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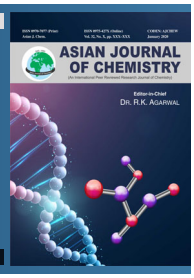

Enhanced Biosorption of Pb(II) Ions from Aqueous Solutions onto Citric Acid Treated *Aspergillus niger* Biomass: Equilibrium and Kinetic Studies

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In the present study, initially *Aspergillus niger* was tested for biosorption of Pb(II) ions and then studied the effect of pretreatment for enhanced biosorption. It was found that the maximum biosorption potential was achieved with citric acid treatment (70.56 %) in comparison with the biomass without treatment (65.46 %) at a biosorbent dose of 20 mg/L, pH 4, 100 rpm, 37 ºC for 8 h. The optimized conditions for treated *Aspergillus niger* were determined by optimizing the biosorption parameters such as pH, temperature, biomass dose, incubation time and agitation speed. This study indicates that the citric acid treated *Aspergillus niger* is an effective biosorbent for removal of lead (II) at optimized conditions with the maximum biosorption potential of 83.6 % as compared to previous reported work. SEM-EDX and FTIR analysis showed the structural variations and the functional groups involved in lead biosorption, respectively. Biosorption kinetics showed that pseudo second order kinetic model as the better fit.

Keywords: *Aspergillus niger*, Biosorption, Citric acid, Incubation, Kinetics, Lead.

INTRODUCTION

The growing industrialization and modern agricultural practices leave persistent toxic heavy metals like chromium, nickel, lead, zinc, cadmium and copper, which tend to accumulate and deteriorate the water bodies making it unfit for human consumption. The concentrations of these metals have to be bought down to permissible limits before discharging into the environment. Unlike the existence of several heavy metals, lead has been recognized as one of the most hazardous heavy metal. Its irregular inputs through several industrial activities results in high concentrations of lead in soils [1] as effluents causes a direct hazard to human and animals which draws the attention in removing from contaminated sites. Due to high operational conditions and risk of secondary pollutants the existing conventional methods are inefficient in removal of metal ions [2].

The technology of biosorption has became a cutting edge approach which involves the sorption of metal ions by a biosorbent that can reduces the cost of chemical usage and pollution compared to the other traditional removal processes [3]. During the last few decades, removal of toxic metals using algal, fungal and bacterial biomass has been extensively studied and introduced as an inexpensive, novel method with good biosorption capacities. Because of the high cell wall material percentage, fungi have chosen as the best biosorbent for the biosorption of heavy metals. Many studies have been focused on the application of fungal species for the removal of various metal ions from aqueous solutions [4,5].

In an attempt to increase the biosorption capacity, biomasses have been modified using various modifying agents [6]. Since fungal cell walls are made up of polysaccharides and proteins which contain many functional groups, treating of these fungal biosorbents with various chemicals can enhance the efficiency of biosorption [7]. Several modification protocols of fungal biomass have been reported [7,8]. However, these protocols involved several complex and time consuming steps like dialysis, multiple washing and re-suspension of biomass, which might even contribute to poor performance of biosorbents.

Hence in present study, a fungal biomass *Aspergillus niger*, which is having lead biosorption capacity was chosen for the removal of lead ions. In order to eliminate the lengthy steps to

enhance the biosorption potential, a four step approaches *i.e.* modification of biomass followed by drying, washing and finally drying was used for the biosorption of lead ions. Modification of biomass with maximum biosorption capacity for lead ions was carried out under optimized experimental conditions with *Aspergillus niger* biomass. Kinetic and isotherm studies, SEM-EDX and FTIR analysis was performed for the treated biomass.

EXPERIMENTAL

Microbial strain: Fungal strain *Aspergillus niger* NCIM 616 having lead biosorption activity [9] was procured from National Collection of Industrial Microorganisms (NCIM), NCL, Pune, India. The stock culture was maintained on potato dextrose agar (PDA) plates at 4 ºC and sub-cultured for every 4 weeks at regular intervals. Fresh slants were prepared for running experiments.

Preparation of metal solutions: The stock solution of lead(II) was prepared by dissolving accurate quantity of lead(II) nitrate (159.85 mg) in 100 mL of double distilled water to get a final concentration of 1000 µg/mL. From stock solution, working concentration of 300 µg/mL was prepared for biosorption experiments.

Inoculum preparation: To a 5 days old slant, 10 mL of sterilized 0.1 % Tween-80 solution was added and the culture was scraped with inoculating loop. From this required volume was pipetted into each flask containing basal medium.

