J. Ecophysiol. Occup. Hlth. 3 & 4 (2013) 21-28

Biosorption of Lead by *Rhizopus stolonifer* Biomass: Role of Functional Groups

***Shahana Khan, Ansari Sarah Mohammed Umer and Wahida Rehman**

Department of Botany, Institute of Science, 15, Madame Cama Road, Mumbai-400032

Abstract : *Biosorption is the ability of biological materials to absorb/ adsorb heavy metals from waste water. The major advantages of biosorption over the conventional treatment method includes low cost, high efficiency, minimization of chemicals in sludge, no additional nutrient requirement, regeneration of biosorption and possibility of metal recovery. With regard to this, the objective of the study was to examine the possible role of the functional groups on biomass of Rhizopus stolonifer for removal of lead from water. The role of amines and carboxylic groups in the lead adsorption was studied. Result indicates that carboxylic groups are more efficient in removal of lead as compared to amino groups as indicated by percent reduction in the sorption efficiency of biomass ,after modification of functional groups. Sorption efficiency of the said functional groups was confirmed further by observation of biomass after sorption studies under an inverted microscope.*

Keywords: Lead (Pb) (II), Biosorption, Functional Groups, Rhizopus stolonifer.

Introduction

Many industrial processes produces large amount of waste water which may contain heavy metal ions that have toxic detrimental effects on human life and environment. Among others, lead is a major pollutant in both terrestrial and aquatic ecosystems, due to its importance in industrial use. Because of its high toxicity, disposal of lead is of special concern. Thus, development of new technologies is required to detect and eliminate the toxic contaminants from the waste waters as an alternative to traditional physiochemical processes. Biosorption, the process of passive cat ion binding by dead or living biomass, represents a potentially cost effective way of eliminating toxic heavy metals from industrial waste waters.

While the abilities of biological materials or microorganism to remove metal ions in solutions have been extensively studied, fungi, algae, bacteria, and yeast have proved to be potential metal biosorbents. Fungi have been recognized as promising class of low-cost adsorbent for removal of heavy metal ions from aqueous waste streams (Awofolu *et al.,* 2006 and Bai and Abraham, 2002). Fungi are able to remove heavy metals from aqueous solutions in rather substantial quantities. In certain instances removal of heavy metal ions by fungal biomass has been observed to be more than that by conventional adsorbants. Fungal biosorption has been studied more extensively because of the availability of large amount of waste fungal biomass from fermentation industries and the amenability of the microorganism to genetic and morphological manipulation. Dead biomass can also be subjected to physical and chemical treatment to enhance its performance.

Strong biosorbent behavior of certain microorganism towards metallic ions is a function of the chemical makeup of the fungal cells. The mechanism of biosorption is complex, it mainly involves ion exchange, chelations, adsorption by physical forces, entrapment in inter and intrafibrilliar capillaries and spaces of the structural polysaccharide network as a result of the concentration gradient and diffusion through cell walls and membranes. The

*Email : greatzaf@yahoo.co.in

sequestering of metallic species by fungal biomass has mainly been traced to the cell wall; however, the cell wall is not necessarily the only site where the sequestered metals are located. Biosorption of heavy metals on the fungi occurs as a result of physico-chemical interactions, ionic interactions, complex formation between the metal ions and the functional groups present on the fungal cell wall. Adsorption depends on its chemical structure and hydrophilic or hydrophobic characters of the cell wall. Numerous chemical groups have been suggested to contribute to biosorption by binding the metal ions. these groups comprise acetamido groups of chitin, amino and phosphate groups in nucleic acid, sulphhdryl and carboxyl groups in proteins, carboxyl and sulphate in polysaccharides of marine algae that belong to the divisions phaeophyta, Rhodophyta, and chlorophyta, polysaccharides and proteins of the fungal cell walls that contain many functional groups such as carboxyl, hydroxyl, phosphate and amino groups that can bind with metal ions. (Awofolu *et al.,* 2006).

This study was to explore the biosorption mechanism by identifying the functional groups involved and investigating their role in Lead sorption and to elucidate the adsorption by employing Inverted microscopy.

Materials and Methods

Media and chemicals:

The media and chemicals were analytical grade, purchased from Hi media Laboratories, India.

