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Full Length Research Paper

Process optimization and mechanistic studies of lead (II): As*pergillus caespitosus* interaction for industrial effluent treatment

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The lead (II) accumulation potential of various biosorbent had been widely studied in the last few years, but an outstanding Pb(II) accumulating biomass still seems crucial for bringing the process to a successful application stage. This investigation describes the use of non-living biomass of *Aspergillus caespitosus* for removal of Pb from $Pb(NO_3)_2$ solution in a batch system under different experimental conditions. The highest Pb(II) sorption (351.7 ± 5.7 mg/g biomass) was observed at 600 µg/ml initial Pb concentration. Biosorption data were well defined by pseudo-second order, saturation mixed order and Langmuir isotherm models. The thermodynamic parameters: G (303 K), H and S were determined to be 4.64 kJ/mol, 75.4 kJ/mol and 26.2 J/mol-K respectively. The Pb uptake from binary solution was inhibited in the order of copper > nickel > zinc > manganese. Fourier transform infrared spectroscopy (FT-IR) characterization of Pb biosorption revealed the involvement of $–SO_3$ and –CN groups along with other groups. The biosorbed Pb was stripped out (85.5%) using 0.01 M HCl and about 12% loss in Pb(II) sorption capacity was observed after five sorption-desorption cycles. High Pb (II) uptake (351.7 ± 5.7 mg/g biomass) by *A. caespitosus* proved it to be an outstanding biomaterial until now reported in literature for accumulating from solutions.

Key words: *Aspergillus caespitosus*, Pb, Langmuir isotherm, pseudo-second-order kinetic model, FTIR, SEM, EDAX.

INTRODUCTION

According to US agency for toxic substance and disease registry (ASTDR), lead (Pb) is the most abundant toxic heavy metal. Since the industrial revolution, its production has increased exponentially (Malik, 2004). The world Pb(II) production has exceeded 3.5 million tons per year. It has been used in the manufacturing of storage batteries, pigments, leaded glass, mining, electroplating, painting, smelting, petrochemical, plumbing, fuels, photographic materials, matches and explosives (Resmi et al., 2010). The Pb released to the environment by these technological activities tends to persist indefinitely, circulate and eventually accumulate throughout the food chain, becoming a serious threat to all kinds of biota. Exposure to Pb above permissible limit (50 ppb in water) can affect hemoglobin synthesis or can be stored in mineralizing tissue (teeth and bones) and released again into blood stream at the time of calcium stress. Also, it has many adverse effects on kidney, liver, gastrointestinal tract, respiratory tract and deposition in mucous membranes causing mental retardness in children, abnormalities in fertility/pregnancy and damage to central and peripheral nervous system (Goel et al., 2005).

Metal ions removal from aqueous solution by various conventional techniques such as precipitation, evaporation, electroplating, ion-exchange and membrane processes is expensive and ineffective especially for

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diluted voluminous effluents. So, with a gradual increase in the Pb content in ecosystem, it seems necessary to develop an efficient technology for its removal. An innovative multidisciplinary technique (biosorption/ bioaccumulation) that combines the metal removal with secondary metal recovery processes is of growing interest and has become the main focus of recent research. It offers several advantages over conventional processes like low operating cost, minimization of disposable chemical and/or biological sludge volume and high efficiency in detoxifying very dilute effluents (Akhtar et al., 2007). This remediation technique makes use of microbial (Akhtar et al., 2008) and non-microbial (Hanif et al., 2007; Nadeem et al., 2009) biomaterials.

However, the major challenge faced by biosorption researchers is the selection of most promising biosorbent. For industrial application, the main factors while selecting a biosorbent for particular application are availability and cheapness of biosorbent. The availability can only be ensured by selecting an organism that can be cultivated or propagated quickly and cheaply specially for biosorption purposes. Biosorption researchers have tested a number of biomass types for their biosorptive capacities under various conditions, but there are no limits to exploration of new biomass types having low cost and high efficiency. Among microbial biosorbents, fungi can be grown easily to produce high yields of biomass or biomass can be available as industrial waste product as they are utilized as producer of economically important substances (Wang and Chen, 2009). The cell wall of fungi is made up of chitin, glucans and mannan that have many potential metal binding sites with oxygen containing groups including carboxylic, phenolic, alcoholic, hydroxyl, carboxyl and methoxyl being particularly important in biosorption (Gadd, 2009). The variation in these contents gives an approach to search out fungal strains having high growth rate and requiring little processing. *Aspergillus caespitosus* is a fungus that is not common to indoor environments. It has been predominantly isolated from soils and also been isolated from sugarcane bagasse. It has been used for production of invertases, alkaline phosphatase and xylanases. This study was carried out to optimize the laboratory conditions for the maximum uptake of Pb (II) from aqueous solutions by non-living, dried powdered biomass of locally isolated fungus *A. caespitosus*. This biomass was selected after screening a wide range of microbes. Although the removal of Pb (II) has been reported earlier, to our knowledge, this is the first comprehensive report on its biosorption by *A. caespitosus*.

MATERIALS AND METHODS

Microorganism growth and media

All fungal cultures used in screening studies were obtained from the Industrial Biotechnology Division, NIBGE. Prior to use, these cultures were revived on Vogal's medium plates. To obtain biosorbent, fungi were grown in liquid Vogal's medium (g/L): trisodium citrate, 2.5; KH_2PO_4, 5.0; NH_4NO_2, 2.0; $(NH_4)_2SO_4$, 4.0; $MgSO_4 .7H_2O$, 0.2; $CaCl_2.7H_2O$, 0.1; peptone, 2.0; glucose, 5.0; at pH 5.5), and at 28 ± 2°C in Erlenmeyer flasks. In order to obtain suspended growth having larger surface area, 10 to 15 glass beads were added to each flask before autoclaving. Cultures were harvested during the stationary growth phase by filtration. Wet biomass was determined after blotting the freshly harvested biomass with commercial grade paper towels to remove excess water. This wet biomass was stored in a screw capped bottle at 4°C. A known weight from this wet biomass was then dried at 80°C in an oven for 24 h and factor to calculate dry weight from wet weight was determined. This freshly harvested biomass stored at 4°C was used for Pb(II) biosorption in screening studies.

Metal solution

Stock solution (1000 mg/L) was made by dissolving 1.598 g of $Pb(NO_3)_2$ (BDH) per liter of distilled water. Further dilutions were made from this stock solution. All other chemicals used were of analytical grade.

Analytical determination

The concentrations of metal ions Pb, copper (Cu), manganese (Mn), nickel (Ni) and zinc (Zn) in solution before and after biosorption were determined using atomic absorption spectrophotometer (Model Varian 240 FS).

Sorption experiments

Biosorption experiments were carried out in batches with control samples containing metal ion solution in the absence of biomass to evaluate the effects of pH of metal solution, contact time, initial metal concentration, etc by suspending known amount of biomass to metal solution. The final volume was 100 ml and other parameters were specified in each experiment. The reaction mixtures were agitated at 180 rpm at 28 ± 2°C in an orbital rotary shaker (Kuhner, Switzerland ISF-1-W). Periodically, 1.0 ml sample was taken and analyzed for residual metal concentration determination. Prior to analysis, sample was centrifuged at 10,000 × *g* for 5 min and cells were discarded. Residual metal concentration was determined in the supernatant. These experiments were conducted in triplicate and average results were reported. The amount of metal ions (mg) biosorbed per gram (dry weight) of biomass (q, mg/g dry weight) was calculated by using following relationship:

$$q = (C_i - C_f)\, V / W \qquad (1)$$

Where, C_i and C_f correspond to the initial and final metal ion concentrations in the supernatant when W (g) of biosorbent was suspended in V (l) of the metal solution. From analysis of Pb solution in control flasks (C_i), losses due to the adsorption to flask walls were found negligible.

Desorption of Pb(II)

Different regenerating solutions (0.01 M) each of HCl, NaOH, $NaNO_3$, Na_2CO_3, $NaHCO_3$, Na_2SO_4, CH_3COONa and distilled water were used to desorb the accumulated metal from Pb loaded biomass (0.025 g/30 ml of regenerating solution). The amount of desorbed metal was analyzed after 1, 2, 4, 6 and 24 h of shaking at

Table 1. Comparison of different biosorbents studied for biosorption of lead.