Cultivation and preparation of biomass for biosorption: The basal medium required for preparation of fungal biomass contained (g/L): ammonium nitrate, 2.06; monopotassium phosphate, 0.55; $MgSO_4 \cdot 7H_2O$, 0.25; sucrose, 50 was made up with distilled water. The medium was swirled while hot and allowed to stand overnight. Then, pH of the medium was adjusted to 5.0 either by using NaOH or HCl and distributed into 250 mL conical flasks and autoclaved. Spore inoculum of 10 mL was added into each flask and incubated in an orbital shaker operating at 100 rpm for 4 days at 28 ºC. After incubation, the harvested cells were washed with deionized distilled water until the pH of the wash solution was in the near neutral range. Then, biomass was powdered by drying at 60 ºC for 24 h and used for biosorption experiments.

Biosorbent treatment procedures: Sodium persulfate treatment was carried out by treating 1 g of powdered biosorbent with 0.2M sodium persulfate and mixture was gently mixed for 30 min. Citric acid treatment was carried out by treating 1 g of powdered biosorbent with 0.1M citric acid and mixture was gently mixed for 30 min. DMSO treatment was carried out by treating 1 g of powdered biosorbent with 1:1 ratio of DMSO and water and mixture was gently mixed for 30 min. At the end of each procedure the treated biomass was separated and washed twice with deionized water and dried at 60 ºC for 24 h.

Biosorption experiments: The biosorption experiments for the control and treated biomass were carried out in 250 mL Erlenmeyer flasks with 2 mg of biosorbent with a Pb(II) ion concentration of 30 mg suspended in 100 ml of distilled water, and the pH was adjusted to 4. The flasks were agitated on an orbital shaker with 100 rpm for 8 h at 37 ºC. After incubation the solutions were centrifuged (4000 rpm, 3 min) and the supernatants were assessed.

In order to attain the maximum biosorption potential a series of biosorption experiments were carried for the treated biomass which has shown highest biosorption potential by optimizing the experimental parameters such as pH, incubation time, biomass concentration, temperature and agitation. Residual Pb(II) concentration in the supernatants was assayed by using inductive coupled plasma optical emission spectrometry (ICP-OES).

The equilibrium sorption capacity (q_e) of *Aspergillus niger* biomass was determined using the following mass balance equation:

$$q_e = \frac{(C_i - C_e)V}{m} \quad (1)$$

where q_e is the amount of biosorbed metal ions of biosorbent (mg g^{-1}), C_i is the initial concentration of metal ion in solution (mg L^{-1}), C_e is the equilibrium concentration of metal ion in solution (mg L^{-1}), V is the volume of medium (L), and m is the amount of biomass used in the biosorption process (g).

The biosorption potential (%) of biomass was determined by using the equation:

$$\% = \frac{C_i - C_e}{C_i} \times 100 \quad (2)$$

SEM-EDX and FTIR analysis: The structural and morphological changes of biomass as result of biosorption were evaluated by scanning electron microscope (SEM) (CARL ZEISS SUPRA 55 GEMIN-German Technology Jena, Germany). The biomass before and after biosorption was mounted on individual metal stubs and sputtered with gold (SC7620 'Mini' sputter coater). The elemental composition of the biomass was evaluated by energy dispersive X-ray analyzer equipped with SEM. The EDX spectrum of biomass was obtained at an accelerating voltage of 16 KeV.

The functional groups of biomass involved in biosorption of lead(II) ions were evaluated by Fourier transform infrared spectrometry (FTIR). For this, translucent discs were prepared by mixing the biomass with KBr in 1:100 ratio. The discs were analyzed immediately within the range of 4000-500 cm^{-1} with a resolution of 4 cm^{-1}.

Biosorption kinetics: To evaluate the mechanism of metal biosorption onto *Aspergillus niger* biomass, two kinetics models pseudo first-order and pseudo second order models were tested to fit the experimental data.

The linear form of pseudo first order model is expressed by following equation:

$$\log (q_e - q_t) = \log q_t - \frac{k_1 t}{2.303} \quad (3)$$

The linear form of pseudo second order model is expressed by following equation:

$$\frac{t}{q_t} = \frac{1}{k_2 \cdot q_e^2} + \frac{t}{q_e} \quad (4)$$

where q_e and q_t are the amount of metal ion biosorbed at equilibrium and at time 't' respectively, (mg/L), k_1 is the pseudo first order rate constant (per minute) and k_2 is the pseudo second order rate constant (per minute). The parameters of pseudo-first and second order kinetic models can be calculated from

the linear plots of log ($q_e - q_t$) *versus* t, and t/q_t *versus* t, respectively.