Preparation of adsorbent

Rhizopus stolonifer was cultured in Czepack Dox broth at 27±2°C under static condition. And the Biomass was harvested after 7 Days. Harvested biomass was washed with sterile distilled water. And dried at 60°C in an oven for 24 hours. This biomass was powdered, and was used directly as a biosorbent in metal uptake experiments to determine the biosorbent capacity.

Estimation of lead by titration method:

- Preparation of stock solution (0.02 M):
  1896 mg of lead acetate was dissolved in 250ml distilled water (1896 mg/250 ml).
- Preparation of Burette Solution:
  250 cm^3 of 0.02 M EDTA was prepared by dissolving 1861 mg of EDTA in 250 ml distilled water.
- Preparation of Xylenol Orange Indicator:
  0.5% of xylenol Orange indicator was prepared by dissolving 500 mg/100 ml of distilled water.
- Titration Method:
  10 cm^3 of 0.02 M lead Acetate solution was pipette out in a 250 ml conical flask. 2-3 drops of xylenol Orange indicator were added to the solutions and then the hexamine powder was added pinch by pinch till the colour changed to reddish pink. Thus, the titration was carried out using 0.02 M EDTA solution, till the colour changed from reddish pink to yellowish.

Characterization of surface chemistry of the biosorbent :

Surface chemistry of the biosorbent was characterized by potentiometric titration and chemical modification of the functional groups on the biomass.

Potentiometric titration of the biosorbent:

Ion exchange properties of *Rhizopus stolonifer* with regard to H^+ and OH^- ions were studied by potentiometric titration. Protonation of H^+ and OH^- ions was carried by soaking 1g of biosorbent in 50ml of 0.1 mol/L HCl and agitating for 2 hours on a rotary shaker. The titration procedure was carried out slowly, by stepwise addition of the titrant (0.1 mol/L NaOH) to the biosorbent slurry. After each 0.3 ml addition of the titrant it was allowed to equilibrate until a stable reading was obtained. The potentiometric titration curve was obtained by plotting the volume of titrant against pH 4.

Chemical modification of the functional group:

Functional groups revealed by the potentiometric titrations, were modified by certain chemical treatment.

Methylation of amines:

Methylation of amines was carried out by treating 0.25 g of raw biosorbent with 5ml of formaldehyde and 10 ml of formic acid. The mixture was agitated on a rotary shaker for 5 hrs at 120 rpm.

Estrification of carboxylic acid:

Estrification of carboxylic acid was carried out by suspending 0.25 g of raw biosorbent in 16.25 ml of ethanol and 0.15 ml of concentrated hydrochloric acid. The mixture was shaken for 5 hrs at 120 rpm.

After all the treatments, the solutions were filtered using whatmann No. 42 filter paper by vaccume filtration and washed with distilled water to remove all the traces of chemicals. The biosorption was dried at 60°C for 8 hrs.

Biosorption studies:

• 1 gram of biosorbent was suspended in 10 ml of 0.02 M of lead acetate solution in 5 separate 250 ml Erlenmeyer flasks. Agitated at 120 rpm at 27±2 on a rotary shaker and the control was maintained without the organism. The biomass was harvested after every two hrs by filtration. The 2-3 drops of Xylenole Orange Indicator (0.5%) was added to the culture filtrate, followed by hexamine powder till the colour changed to reddish pink. It was then titrated against 0.02 M EDTA solution till the colour changed from reddish to yellowish. Removal efficiency of the metal ions was calculated by using the formula

$$10\ cm^3\ of\ 0.02\ M\ EDTA = \frac{207.2 \times 0.02 \times 10Y}{1000}$$

The % of sorption capacity of metal ions is the concentration of metal ions on the fungal biomass, calculated,

$$\frac{X - Y}{X} \times 100$$

Where as,

X=control reading of lead in (g)

Y = actual reading of lead in (g).

FT-IR spectroscopy:

The spectra of biomass of *Rhizopus stolonifer* were obtained using a Perkin Elmer spectrum-100 employing a DRS (Diffuse Reflectance Spectroscopy). The infrared spectra were obtained and averaged over 40 scans in transmission mode. The IR pattern obtained for the *R. stolonifer* biomass before sorption was compared with IR pattern of Lead-laden *R. stolonifer* biomass in order to determine which functional groups participated in the lead sorption.