Biosorbent	C_o (mg/L)	q_m (mg/g)	Reference
Aspergillus caespitosus	100	174.2±4.4	Present study
Arthrobactor sp.	250	130	Fourest et al. (1992)
Rhizopus arrhizus	600	55.6	Fourest et al. (1992)
Sargassum natans	10-300	310	Fourest et al (1992)
Aspergillus niger	500	34.4	Dursan et al. (2003)
Wild Cocoyam	100	49.53	Jnr et al. (2006)
Cystein modified biomass	30	29.95	Tong et al. (2007)
Spirogyra sp.	100	52.00	Gupta and Rastogia (2008)
B. vesicularis	80	18.15	Resmi et al. (2010)
Egg shell	100	24.95	Kalyani et al. (2010)
Pine cone powder	120	25.45	Ofomaja et al. (2010)

150 rpm at 28 ± 2°C. All experiments were carried out in triplicate. The values of q_{des} were calculated from C_{des} as follows:

$$q_{des} = C_{des}\, V/W \quad (2)$$

The percentage of desorbed metal was evaluated as:

$$\text{Percentage desorption} = [q_{des} / q]\, 100 \quad (3)$$

Fourier transforms infrared (FTIR) studies

FTIR spectra of biomass (before and after adsorption) were recorded using BX-FTIR (Perkin Elmer) spectrophotometer according to Akhtar et al. (2009).

Scanning electron microscopic (SEM) and energy dispersive X-ray analysis (EDAX)

SEM and EDAX studies of *A. caespitotus* before and after metal sorption were carried out at the National University of Science and Technology, Islamabad, Pakistan. For this purpose, the biomass used was oven-dried till constant weight and were ground to fine powder in a pestle and mortar.

RESULTS AND DISCUSSION

Screening for lead sorption

15 fungal isolates were screened for Pb(II) biosorption potential at initial pH value of 4.5 and temperature 30°C by incubating freshly harvested wet biomass corresponding to 0.05 g dry weight per 100 ml of metal bearing solution. The biomasses harvested after 3 days were used and significant differences varying from 55.6 ± 5.7 to 164.5 ± 3.5 mg/g dry weight were observed in biosorption capacity (q) for Pb(II). These differences in q may be due to morphological or composition differences in the polysaccharide structures of cell walls of these strains. The *A. caespitosus* (identified by amplifying ITS1 and ITS4 regions) showed the highest potential for Pb(II) biosorption (164.5 ± 3.5 mg/g). The value was considerably higher than reported for other fungi, yeast or bacteria in literature (Table 1). Therefore, this isolate was used for optimization of other parameters for Pb (II) biosorption. The Pb (II) uptake capacity of this biosorbent was greater with dried biomass (174.2 ± 4.4 mg/g) compared to that of wet (164.5 ± 3.5 mg/g). This may be due to the fact that after heat and crushing, the intracellular components (potential binding sites) become exposed for metal biosorption (Park et al., 2010). Another variable is the age of biosorbent that affects the biosorption efficiency. The biomass showed increasing biosorption capacity (133.5 ±1.1 to 174.2 ± 4.4 mg/g) up to the age of 72 h incubation, and then there was a decrease in its biosorption potential. This may be due to the increasing content of glucan and mannan than chitosan in the cell wall structure as the incubation period prolonged, which proved better biopolymer in metal uptake (Gadd, 2009). In further experiments, biomass harvested after 72 h and powdered after drying at 80°C was used.

Effect of pH and temperature on sorption

To investigate the effect of initial pH of solution and incubation temperature (25 to 50°C) on Pb(II) biosorption by *A. caespitosus*, time course studies were carried out at initial pH values of 3, 3.5, 4.0, 4.5 and 5.0 (Figure 1). Among physico–chemical factors, initial pH of metal solution is the most important parameter that critically affects the metal sorption. The pH affects the solution chemistry of the metal ions, the activity of functional groups in the biosorbents and the competition with coexisting ions in solution (Akhtar et al., 2009; Vijayaraghavan and Yun, 2008). In the present studies, with increase in pH from 3 to 4.5, the value of sorption capacity gradually increased giving maxima at pH 4.5. At pH 5, the sorption capacity value remains almost practically constant. The present results indicate that the pH value in the range 4.0 to 5.0 was suitable for Pb(II)

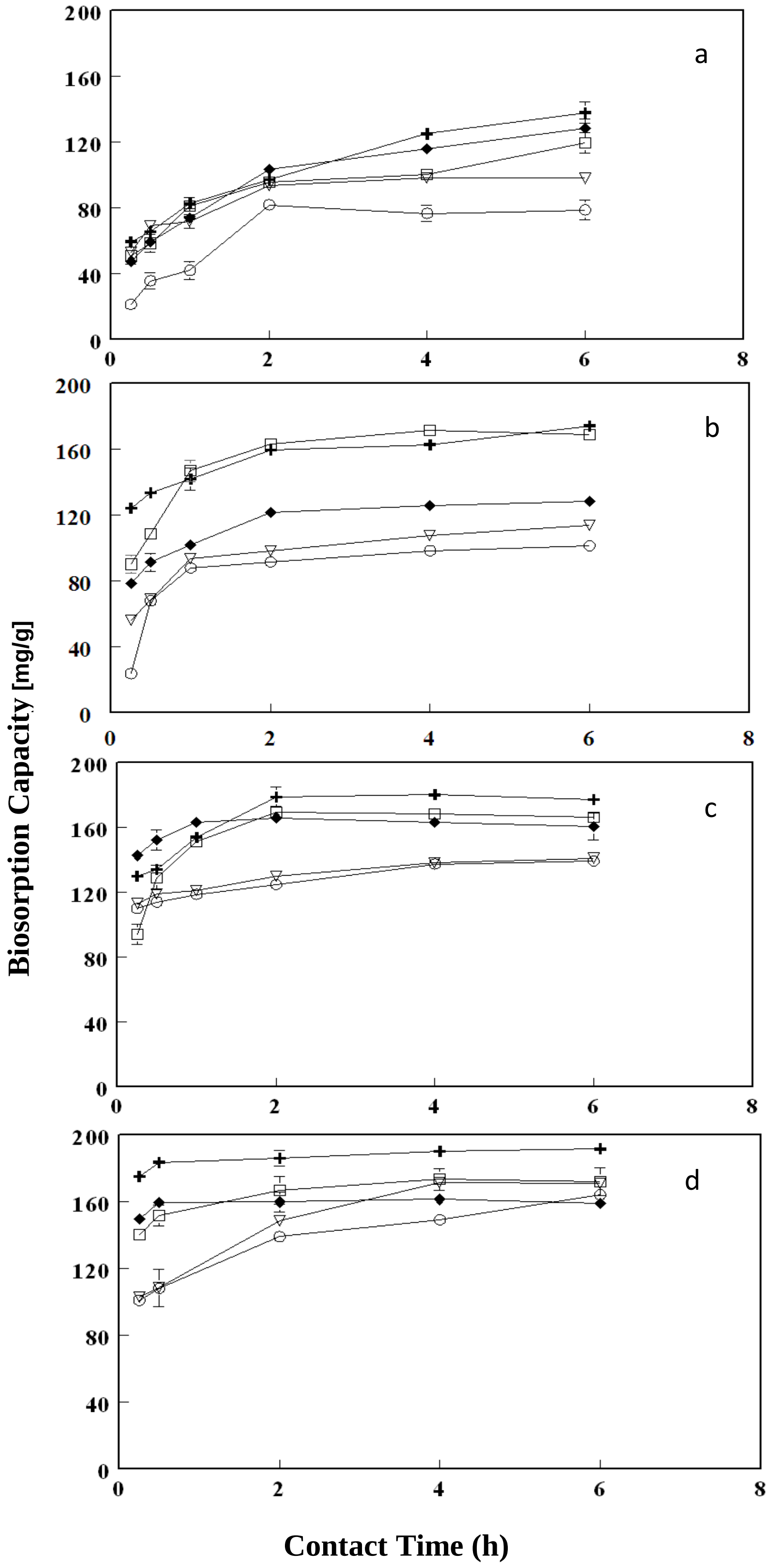

Figure 1. Biosorption of Pb (II) as a function of initial pH 3.0 (○),3.5 (▽), 4.0 (◆),4.5 (✚) and 5.0 (☐) of solution by *A. caespitosus* at (a) 25°C, (b) 30°C, (c) 40°C and (d) 50°C. W = 0.05 g; C_i = 100 mg/L; V = 100 ml; agitation rate = 180 rpm.

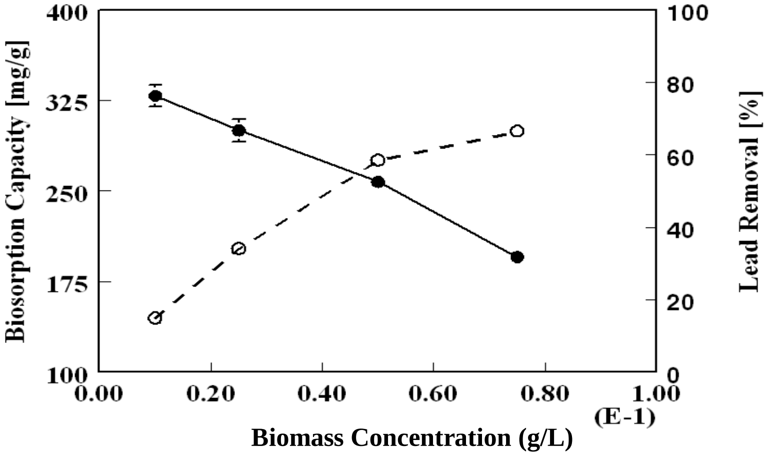

Figure 2. Effect of biosorbate amount on Pb (II) biosorption (●) and percentage removal (○) by *A. caespitosus.* C_i = 200 mg/L; agitation rate = 180 rpm; temperature =28 ± 2°C, pH = 4.5.

binding. At lower pH value (pH 3.0), Pb(II) removal was inhibited possibly as a result of competition between proton and Pb(II), while an increasing metal biosorption at higher pH (pH 4.5 to 5) is due to deprotonation of metal binding sites yielding more ligands with negative charge which results in increased binding of cations (Viera et al., 2007). However by increasing initial pH to 6.0, Pb precipitated out in the form of lead hydroxide due to higher concentration of OH^- in the biosorption medium. The same trend about pH was observed almost at all values of temperature studied. Our findings are in accordance with those already reported in literature for Pb(II) sorption (Hawari aqnd Muligan 2005).