Statistical analysis: All the experiments were carried out in triplicates. The graphs were plotted by using Graph Pad Prism 5. Statistical analysis was done by using one way Anova and the values are presented as mean ± standard deviation.

RESULTS AND DISCUSSION

In the present study, *Aspergillus niger*, a fungal biomass was used for the biosorption of lead ions. Several studies evaluated the biosorption capacity of *Aspergillus niger* in removal of metal ions from contaminated water effluents [10,11]. The treatment of biomass with physical and chemical processes appeared to enhance the metal biosorption efficiency [12]. However scanty information is available regarding biosorption potential of treated *Aspergillus niger* biomass.

Biosorption studies: Based on the results obtained by ICP-OES, the percentage biosorption capacity of *Aspergillus niger* biomass was 65.4 % with an initial Pb(II) ion concentration of 0.3 g /L which was in accordance with the other studies, where the maximum Pb(II) biosorption capacity of *A. niger* biomass has been found to be 60.7 % under the same experimental conditions. Some studies reported that *Saccharomyces cerevisiae* and *Pencillium* sp. achieved a removal percentage of about 86.4 and 52.09 % [1,13].

Aspergillus niger biomass was treated with different reagents. Highest percentage of biosorption was achieved with citric acid (70.5 %) compared with ammonium persulfate (24.6 %) and DMSO (68.6 %) treatments. Studies suggests that an increase in biosorption after treatment could be due to the modifications of the cell wall components or exposure of the active metal binding sites embedded in the cell wall or by removing the surface impurities and exposing the available binding sites for metal biosorption. The increase in biosorption with the citric acid treated biomass may be attributed due to presence of carboxyl groups within the chemical structure of citric acid, which contributed for the creation of additional adsorption sites for metal ions [14]. The changes in results after specific treatment have a significant impact on biosorption capacity.

Optimization of biosorption process: The biosorption of citric acid treated biomass was further optimized by varying the experimental conditions.

Effect of pH: The influence of pH was measured in the pH range of 4.5-7.5. Fig. 1 shows that biosorption was low at pH 4.5, but the metal was well biosorbed in the pH range of 5.0-7.5. Maximum biosorption was achieved at 76.8 % was observed at pH 7. It states that optimal pH for biosorption of heavy metals by fungal biomass is in between 5-5.2 [11]. An increase in pH, results in increase of negative charge density on the surface of the cells, which leads to deprotonating of the metal binding sites and thus increases the biosorption [6]. The low biosorption at pH 4 may be due to competition of metal ions with hydronium ions in the biosorption mechanism. Such an effect of pH on biosorption of Cu(II) and Pb(II) had also been observed for *A. niger* biomass and *A. flavus* [15].

Effect of temperature: Fig. 2 shows the effect of different temperatures ranging from 25-55 ºC on the biosorption of Pb(II) ions. There was passive biosorption of Pb(II) ions onto biomass

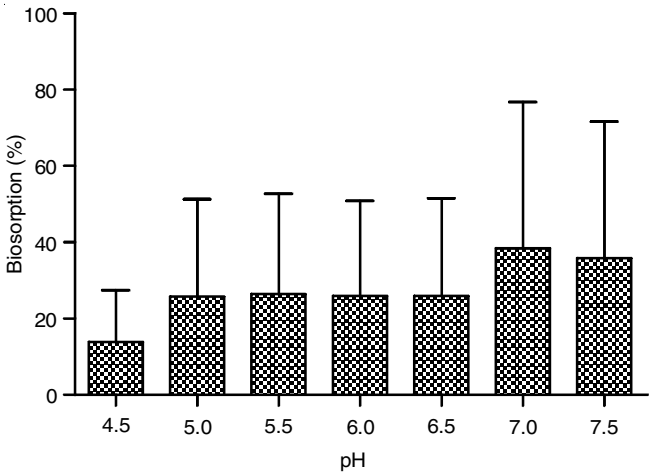

Fig. 1. Effect of pH on biosorption of Pb(II) by citric acid treated *A. niger* biomass

