing low sorption capacity at acidic pH, as seen in the results indicated above. The increase in peak intensity as well as a shift in the stretching vibration of the C–O or C–N of proteins at 1,155 cm^{-1} and the P–O–C linkage of the organo-phosphorous groups at 1,030 cm^{-1} were more pronounced when exposed to Cu^{2+}.

This mechanism of ion exchange in Pb^{2+} and Cu^{2+} sorption by the *A. versicolor* isolate is in accordance with reports on metal sorption by aspergilli: *A. parasiticus*, *A. niger* and *A. versicolor* (Akar et al. 2007; Akhtar et al. 1996; Bairagia et al. 2011) as well as earlier results obtained (unpublished data).

Fatty acids composition of fungus biomass

Aspergillus versicolor showed a marked increase in most of the fatty acids when grown in presence of Pb^{2+} and Cu^{2+} in comparison to the control grown in absence of the metal, being enhanced more by Cu^{2+} than by Pb^{2+} (Table 3). The increase in fatty acids in presence of metals was particularly seen with the saturated stearic acid (C16:0) and the unsaturated oleic acid (C18:1n9c, C18:1n9t) and linoleic acids (C18:2 n6t), the latter being more intense in presence of Pb^{2+}. Similar results were also obtained with

Table 3 Changes in fatty acid composition of *A. versicolor* in response to Pb^{2+} and Cu^{2+} stress

Fatty acids		Fatty acid of mycelia grown in presence of metal (μg g^{-1})		
		Control	Pb^{2+}	Cu^{2+}
Saturated				
Myristic acid	C14:0	0.178 ± 0.02	2.844 ± 0.12	2.006 ± 0.1
Pentadecanoic acid	C15:0	0.173 ± 0.03	0.1071 ± 0.01	4.234 ± 1.2
Palmitic acid	C16:0	5.416 ± 0.2	2.379 ± 0.3	81.063 ± 2.1
Margaric acid	C17:0	0.210 ± 0.01	0.1395 ± 0.05	5.544 ± 0.99
Stearic acid	C18:0	2.102 ± 0.09	28.737 ± 1.4	39.542 ± 1.5
Arachidic acid	C20:0	0	1.3395 ± 0.7	1.717 ± 0.7
Behenic acid	C22:0	0	1.698 ± 0.8	1.784 ± 0.4
Lignoceric acid	C24:0	0	1.698 ± 0.4	2.604 ± 0.24
Unsaturated				
Methyl *cis* 10 pentadecenoic acid	C 15:1	Tr.	0.775 ± 0.1	2.813 ± 0.41
Plamitoleic acid	C16:1	Tr.	1.527 ± 0.33	1.626 ± 0.12
Cis 10 heptadecenoic acid	C17:1	0.677 ± 0.04	Tr.	1.099 ± 0.09
Oleic acid	C18:1 n_9c	2.946 ± 0.18	37.014 ± 3.02	35.461 ± 4.2
Elaidic acid	C18:1 n_9t	4.797 ± 0.22	79.813 ± 2.88	81.840 ± 2.5
Linoleic acid	C18:2 n_6t	4.492 ± 0.3	100.89 ± 3.1	21.190 ± 0.4
Saturation index (SI)		0.62	0.177	0.96

Data are expressed as μg g^{-1} lyophilized cell biomass. ±SE of two investigations