It was also observed that the biosorption capacity increased with an increase in temperature from 25 to 30°C at all the pH values. Biosorptive removal of most of the heavy metals is endothermic, therefore higher temperature usually improved biosorptive removal of adsorbate through increase in its surface activity and kinetic energy (Vijayaraghavan and Yun, 2008). Further increase in temperature from 30 to 50°C resulted in non-significant increase in biosorption capacity, which may be due to physical damage to the biosorbent at higher temperature (Akhtar et al., 2009).

Moreover, at higher temperature, the equilibrium was attained faster compared to that at lower temperature (maximum removal at 30°C was attained after 6 h, while at 50°C it was achieved after 1 h only). However, with increase in temperature, the difference in sorption capacity at different pH values tends to be smaller. This rapid increase in uptake capacity at higher temperature may be due to increased surface activity and kinetic energy of the solute (Vijayaraghavan et al., 2005).

Effect of biosorbent concentration

To determine the effect of the biosorbent dosage on the biosorption capacity of *A. caespitosus*, experiments were conducted at pulp densities 0.1, 0.25, 0.50 and 0.75 g/L (Figure 2). With increase in the biosorbent dosage from 0.1 to 0.75 g/L, the percentage of Pb removal from aqueous solution increased from 14.9 to 66.5%. This may be due to the availability of more active sites for metal binding with increase in pulp density as the quantity of biosorbed pollutant per unit weight of biosorbent decreases with increase in biosorbent dosage (Park et al., 2010). The maximum value of biosorption capacity obtained during these studies was 328.8 ± 8.8 mg/g with 0.1 g/L dry weight biomass. Further increase in biomass concentrations lowered this value to 195.3 ± 0.9 mg/g with 0.75 g/L dry weight of biomass.

The lowering in q value with increase in biomass concentration may be due to the formation of cell aggregates at high biomass concentrations that resulted in reducing the effective biosorption area or due to low metal concentration in solution or an increase in biomass concentration which leads to interference between binding sites. Similar results on the effect of biomass concentration on the biosorption of different metal ions have been reported for various microorganisms by various researchers (Gong et al., 2005).

At biomass concentration of 0.05 g/L, the good agreement between biosorption capacity and percentage removal was observed and this pulp density was used for further experiments.

Effect of initial metal ion concentration

The effect of initial Pb(II) concentration (100 to 600 mg/L) was investigated at optimized pH and temperature (Figure 3). The Pb (II) biosorption capacity of the biomass increased from 173.3 ± 1.0 to 341.6 ± 7.1 mg/g dry weight with increase in the initial Pb (II) concentration from 100 to

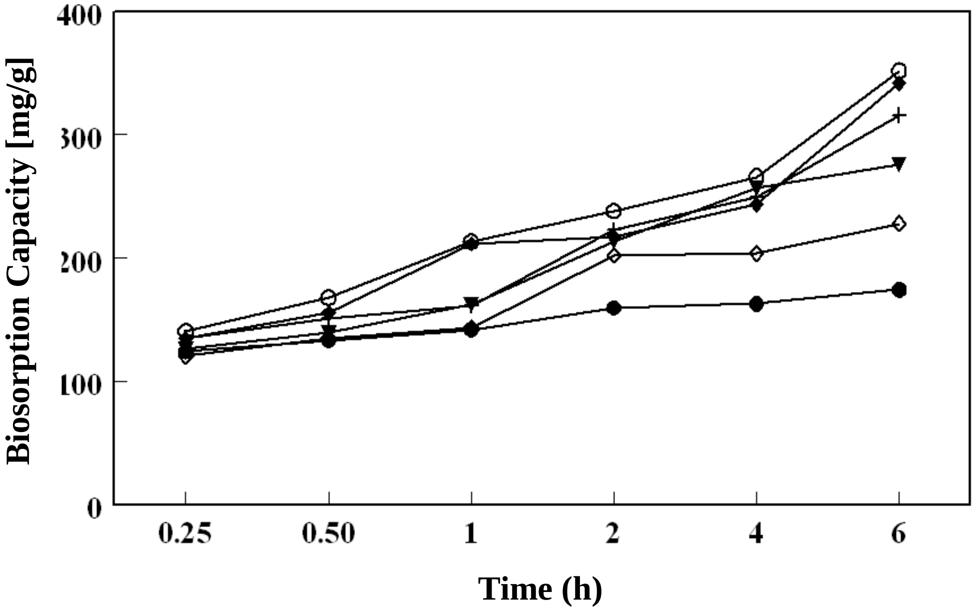

Figure 3. Effect of initial Pb (II) concentrations, 100 (●);200 (◇),300 (▼), 400 (+), 500 (◆) and 600 (○) mg/l on Pb (II) biosorption by *A. caespitosus*. W = 0.05 g; V = 100 ml; agitation rate = 180 rpm; temperature =28 ± 2°C; pH = 4.5.

500 mg/L. With increase in initial pollutant concentration, the quantity of biosorbed pollutant per unit weight of biosorbent increased but its removal efficiency decreased (Park et al., 2010). Further increase in concentration from 500 to 600 mgL^{-1} resulted in non-significant increase in sorption capacity (341.6 ± 7.1 to 351.7 ± 5.7 mg/g), indicating the saturation of biomass sites involved in Pb (II) biosorption. The increase in biosorption capacity with the increase in initial Pb (II) was also observed for removal of Pb (II) by loofa sponge immobilized *Aspergillus terreus* (Sun et al., 2010). The relatively higher biosorption capacity at lower initial metal concentrations indicated the suitability of biosorbent for treatment of dilute solutions (Sun et al., 2010).

Biosorption of Pb(II) and co-cations in binary system

The Pb(II) biosorption by *A. caespitosus* in the presence of other co-cations in binary system along with biosorption of co-ions as a function of their concentration is depicted in Figure 4. The initial concentration of Pb(II) used was 2 mM (200 ppm) and the effect of cations Mn, Zn, Ni and Cu was investigated at Pb(II) to cation ratio of 1:0.5, 1.0:1.0, 1.0:2.0 and 1.0:5.0. The presence of Mn, Zn, and Ni increased Pb biosorption, while the presence of Cu decreased it significantly. With an increase in concentration of co-ions from 1 to 4 mM keeping Pb concentration constant (2 mM), the uptake of Pb(II) by *A. caespitosus* increased to different degrees in the presence of Zn, Ni, and Mn. Maximum inhibition of Pb(II) uptake was seen to have been due to the presence of Cu in the solution. The uptake of Pb(II) decreased by increasing Pb:Cu from 1:0.5 to 1:1. Further increase in ratio resulted in increase in Pb(II) biosorption although it is significantly less compared to other cations. At Pb to cation ratio of 1:5, the inhibition of Pb(II) uptake was observed in the presence of all cations studied. With increase in cation ratio, the biosorption of other cations increased significantly.

This progressive intrusion in sorption by the co-cations indicated an extent of overlap in the sorption site function at higher equilibrium total metal concentration. However, the total metal uptake was higher from binary metal solutions than from single metal solutions. This may have been due to the higher cumulative concentration of metal ions in the binary metal system. All these cations showed higher biosorption capacity than for Pb (II) at higher Pb to cation ratio. This may be due to reason that all these cations belong to borderline metal ions and these could bind with all types of ligands with different preferences (Wang and Chen, 2009).