Inverted microscopy:

The change in the surface structure of the substrate after biosorption was further elucidated with the help of inverted microscopy. The sorption efficiency of functional groups and the surface structure of biosorbent were analyzed under inverted microscope using OLYMPUS CX31, which offers clear bright field imaging with excellent flatness .The high intensity 6V 30W halogen lamps illumination assures the bright imaging and even color.

Results and Discussion

Characterization of the surface chemistry of the biosorbent:

A possible preliminary test, which may be used for identification of functional groups, is the surface titration of biomass. With this experimental procedure rough characterization of selected biomass can be obtained, mainly when ionic exchange is the prevalent mechanism in the removal of heavy metals from waste water. In order to gain a closer insight into the biomass surface properties, a suspension of *Rhizopus stolonifer* was potentiometrically titrated applying 0.1 N NaOH. (Fig. 1) The result

of potentiometric titration experiments permits the quantitative and semi-quantitative determination of the nature of acidic sites present on the cell wall of *R. Stolonifer*. Similar method had been used earlier by other workers as well. (Narvekar and Vaidya, 2009.)

The curve showed three inflexion points: Two at approximately pH 5.0 and pH 6.0 corresponding to the pKa values of acidic groups, and one at approximately pH 9.0 corresponding to alkaline groups. It may be inferred that the two acidic groups are carboxyl and phosphate. The inflexion point around pH 9.0 is comparable to the values reported by Parvathi *et al.* (2007) for saturated amines. (8.5-12.5). The results are shown in (Table 1).

The present study also revealed presence of these groups on the biomass of *R. stolonifer* indicating its potential to be used as biosorbent in the removal of lead.

Biosorption studies:

Biosorption by unmodified biomass:

Results of biosorption studies with unmodified biomass of *R. stolonifer* are depicted in the Table 2. There was steady increase in the adsorption of lead by the fungal biomass with increase in time. Adsorption percentage increased from 17.6% at 2 hrs to 88.66% at 8hrs.In earlier studies, it was reported that as time increases, more amount of lead gets adsorbed onto the surfaces of the adsorbent and surfaces area available decreases.

Chemical modification of biomass:

To elucidate the role of the functional groups in the sorption of lead, they were modified by various chemical treatments and the percent reduction in the efficiency of the lead sorption by the chemically modified biomass was studied (Table 3). There was 30.23% and 49.12% reduction in biosorption after methylation of amines and esterification of carboxylic groups respectively, thus amines and carboxylic groups on biomass play a role in sorption of lead, role of

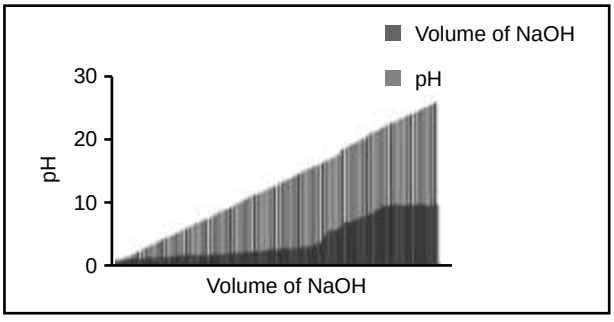

Fig. 1 Potentiometric titration curves.

Table 1 Functional groups present on the cell wall of *R. stolonifer.*

Pk_H value		Functional groups
Parvathi et.al 2007	Present study	
5.6	5.8	Carboxyl
6.63	6.8	Phosphate
9.51	9.4	Amine

Table 2 Percent removal of Lead by *R. stolonifer* biomass at different time interval.

Contact time(hrs)	% Removal
0	0%
2	17.6%
4	47.8%
6	68.7%
8	88.66%

Table 3 Percent reduction in biosorption efficiency of *R. stolonifer* biomass after chemical modification after eight hours.

Treatment to the cell wall	% Reduction in sorption efficiency
Methylation of amines	30.23%
Esterification of carboxylic acids	49.12%

carboxylic groups being greater than amines. The effects of chemically modifying the biosorbent are shown in the Table 4 and discussed in a step-wise manner in the following paragraph.