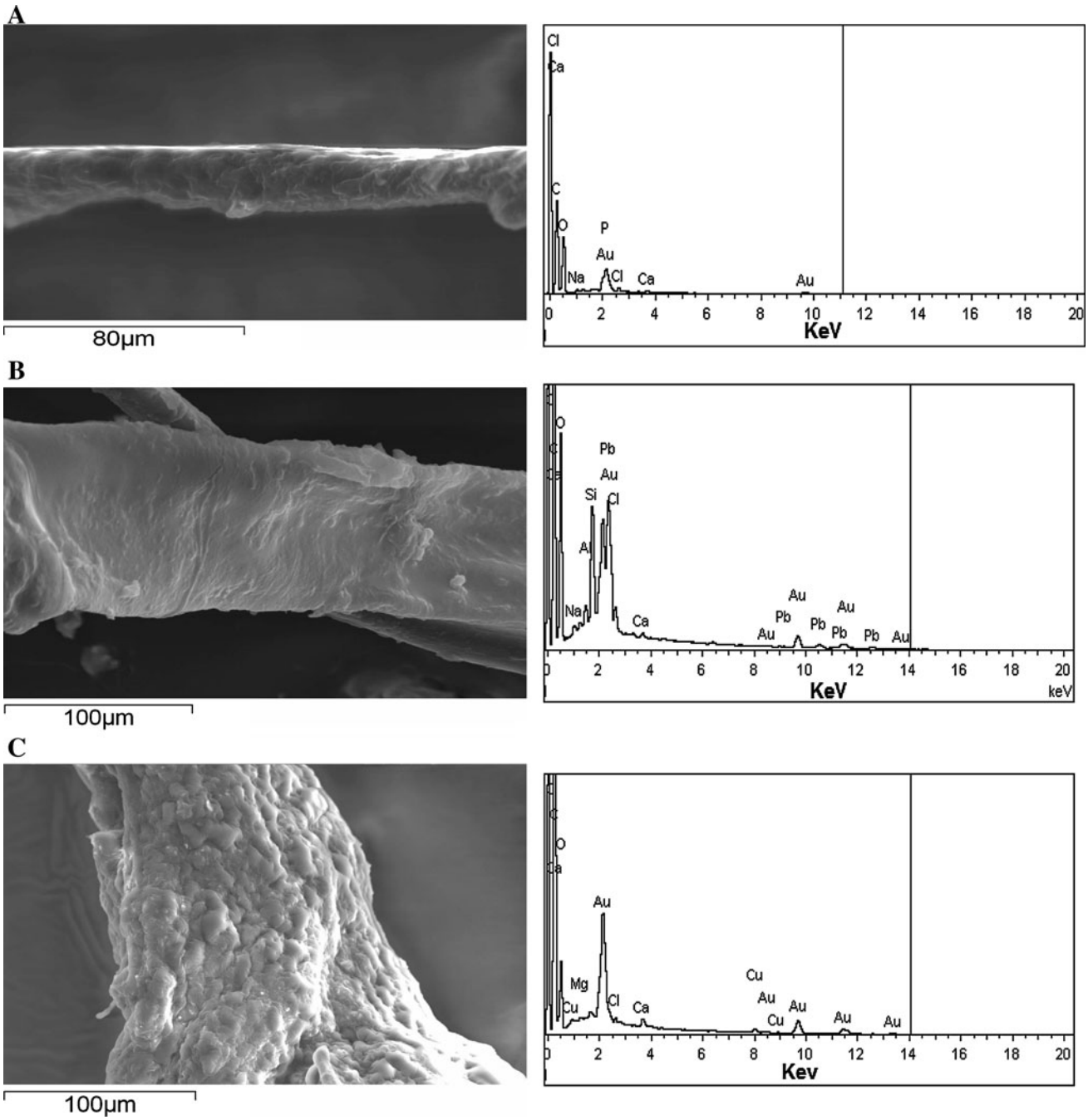

Fig. 10 Scanning electron micrograph and EDX spectra of *A. versicolor* biomass: **a** pristine (control); **b** after sorption of lead and **c** after sorption of copper

Aspergillus terreus in response to Cu^{2+} (Al Abboud and Alawlaqi 2011) and with *Curvularia lunata exposed to* Ni^{2+} (Paraszkiewicz et al. 2010). In addition, mycelia of *A. versicolor* grown in presence of Cu^{2+} also showed a high increase in palmitic acid (C18:0), whereas there was a decrease when grown in presence of Pb^{2+}.

The saturation index of fatty acids decreased from 0.626 seen in the control biomass, to 0.177 in biomass cultured with 2 mM of Pb^{2+} but was increased to 0.96 when cultured with 1 mM of Cu^{2+}. This increase in saturation index in presence of Cu^{2+}, corroborates earlier findings on *Stachybotrys chartarum* by Hefnawy et al. (2010), who conclude that this results in a decreased membrane fluidity or increased rigidity, thus enabling the organism to tolerate toxic metals up to certain concentrations and might be one of the tolerance mechanisms to heavy metals stress by filamentous fungi. Their observation that the saturation index decreases at higher metal concentration, could be a

reason for decreased tolerance of the fungus to high concentrations of metal ions.

SEM and EDX analysis

The mycelium grown in presence of metal ions, showed considerable changes as seen in the SEM micrographs (Fig. 8). The ridges and grooves seen on the pristine biomass (control), were intensified when the culture was exposed to metal and are thought to aid in metal sorption (Khambhaty et al. 2009; Srivastava and Thakur 2006). The mycelium in presence of lead ions became broadened, with a shiny appearance, attributable to the deposition of metal on the surface (Bansal et al. 2009). On exposure to copper ions, the mycelium was greatly thickened and showed a highly wrinkled surface. The thickening of the mycelia in presence of metal ions could be a result of chitin deposition in the cell wall, which contributes ligand molecules for the sorption of metal (Nazareth and Marbaniang 2008; Ram et al. 2004). The sorption of metal ions onto the cell surface ligands has been indicated by the IR analysis.

The EDX spectra of the biomass before and after exposure to Pb^{2+} and Cu^{2+} (Fig. 10) showed the presence of a strong signal due to Pb^{2+} at 2.2 keV (Fig. 10b) and a signal of Cu^{2+} between 0.9 keV (Fig. 10c), indicating the sorption of Pb^{2+} and Cu^{2+} ions respectively on to the mycelial biomass and confirming the results obtained by FTIR and SEM analysis. The phosphorous signal at 2.1 keV disappeared after sorption of metal, indicating the involvement of the phosphate group on the biomass in the complexation with metal ions, as was also seen in FTIR analysis. These signals for Pb^{2+} and Cu^{2+} ions on the biomass, indicating the accumulation of metal ions on the fungal cell walls, were also recorded by Akar et al. (2007) and Bairagia et al. (2011) using different species of fungi such as *A. parasiticus, A. versicolor* and *Botrytis cinerea.*

Conclusion

The results obtained indicated that metal sorption by *A. versicolor* occurred rapidly in the first 5–10 min, favoured by a pH of 6.0. The equilibrium data fitted the Freundlich adsorption isotherm model, indicating the involvement of a heterogeneous surface in metal sorption, following a pseudo-second order kinetics reaction. The fungus showed a higher accumulation of fatty acids when grown in presence of metals as compared to the mycelium grown in absence of the metal; there was also an increase in the saturation index of fatty acids in presence of Cu^{2+} which serves as a protective mechanism for the fungus. Metal removal from solution occurred by a passive adsorption by the fungal cell surface, involving an ion exchange mechanism as seen by FTIR, SEM and EDX analysis, which explains the rapid rate of removal from solution. The isolate *A. versicolor* EM2wt41 obtained from an estuary that is exposed to metal contamination and has consequently developed a good mechanism of metal sorption, serves as a potential culture in bioremediation measures for removal of metal from aqueous waste.

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