Regeneration study

The usefulness of a specific biomass as a biosorbent depends not only on its biosorptive capacity, but also on the ease of its regeneration and reuse (Bishnoi and Garima, 2005). In the present investigation, eight different desorbents having pH in the range of 2.5 to 12.0 were screened for their potential to desorb Pb from loaded biomass. These desorbents were selected keeping in

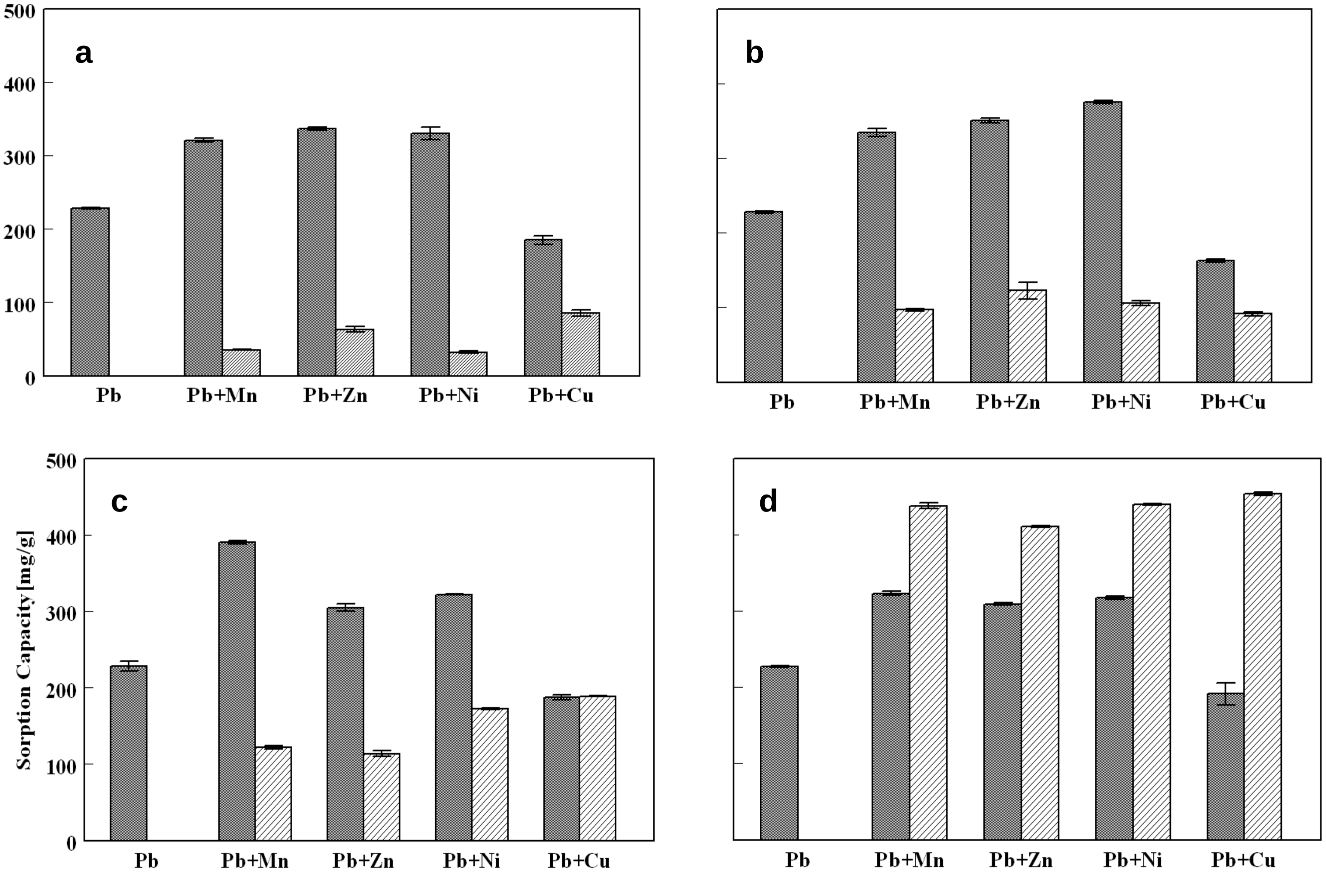

Figure 4. Biosorption of Pb (II) and other cations from binary mixture at (a) 1: 0.5 (b) 1:1 (c) 1: 2 and (d) 1: 5 Pb (II) to cation ratios.

mind that the solution pH has a strong influence on biosorption, thus simple manipulation of the pH of the desorbing solution can be a good method for recovery of the metals and regeneration of the biosorbent. The order of desorption efficiency of different desorbents was found to be HCl > Na_2CO_3 > Na_2SO_4 >NaOH >$NaHCO_3$ >CH_3COONa> $NaNO_3$> distilled water. The desorption values decreased from 85.5 ± 0.2 to 14% with increase in pH from 2.5 to 8.7 and then at pH 11.0 the percentage desorption again increased to 68%. The regeneration data show that adsorption of Pb (II) to *A. caespitosus* was a reversible process. To reduce the process cost and continuous supply of the sorbent, sorption–desorption cyclic study was carried out. The value of sorption capacity decreased from 174.2 to 152.9 mg/g with 12% loss in Pb(II) sorption capacity up to five cycles of reuse of *A. caespitosus.*

Application of kinetic and equilibrium models

Kinetic models

Batch kinetic modeling is used to have detailed information on adsorbate uptake rates and on rate limiting steps such as external mass transfer, intraparticle mass transfer and biosorptive reactions (Liu and Liu, 2008). In the present studies, ten kinetics models were used to analyze adsorption kinetics at initial Pb(II) concentration (100 mg/L), pulp density (0.5 g/L) and at temperature values of 25, 30, 40 and 50°C (Table 2). The adsorption kinetics data fit very well to pseudo-second order and saturation-mixed order kinetic models (R_{val} 0.99, Figure 5) that are presented by the following equations:

$$[t / q_t] = 1/ K_2q_e^2 + t /q_e \quad (4)$$

$$1/t \ln[C_o] /[C] = K_o /K – 1/K(C_o - C_t)/t \quad (5)$$

The values of K_2, rate constant of pseudo second-order (g/ mg.min) and q, adsorption capacities at equilibrium (mg/g) were determined from the slope and intercept of straight line that was obtained by plotting t/q against t (Figure 5a). Comparison of experimental and theoretical q values (Table 2), revealed that the adsorption system adhered to the pseudo second-order kinetics as compared to other models applied and provided the best correlation of the data. This model is based on the

Table 2. Comparison of adsorption rate constants and q_e estimated from different kinetic models for Pb(II) biosorption by *Aspergillus caespitosus.*

Kinetic model		Temperature ($^{\circ}$C)			
		25	**30**	**40**	**50**
Experimental q		125.0±2.64	174.2±4.41	176.7±2.3	183.6±2.66
Zero order	C_o	52.3	26.5	12.7	10.7
	K	-1.75×10^{-2}	-2.5×10^{-3}	-5.9×10^{-3}	-3.9×10^{-3}
	R val	0.61	0.14	0.33	0.29
First order	C_o	52.3	24.9	8.15	8.48
	K	-4.1×10^{-4}	-7.4×10^{-4}	-2.5×10^{-4}	-2.9×10^{-4}
	R val	0.61	0.09	0.154	0.16
Second order	C_o	50.5	23.3	23.3	5.9
	K	9.0	2.19	2.19	1.0
	R val	0.61	0.06	0.06	0.05
Pseudo first order	q_e	72.4	50.0	48.6	36.2
	K	-2.8×10^{-3}	-8.9×10^{-4}	-4.9×10^{-4}	1.6×10^{3}
	R val	0.97	0.43	0.207	0.47
Pseudo second order	q_e	131.5	186.2	169.4	188.6
	K_2	3.49×10^{-4}	3.99×10^{-4}	-8.835×10^{-4}	1.4×10^{-2}
	R val	0.99	0.99	0.99	0.99
Second order	q_e	51.5	21.6	8.2	9.1
Rate equation	K	7.9×10^{-5}	7.6×10	-3.7×10^{-5}	2.7×10^{-5}
	R val	0.7	0.01	0.09	0.06
Bangham's model	K_γ	35.6	115.8	125.7	161.0
	M	49.1	19.3	18.9	45.1
	R val	0.93	0.67	0.73	0.49
Elovich model	β	0.05	0.13	0.12	0.25
	α	29.27	3×10^{7}	2.3×10^{7}	1.7×10^{18}
	R val	0.90	0.65	0.72	0.50
Intrapartical diffusion	K_1	19.2	57.3	67.9	80.1
Model	a	0.19	0.05	0.05	0.02
	R val	0.93	0.67	0.73	0.48

K, Rate constant; R_{val} , correlation coefficient; $q_{e:}$, calculated sorption capacity; β, surface coverage; α, chemisorption capacity.

assumption that the rate limiting step might be chemical biosorption involving valency forces through sharing or exchange of electrons between sorbent and sorbate (Ho and McKay, 2000).