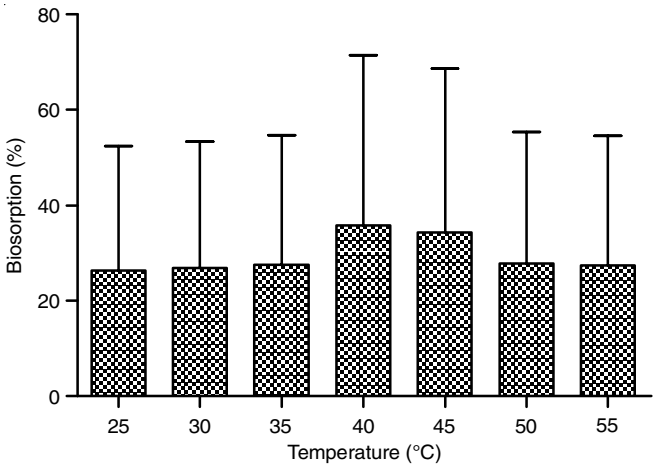

Fig. 2. Effect of temperature on biosorption of Pb(II) by citric acid treated *A. niger* biomass

at low temperatures. Maximum biosorption was obtained at 40 ºC. A decrease in biosorption was observed at temperatures higher or lower than the obtained optimum temperature. Temperature is known to effect the configuration and stability of the cell wall, and also causes the ionization of chemical moieties. These factors ultimately affect the binding sites of the biomass causing reduction in biosorption [15]. Usually optimal temperatures (35-45 ºC) enhance the biosorption due to increased kinetic energy and surface activity of the solute. High temperatures can cause physical damage to the biosorbent thus reducing the biosorption capacity of biomass [11]. Other studies also reported that maximum absorption of Cr (86.49 %) by *Trichoderma* sp. was observed at 35 ºC [16].

Effect of contact time: Fig. 3 showed that the rate of biosorption is rapid with the biosorption capacity of 74 % within 8 h of incubation. It was also observed that in 9 h the biosorption capacity reached 78 %. After this, amount of metal biosorbed did not change with contact time (saturated) and hence it is assumed a shaking time of 9 h would be the optimum temperature. This trend is probably due to the availability of the binding sites located on the surface of the cells in the first stage, and with gradual occupancy of these sites, rate of biosorption becomes less efficient and attains saturation at the final stage [17]. Similar results were obtained with the biosorption

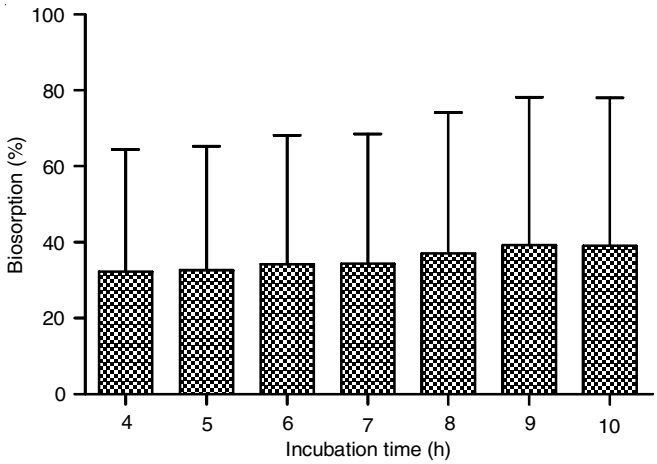

Fig. 3. Effect of incubation time on biosorption of Pb(II) by citric acid treated *A. niger* biomass

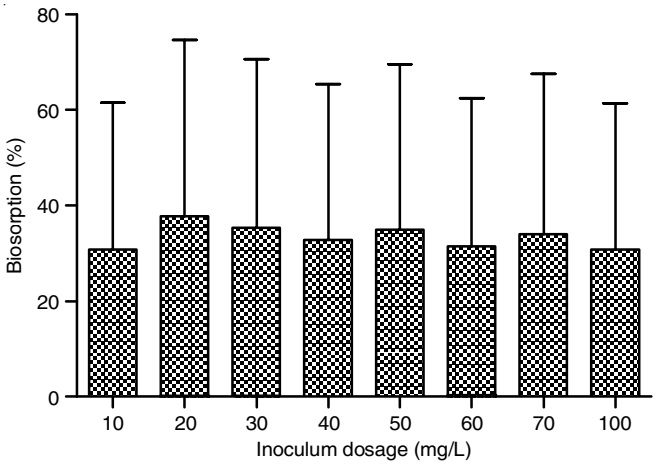

Fig. 4. Effect of inoculum dose on biosorption of Pb(II) by citric acid treated *A. niger* biomass

of Zn(II), Cd(II) and Co(II) by *A. niger* [17], while in some other studies biosorption attained maximum in first 15 min of incubation time [18].