Equilibrium isotherms

The equilibrium biosorption isotherms are one of the most important data to understand the mechanism of the biosorption. In the present studies, experimental biosorption data obtained from the batch system at different initial metal concentrations were subjected to Langmuir, Freundlich and Dubinin–Radushkevich (D–R) isotherms (Figure 6). The Langmuir isotherm model assumes a monolayer sorption, which takes place at specific homogeneous sites within the biosorbent and mathematically it is;

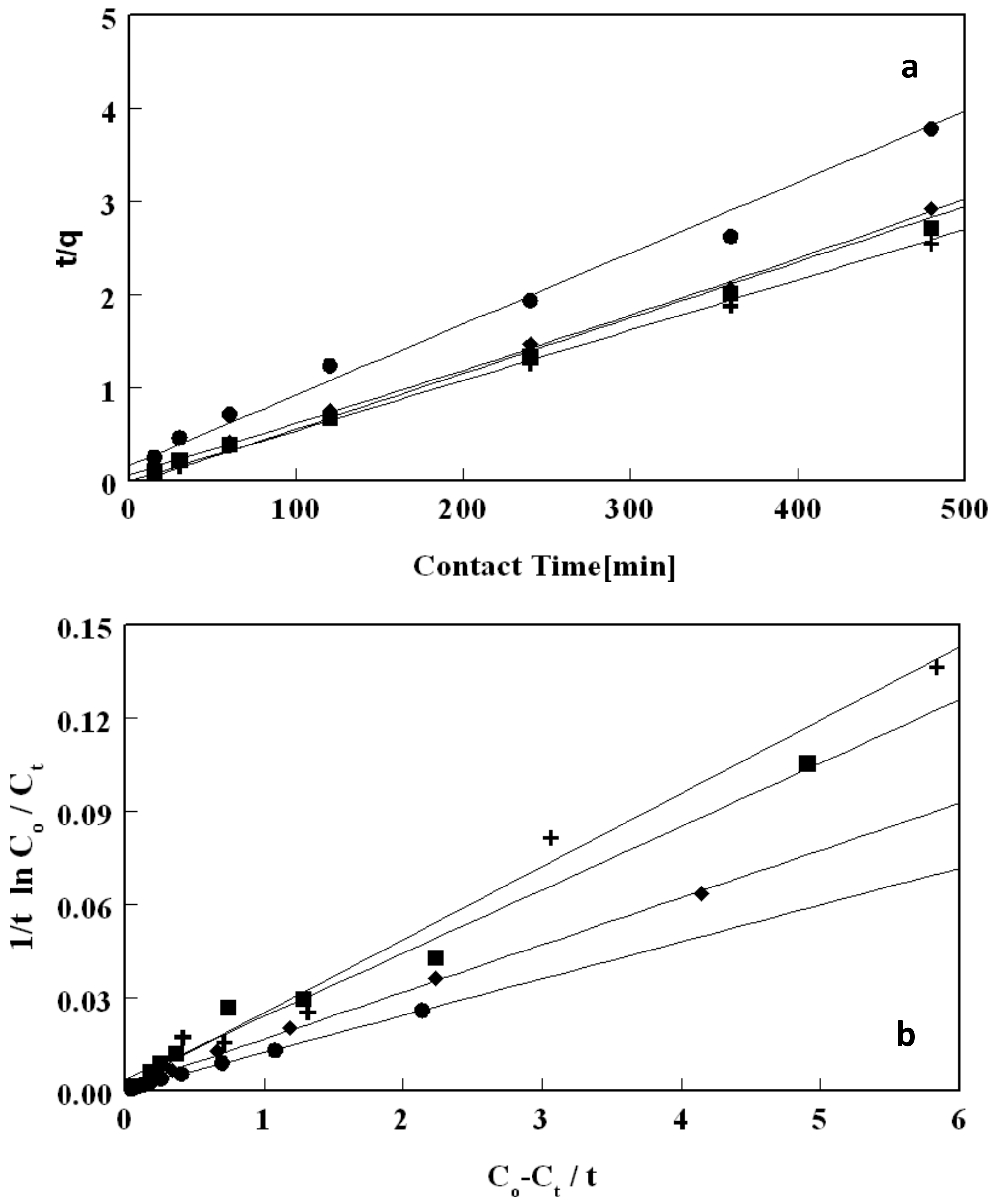

Figure 5. Kinetic model plots. (a) Pseudo-second-order and (b) saturation mixed order for Pb (II) biosorption by *A. caespitosus* at 25°C (●), 30°C (◆), 40°C (■) and (+) 50°C.

$$1/ q_e = 1/q_{max} + (1/q_{max} K_L)\ 1/C_e \qquad (6)$$

Where, q_e and C_e are the equilibrium Pb(II) concentration on the biosorbent (mg/g) and in the solution (g/L) respectively, q_{max} the maximum monolayer biosorption capacity of the biosorbent (mg/g), and K_L is the Langmuir adsorption constant (L/g). The values of K_L and q_{max} can be calculated from slope and intercept of the straight line obtained by plotting $1/q_e$ versus $1/C_e$ (Figure 6a).

The Freundlich isotherm is employed to describe heterogeneous systems and is represented as:

$$\ln q_e = \ln K_F + 1/ n \ln C_e \qquad (7)$$

Where, K_F and n are constants indicating extent of the biosorption and the degree of non-linearity between solution concentration and adsorption respectively. The plot of log q_e versus log C_e was employed to generate K_F and n from the intercept and the slope values respectively (Figure 6b). According to Halsey (1948),

$$K_F = q_m / C_i^{1/n} \qquad (8)$$

Where, C_i is the initial concentration of the solute in the solution (mg/L) and q_m is the Freundlich maximum adsorption capacity (mg/g). The Dubinin–Radushkevich (D–R) isotherm that distinguishes the nature of biosorption

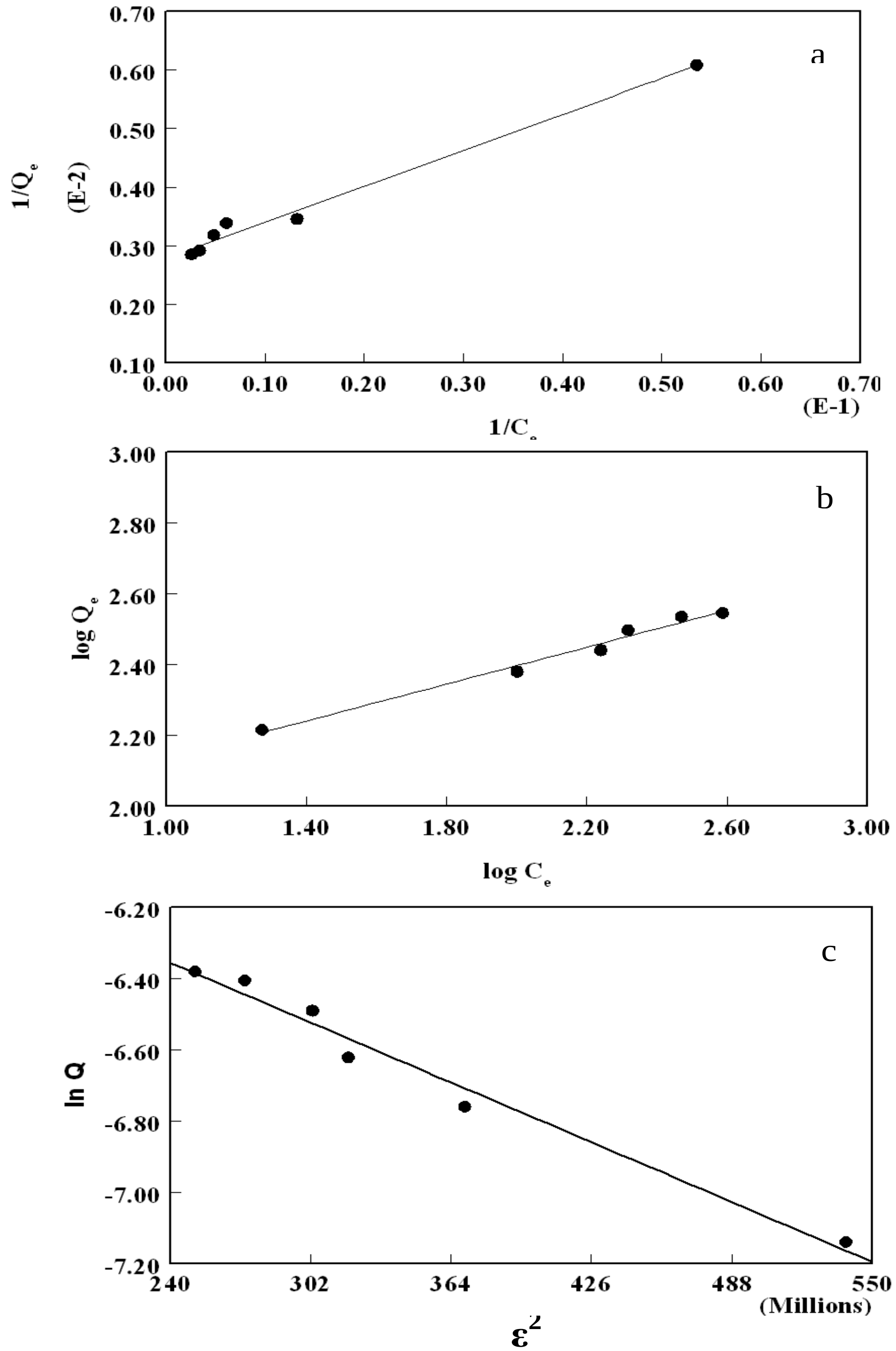

Figure 6. The adsorption isotherms plots: (a) Langmuir, (b) Freundlich and (c) Dubinin-Radushkevich for Pb (II) biosorption by *A. caespitosus*.

as physical or chemical is presented as:

$$\ln q_e = \ln q_m - ß\varepsilon^2 \quad (9)$$

By plotting ln q_e against ε^2 [Polanyi potential = RT ln(1 + (1/Ce)], where T is temperature in Kelvin and R is the gas constant 8.314 g/mole-K, the value of q_m (mole/g) and ß (mole^2/J^2) can be calculated from the intercept and slope of the straight line (Figure 6c).

The constant ß gives an idea about the mean free energy (kJ /mole) of biosorption that can be calculated using the relationship:

$$E = 1 / (2ß)^{1/2} \quad (10)$$

Table 3. Comparison of q_{max} obtained from Langmuir, Freundlich and Dubinin-Radushkevich adsorption isotherms for biosorption of Pb(II) by *Aspergillus caespitosus.*

Isotherm model	**Parameter**						
	q_{max} **(mg/g)**	**R** $_{valu}$	*K* **(l/g)**	R_L	*n*	*β* **mol^2/ J^2**	*Es* **J/mol**
Langmuir isotherm	351.2	0.99	4.6×10^{-2}	0.17	----	----	-----
Freundlich isotherm	403.5	0.95	87.4	----	4.14	----	----
D–R isotherm	689.1	0.98	-----	-----	----	-2.71×10^{-9}	-38.68

Experimental q_{max} = 351.2±5.7; q_{max}, maximum sorption capacity from isotherm model; R $_{val,}$ correlation coefficient; K, adsorption capacity; $R_{L,}$ separation factor; n, adsorption intensity; β, free energy of biosorption; Es, sorption energy.

Table 4. Comparison of $R_{L,}$ D, and θ values obtained from Langmuir adsorption isotherms for biosorption of Pb(II) onto fungus *Aspergillus caespitosus* at 30 °C.

Initial metal concentration (mg/l)	**R_L**	**D (mg/ ml)**	**$\theta = KC_i/1+KC_i$**
100	0.175	8749.1	0.83
200	0.088	2793.5	0.91
300	0.064	1650.2	0.94
400	0.055	1519.4	0.94
500	0.044	1157.4	0.95
600	0.037	908.8	0.96

The value of E gives information about biosorption mechanism as chemical ion-exchange or physical adsorption. The value of E in this studies is -38.68 J/mole that corresponds to chemical ion-exchange mechanism. The Langmuir, Freundlich and D–R parameters for the biosorption of Pb(II) are listed in Table 3.

Separation factor (R_L), surface coverage (θ) and distribution coefficient (D)

The essential feature of Langmuir isotherm can be expressed by means of a separation factor (R) that is calculated using:

$$R_L = 1/ (1 + K_LC_i) \quad (11)$$

Where, C_i is the initial Pb (II) concentration (g/L). The R_L values between 0 and 1 indicate the favorable biosorption process (Nasir et al., 2007). The R_L value for this study at different initial concentrations ranged from 0.037 to 0.175 (Table 4) therefore, biosorption of Pb(II) was favorable even for higher initial metal concentrations.

Adsorption behavior of Pb(II) on *A. caespitosus* was also estimated by calculating surface coverage (θ) values at different initial metal ion concentrations using following equation:

$$\theta = K C_i / 1 + K C_i \quad (12)$$

Where, C_i and K are initial metal concentration and adsorption coefficient, respectively. The value of θ increased from 0.837 to 0.96 with an increase in C_i from 100 to 600 mg/L (Table 4). The same trend was also reported for Pb biosorption by modified distillation sludge of rose (Nasir et al., 2007) and pyrolysed *Pongamia pinnata* pods carbon (Nadeem et al., 2009). The adsorption distribution coefficient (D) that is the ratio of the metal ion concentration in the adsorbent phase, to the concentration in the aqueous phase [(mg metal / g biosorbent)/ (mg metal/ ml solution)] or (ml/ g biosorbent)] can be used to evaluate the biomass relativeness in sequestration of Pb (II) ion from aqueous solution. A high value of distribution coefficient is the characteristic of a good biosorbent. The D value for Pb(II) adsorption by *A. caespitosus* (8749.1 mg/ml) (Table 4) was almost comparable to uranium distribution coefficient value (10,000 ml/g) of *A. fumigatus* (Akhtar et al., 2007). Since many industrial separation processes utilized adsorbents with distribution coefficient as small as 10 ml/g adsorbent, therefore *A. caespitosus* appeared to be the potential microbial biosorbent for Pb(II) biosorption from aqueous streams.

Thermodynamic control

Temperature is an important parameter affecting the sorption capacity of biosorbent for a particular metal by enhancing the solubility and diffusion rate of adsorbate molecules from the solution. The adsorption characteristics of a biosorbent can be expressed by thermodynamic parameters $\Delta G°$, $\Delta H°$ and $\Delta S°$ that are highly helpful to understand the sorption mechanism. The value of $\Delta G°$ addresses the possibility and feasibility of a certain reaction, $\Delta H°$ shows the route of energy in the system, and $\Delta S°$ suggests increasing or decreasing randomness at the solid/solution interface in the system. In present studies, the biosorption data at C_i 100 ppm and temperature 298, 303, 313, 323°K were used to determine these parameters (Babarinde et al., 2008).

$$\Delta G° = RT \ln K_D \quad (13)$$

Where, $\Delta G°$ is standard free energy change, R is universal gas constant, T is temperature in Kelvin and K is the equilibrium constant and it is calculated from

$$K_D = C_e / C_i \quad (14)$$

The $\Delta G°$values decreased from -2.08 to - 8.92 KJ/mol with an increase in temperature from 298 to 323°K. The negative values of ΔG at all temperatures studied are due to the fact that biosorption process is spontaneous and these values also indicated the mechanism of physical adsorption (Horsfall and Spiff, 2005). The decrease in $\Delta G°$ value, with increase in temperature indicates the endothermic nature of the biosorption process and it was thereby favored with increase in temperature (Babarinde et al., 2008). The values of $\Delta S°$ and $\Delta H°$ calculated from the slope and intercept of the plot between $\Delta G°$ and T (R^2 = 0.981) were 26.2 J/mol-K and 75.37 KJ/mol by using the following equation (Horsfall and Spiff, 2005).

$$\Delta G° = \Delta H° - T\Delta S° \quad (15)$$

The positive $\Delta H°$ value showed an endothermic process. This information also suggests whether a certain biomass can be used for the removal of metal ions at elevated temperature or not. The positive value of $\Delta S°$ revealed increase in the disorderliness of the system and decreasing trend at high temperature causing a change in biomass structure during the sorption process.

FTIR studies

The nature of biosorbent - Pb(II) interactions was elucidated by recording the FTIR spectra of unloaded and metal loaded *A. caespitosus* in the range of 400 to 4000 cm^{-1} (Figure 7a, b). The broad band positioned around 3430 cm^{-1} was assigned to the stretching vibration of hydroxyl groups. The presence of multiple absorption bands at 3000 to 3400 cm^{-1} revealed the existence of –OH and –NH groups of the biomass. The absorption band at 2927 cm^{-1} is attributed to –CH stretching modes of –CH_2 and –CH groups. The absorbance at 1638 cm^{-1} is due to C = O of carboxylic acids. Absorption at about 1412 and 1032 cm^{-1} are indicative of the bending of CH_3 and C–N stretching vibrations respectively (Kiran and Kaushik, 2008). The bands centered on 1234 and 1080 cm^{-1} can be assigned to the –SO_3 and –CN stretching vibration. The FTIR spectra of biomass exposed to Pb(II) ions indicated no significant shifts or change in any of the characteristic absorbance bands at 3341, 2926, 1638, and 1406 cm^{-1} exception of a peak shift at 1071 to 1030 cm^{-1}. However, a new band at 1539 cm^{-1} appeared and the band at 1233 cm^{-1} disappeared after Pb(II) biosorption. These results imply the involvement of –SO_3 and –CN groups in biosorption of Pb(II) ions.