Effect of biomass dosage: Biosorption of Pb(II) ions onto treated *A. niger* was studied using different biomass dosages of 10, 20, 30, 40, 50, 60, 70 and 100 mg/L with 0.3 g/L initial metal ion concentration and at pH 4. From Fig. 4, there was an increase in biosorption capacity with increase in biomass dosage. The maximum biosorption of 74.7 % for Pb(II) ions was achieved at 20 mg/L. A decrease in biosorption capacity from 30-100 mg/L was observed due to cell aggregation causing a decrease in availability of binding sites. Also this reduction may be attributing to a shortage of metal ions at higher concentration and to an excess at low concentration, where all the binding sites are saturated with the metal ion biosorption [18]. Thus at a given metal concentration, increasing of biomass dose will not enhance the metal/biosorbent ratio [18]. In a similar study for lead(II) biosorption by *Aspergillus niger*, 2 g/L concentration of biomass was employed for maximum biosorption of lead ions [19].

Effect of agitation speed: A maximum biosorption capacity of 75.5 % was obtained with agitation speed of 60 rpm (Fig. 5). An increase in biosorption capacity was observed up to 60 rpm and thereafter there is no proportionate increase. Lower biosorption capacity at lower agitation speed (20 rpm) might be due to agglomeration of biomass particles. Increase in agitation speed up to 60 rpm might have facilitated complete contact between the Pb(II) ions and biomass binding sites and hence promoted the effective transfer resulted in maximum biosorption. The observed reduction in biosorption beyond 60 rpm might be due to the increased turbulence promoted desorption of biosorbates into the solution resulting in increased residual concentration of metal ions. In similar studies with lead(II)

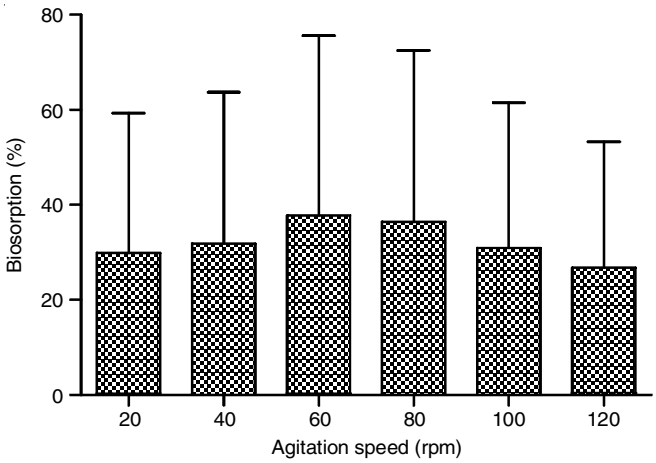

Fig. 5. Effect of agitation speed on biosorption of Pb(II) by citric acid treated *A. niger* biomass

biosorption, maximum biosorption was attained at 200 rpm per gram of biosorbent used [19].

Effect of pretreatment on biosorption: Biosorption experiments were carried out at optimized conditions of 0.3 g/L of metal solution contacted with biomass at dosage of 20 mg/L at pH 7 agitated with a speed of 60 rpm at 40 ºC for 9 h. Citric acid treated biomass improved the biosorption of Pb(II) ions to 83.6 %. Untreated biomass had biosorption capacity of 65.4 %. The results indicate that at the optimum experimental conditions and pretreatment influences the biosorption of lead(II) ions. Pretreatment can improve the heavy metal biosorption due to the fact that it may eliminate the lipids and proteins which mask the binding sites, liberate certain fungal cell wall polymers like chitin, and thus enhance the affinity for metal ions. Table-1 shows the increase in biosorption efficiency of different bio-

TABLE-1
COMPARISON OF BIOSORPTION EFFICIENCY OF DIFFERENT BIOSORBENTS AFTER PRETREATMENT WITH DIFFERENT CHEMICALS

Biosorbent	Modifying agents	Metal biosorbed after pretreament (%)	Ref.
Mucor rouxii	0.5 N NaOH	66	[25]
Aspergillus niger	0.5 N NaOH	75-80	[3]
Aspergillus niger	Malic acid and EDTA	41.23 and 39.83	[26]
Penicillium lanosa-coeruleum	NaOH	27	[27]
Aspergillus niger	Citric acid	83.6	Present study

sorbents after treatment with different chemicals. From the data, it can be concluded that biomass treated with citric acid showed maximum biosorption efficiency when compared with other chemically pretreated biosorbents. Hence citric acid treated *Aspergillus niger* can be used an alternative biosorbent for biosorption of Pb(II) ions with better efficiency.