Pb belongs to Class B metal ions that show high affinity for III types of ligands but also form strong binding with II types of ligands (Wang and Chen, 2009). In our case, -S O_3 and –CN groups belong to II and III types of ligands respectively. The binding of Pb (II) with – SO_3 and –CN groups is due to the covalent nature (Wang and Chen, 2009). The interference of metal ions Mn, Cu, Ni and Zn on biosorption of Pb(II) by *A. caespitosus* was also elucidated by FTIR spectra (Figure 7c,d,e and f). This biomass also biosorbed co-cations from binary metal solutions that was evident from detailed examination of corresponding FTIR spectra. The spectrum of Pb and Mn loaded biomass from binary solution exhibits a band shifting from 3572 to 3643 cm^{-1} and 3267 to 3154 cm^{-1} due to the stretching of hydroxyl groups, carboxylic groups and double bonded carbon oxygen groups on the biomass surface. Two new bands appeared at 2403 and 2128 cm^{-1}. However, the absorption band at 1543 cm^{-1} that appeared in Pb loaded biomass disappeared in this spectrum. The FTIR spectrum of biomass after exposure to binary mixture of Pb + Zn, closely resemble that obtained from Pb + Mn loaded biomass with new bands at 2116 cm^{-1} instead of 2128 cm^{-1}.

These spectra also revealed almost the same effect in the region 1600 to 400 cm^{-1}.The comparison of spectra after exposure to binary solutions of Pb + Ni and Pb + Cu with unloaded biomass showed the appearance of two new peaks at 2854 and 2305 cm^{-1} and one new peak at 2185 cm^{-1} respectively as well as disappearance of the peak at wave numbers 3572 and 3267 cm^{-1}. In addition to these results, the observations of bands for hydroxyl, carboxylic acid groups and alkanes (symmetrical bending of CH_2) revealed that the vibrations associated with these bonds or groups were shifted in the presence of all the cations.

Scanning electron microscope (SEM) and energy dispersive X-ray analysis (EDAX)

To investigate the adsorption process further, the unloaded and Pb(II) loaded biomass were observed under the scanning electron microscope (SEM) and EDAX (energy dispersive X-ray analysis). SEM micrographs of powdered dead fungal biomass showed a smooth surface before adsorption (Figure 8a) and after Pb adsorption, the

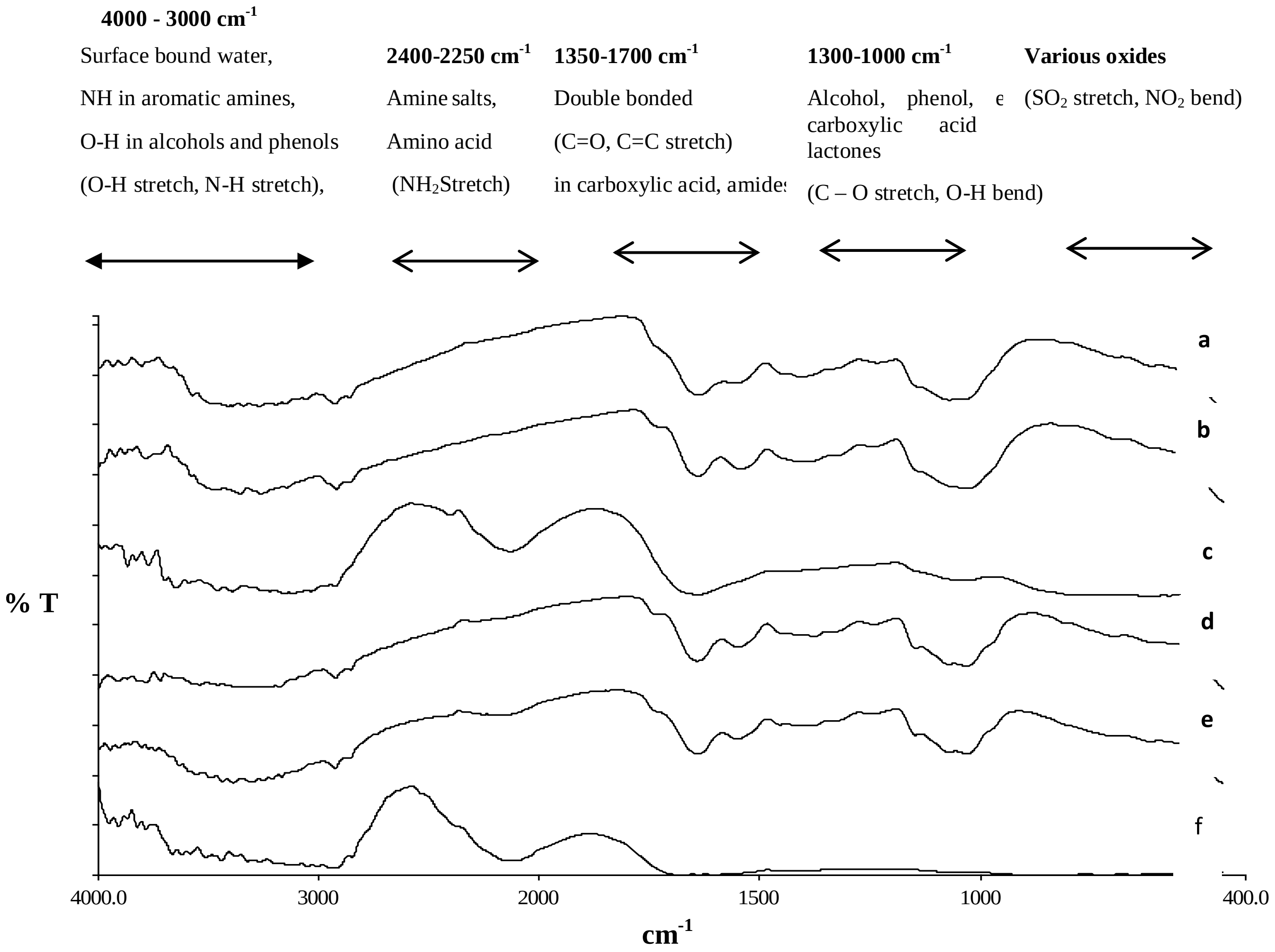

Figure 7. FTIR characterization of *A. caespitosus* biomass before (a) and after (b-f) Pb (II) biosorption from single metal solution (b), from binary metal solution in the presence of Mn (c), Cu (d), Ni (e) and Zn (f).

surface of the biomass appeared to be prominently rough as it was covered with many clusters of small Pb crystals (Figure 8b). Wang et al. (2010) showed the SEM images of biosrption of uranium (IV) by immobilized *A fumigatus* beads. Chakravarty et al. (2010) studied the biosorption of Pb(II) by native and Pb loaded bael leaves. SEM image of the powdered bael leaves showed a regular symmetry with hollow tubular structures before adsorption. After Pb adsorption, the tubes appear to be prominently swollen as Pb enters the fibers of the bael leaves.

Energy dispersive X-ray analysis (EDAX) studies of Pb biosorption by dried powdered *A. caespitosus* biomass after 24 h of contact time with 100 mg l^{-1} $Pb(NO_3)_2$ solutions at pH 4.5 are shown in Figure 9. The spectra revealed the presence of mass percent elements C (44.13), O (48.26), P (2.22), Cl (0.96) and K (4.44) with no Pb deposits in native biomass (Fig 9a) while after Pb exposure the spectra showed the major peaks of Pb with the 30.95 mass percent by replacing the peaks for K and Cl and decreased the mass percent of other elements as C (39.27), O (28.54), P (1.24).

These findings suggest that sorption, precipitation and ion exchange on the surface might be the major mechanisms for removal of Pb from waste water using *A. caespitosus*.

Case study: Removal of Pb from real industrial effluents

The feasibility and efficiency of a biosorption process depends not only on the properties of the biosorbents, but also on successful application of biosorbent for industrial effluents. Therefore, perusal of data obtained from aforementioned findings was used to conduct experiment with wastewater of the paint industry. The biomass of *A. caespitosus* removed almost 78% of the Pb(II) present in waste water (3.4 ppm) in a batch experiment using pulp density (0.5 g/L). The biosorbent exhibited good potential for Pb(II) removal from real effluent as was obtained with