SEM-EDX analysis: SEM images of *Aspergillus niger* biomass before and after biosorption of Pb(II) ions can be evaluated from Figs. 6a and 7a. Interaction of biomass with Pb(II) ions resulted in formation of flake like deposits on its surface. Control biomass had smooth hyphae like structure where it changed to rough texture upon deposition of Pb(II) ions. The results are in accordance with other studies who visualized the conformational changes of *Aspergillus flavus* biomass after lead biosorption [20].

EDX analysis confirmed the biosorption of Pb(II) ions (Fig. 7b). Characteristic peak for Pb(II) appear distinctly in the spectra and confirmed that the biomass has well fixed the metal ions while no such peak was observed on the surface of untreated (Fig. 6b) biomass. Several studies reported the biosorption of metals by SEM-EDX techniques to highlight of presence of ions on different biomasses [21].

FTIR analysis: Determination of characteristic functional groups responsible for biosorption of Pb(II) ions before and after treatment with citric acid was done by FTIR analysis. For comparative study the spectra of untreated and biomass treated with citric acid were initially examined. In both the spectra (Fig. 8a-b), a band 3550-3200 cm^{-1} corresponds to the stretching of hydroxyl groups (-OH) associated with N-H bond of amino groups. Two peaks at 2925 and 2373 cm^{-1} are attributed to -CH asymmetric and symmetric vibrations of methylene hydrogen. The peak at 1644 and 1647cm^{-1} was attributed to the aromatic C=C, C=O and conjugated ketones or C=N amide stretching. The peak at 1560 cm^{-1} corresponds to amide II band. The peaks at 1371 and 1349 cm^{-1} were due to stretching vibration of -C-O bond. The peaks at 1072 and 1039 cm^{-1} corresponds to C-O stretching of alcohol or polysaccharide like substance. The peaks at 620, 627, 495 and 484 cm^{-1} indicate the presence of nitro compound and disulfide groups. Additional peak at 1150 cm^{-1} in the treated biomass corresponds to -S=O stretching. The peak at 799 cm^{-1} which is present in untreated spectra was not observed in the treated spectra. From the above comparison, it revealed that after citric acid treatment there was increase or decrease in the number of peaks with the peaks appearing sharper.

By comparing the spectra of untreated and treated biomass of *A. niger* with Pb(II) ions (Fig. 8c-d), a minute shift in the peak positions was observed. It can be seen that the stretching vibration of -OH group shifted from 3423 cm^{-1} to 3437 cm^{-1} revealed the chemical interaction between the lead ions and hydroxyl groups occurred on biomass surface. The minute shift from 2924 cm^{-1} to 2923 cm^{-1} indicated the involvement of -CH bond in biosorption. The peaks at 1349 cm^{-1} and 1039 cm^{-1} shifted to 1369 cm^{-1} and 1030 cm^{-1} following Pb(II) treatment. The bands at 1647, 1560, and 484 cm^{-1} did not alter. The bands at 2373, 1150, and 627 cm^{-1} did not appear in the lead(II) treated biomass spectra. Disappearance of bands at 2373, 1560, and 620 cm^{-1} in the spectra of lead biosorbed untreated biomass indicate that they were involved in the biosorption of lead ions. From the analysis, it can be seen that lead biosorption by *A. niger* involves the interaction of lead ions with hydroxyl, amino and carbonyl groups of the fungal biomass surface by clear

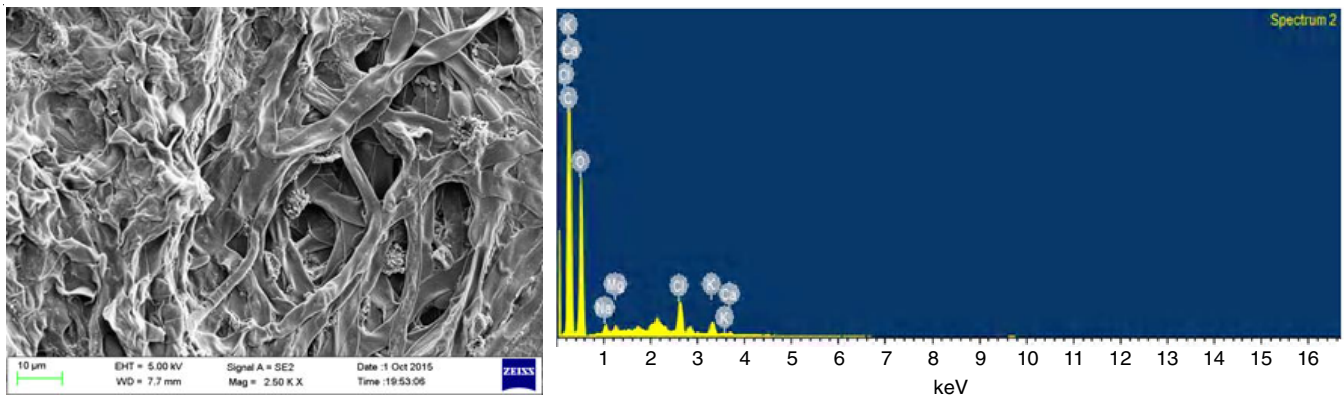

Fig. 6. (a) SEM image and (b) EDX spectra of untreated (blank) biomass

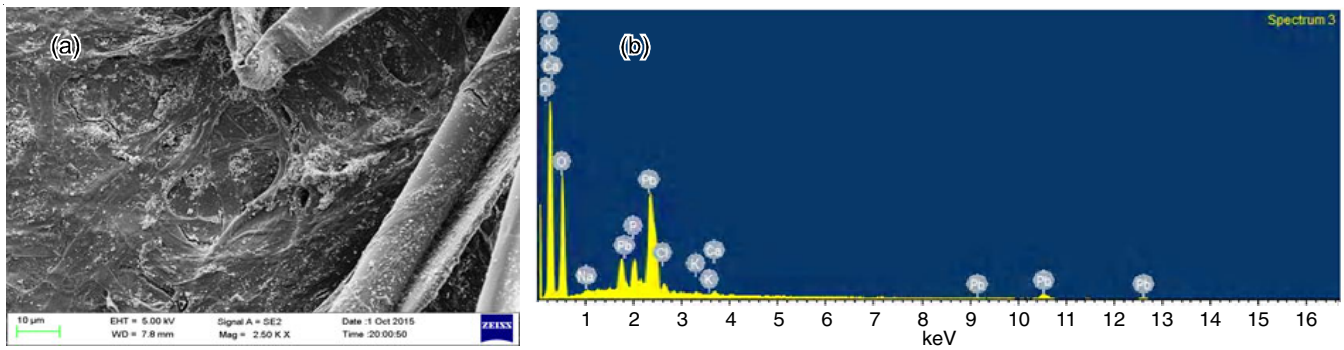

Fig. 7. (a) SEM image and (b) EDX spectra of biomass treated with Pb(II) ions

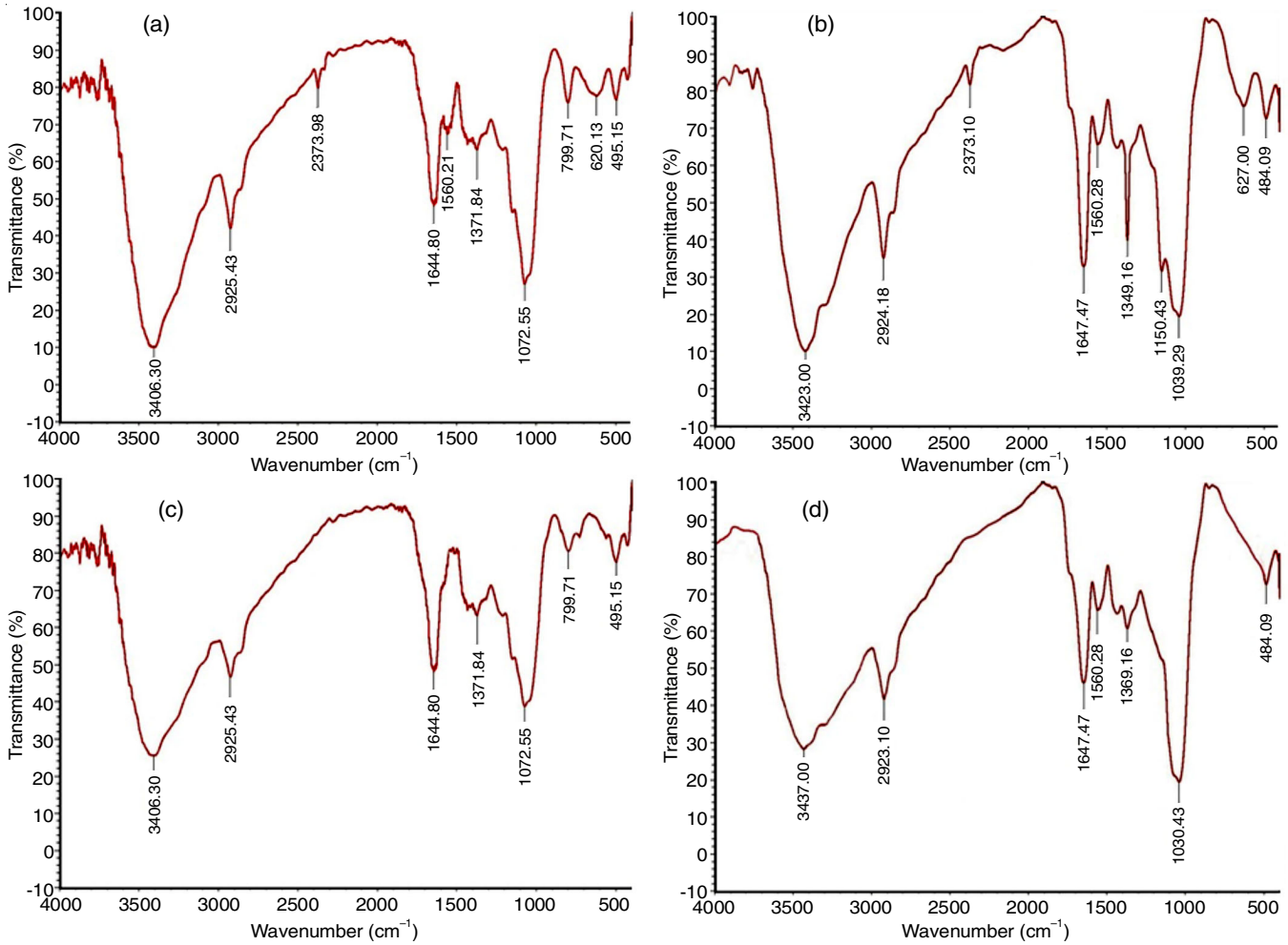

Fig. 8. FTIR spectrum of (a) untreated *A. niger* biomass; (b) citric acid treated *A. niger* biomass; (c) untreated *A. niger* biomass biosorbed with Pb(II) ions; (d) citric acid treated *A. niger* biomass biosorbed with Pb(II) ions

TABLE-2
CONSTANTS OF PSEUDO FIRST AND SECOND ORDER KINETIC MODELS FOR THE BIOSORPTION OF Pb(II) ON TREATED BIOMASS OF *A. niger*

Pseudo first order kinetic model			Pseudo second order kinetic model		
Q_1 (mg/g)	K_1 (min^{-1})	R^2	Q_2 (mg/g)	K_2 (gmg^{-1} min^{-1})	R^2
0.673	0.525	0.7806	2.424	0.029	0.9254

shift in the peaks characteristic to these groups were registered. These results are in line with those reported for the biosorption of Cu(II) onto *Aspergillus oryzae* fungal biomass [22] and Cr(VI) from aqueous solutions by *Aspergillus niger* biomass [23].

Biosorption kinetics: The constants of two kinetic models were determined experimentally by non-linear regression. From Table-2, the R^2 (correlation coefficient) value was high for the pseudo second kinetic model (0.925), which indicates that the experimental data was fitted well with the second order kinetic model and meant to be chemisorption process. Previous studies also showed that biosorption of Cr(VI) and Zn(II) by *Aspergillus niger* biomass showed the better fit with the pseudo second order kinetic model [24].

Conclusion

The biosorption capacity of *Aspergillus niger* biomass was enhanced from 65.4 to 83.6 % by chemical treatment with citric acid at the optimum conditions of 0.3 g/L of metal solution contacted with biomass at dosage of 20 mg/L at pH 7 and agitated with a speed of 60 rpm at 40 ºC for 9 h. SEM-EDX analysis showed the morphological changes as a result of pretreatment and biosorption. FTIR analysis revealed the functional groups which were involved in biosorption of lead ions. Pseudo second order kinetic model was found to be better fit. The results indicate that citric acid treated biomass of *Aspergillus niger* can be used as an effective biosorbent for the removal of lead(II) ions from the aqueous solutions.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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