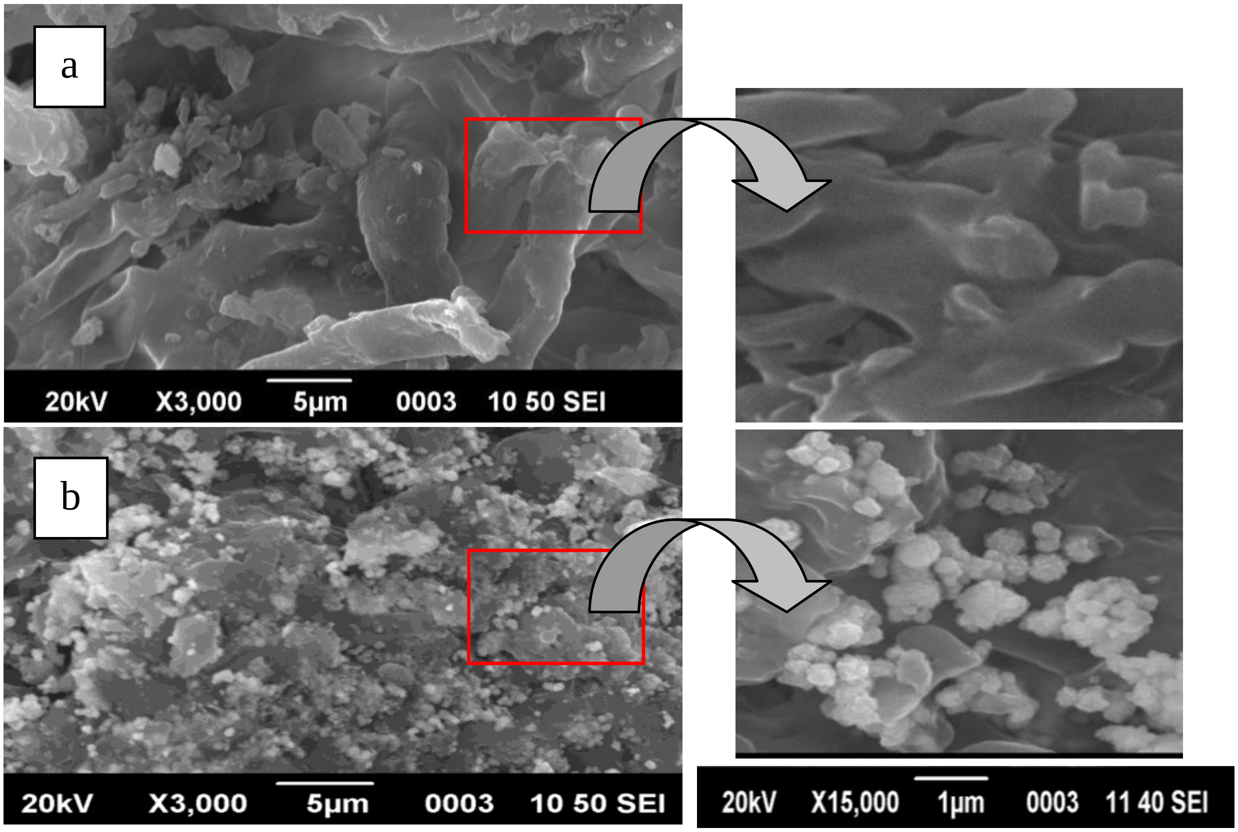

Figure 8.. Scanning electron micrographs of *A. caespitosus* biomass before (a) and after (b) 6 h of exposure to 100 mg/l of lead nitrate solution at pH 4.5

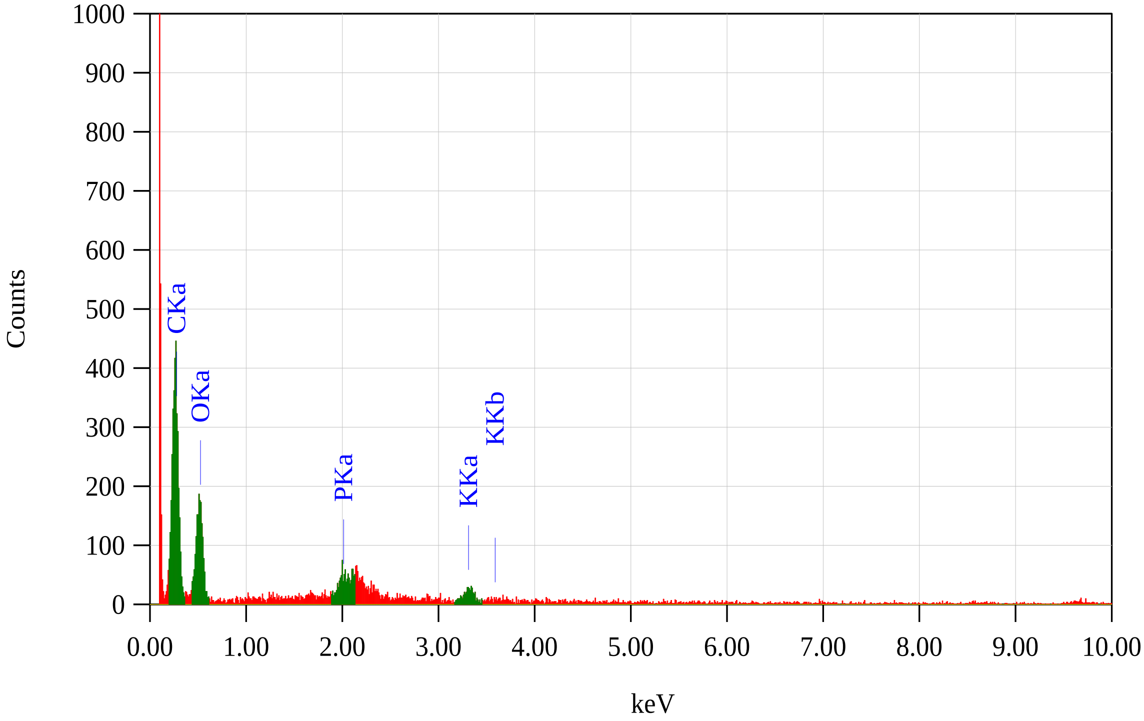

Figure 9. Energy dispersive X-ray (EDX) analysis of *A. caespitosus* biomass before (a) and after (b) Pb (II) biosorption.

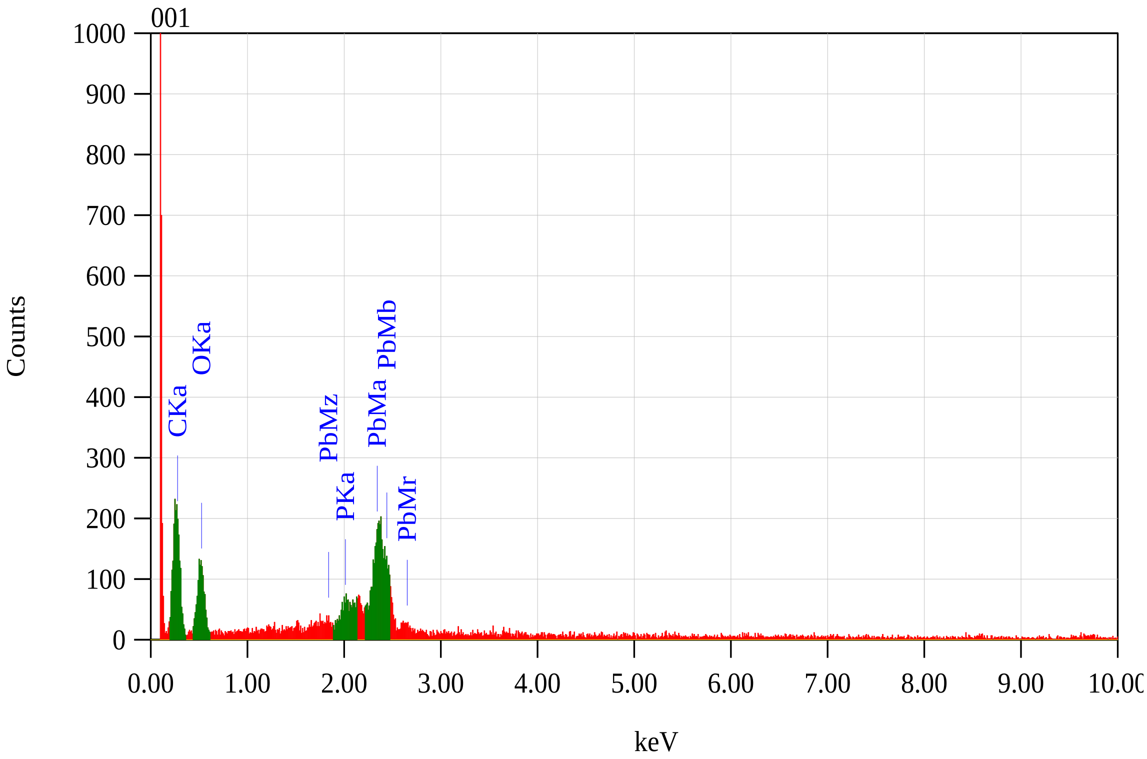

Figure 9. Continued.

synthetic solution.

Conclusions

These studies demonstrate the feasibility of removal of Pb(II) from aqueous stream by *A. caespitosus.* The results endorsed that the sorption performance was strongly affected by parameters such as pH, temperature, Pb concentration and sorbent dosage. The biosorption of Pb by *A. caespitosus* increased in the presence of Mn, Zn and Ni, while the presence of Cu decreased it significantly in binary mixture. The application of the kinetic models to experimental facts showed that the adsorption equilibrium data fitted very well to pseudo-second order kinetic model at all the temperatures. The equilibrium experimental data were found to provide closest fit to Langmuir model with R value of 0.99. The FTIR outcome implied the involvement of $-SO_3$ and –CN groups in biosorption of Pb(II) ions. SEM and EDAX analysis revealed the deposition of Pb in the form of small crystals on the surface of biosorbent. High sorption capacity value and effective elution studies suggested this biosorbent as potential source for removal and recovery of Pb from industrial effluents employing sorption–desorption cyclic studies.

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