Methylation of amines:

The treatment of the biosorbent with formaldehyde-formic acid offers valuable methods for blocking primary and secondary amines by methylation. (Davis *et al.,* 1973).The reaction is as follows:

$$R - NH_2 + HCHO + HCOOH \rightarrow R - N(CH_3)_2 + CO_2 + H_2O$$

The different types of functional groups present on the surfaces of the cell wall are only responsible for binding of metal ions during the adsorption process. Amines and carboxyl groups react with lead during adsorption can be confirmed by blocking functional group from participating in the metal uptake process (Fein, *et al.,* 1997).The results of biosorption studies with modified amine groups of the biomass are depicted in Table 4.

There was steady increase in the adsorption of lead by the fungal biomass with increase in time. The adsorption percentage increased from 15.16% at 2 hrs to 58.43% at 8 hrs. However this was much lesser than adsorption percent (%) of unmodified biomass for the same period indicating participation of amine groups in the adsorption process. The positively charged lead ions get electrostatically attracted towards the amines of the biomass cell-wall.

The ammonium ions are derived from amines, however, normal amines are not charged and it can complex with lead through the lone-pair on the nitrogen atoms. The lone-pair is an area of negative charge and hence can attract the positively charged lead ions. Similar results for lead biosorption have been reported elsewhere and in another study methylation of amines of Ecklonia sp (Park, *et al*., 2005) and by *Aspergillus niger* (Narvekar and Vaidya, 2009) was found to have decreased the sorption of chromium.

Esterification of carboxylic acid:

Ethanol treatment of the biosorbent also reduced the lead removal by the biomass. It has been reported that the treatment of biosorbent with ethanol results in estrification of carboxylic acids present on the cell wall of the biosorbent and the reaction is as follows:

$$RCOOH + CH_3CH_2OH \rightarrow RCOOC_2H_5 + H_2O$$

The result of biosorption studies after esterification of carboxylic groups is depicted in the Table 4.There was a steady increase in the adsorption of lead by the fungal biomass with increase in time .the adsorption percentage increase from 14.60% at 2 hrs to 39.54% at 8 hrs. However, adsorption percent (%) was significantly less as compared to control at the corresponding time. Maximum reduction in removal efficiency being 49-12% indicating the significant role played by carboxylic groups in adsorption of lead. (Parvathi, *et al.,* 2007).

FT-IR spectroscopy:

IR spectra of biomass were obtained to reveal the functional groups present on the surface of *Rhizopus stolonifer* that would contribute to biosorption of lead. The infrared spectrum was highly complex, reflecting the nature of the examined biomass. Despite the complexity, certain characteristic peaks could be assigned. IR spectrum of the biomass is shown (Fig. 2).

The FTIR spectroscopic analysis of the biomass before lead adsorption indicated broad

Table 4 Lead removal efficiency of chemically modified biomass of *R.stolonifer.*

Contact time(t) hr	% Adsorption after eight hours.	
	Methylation of amine	Estrification of carboxylic acid
2	15.61%	14.60%
4	45.84%	21.91%
6	53.14%	32.24%
8	58.43%	39.54%

adsorption band at 3700-3000 cm^{-1}, representing -OH groups of the glucose and -NH stretching of protein and chitisan-chitosan strong absorption bands at 2927 cm^{-1} and 2853 cm^{-1} can be assigned to the -CH stretch. The similar result was reported (Loukidou, *et al.,* 2003). The absorption band at 1628.2 cm^{-1} could be attributed to -NH stretch bending, have two unsubstituted bands. That is amide I and amide II groups of protein peptide bonds of chitisan and chitosan. The band present at 1554.6 cm^{-1} indicates the presence of -NH amide, and the C=C stretch. In earlier studies, it was reported that the absorption band at 1639 cm^{-1} could be attributed C=O stretching conjugated to -NH deformation of amide I group of protein peptide bond of chitisan-chitosan and band present at 1546 cm^{-1} indicated the presence of amide and the result from -NH deformation mode conjugated to C=N deformation mode. (Kapoor and Viraraghvan, 1997). These two band attributed to the amide I and amide II bands of protein peptide bonds and thus confirm the presence of amide functional in the biomass. (Tsekova, *et al*., 2006). The bands present at 1410.5 cm^{-1} indicates that the presence of C=C amines stretch and the bands at 1327.2 cm^{-1} indicates the presence of C-N stretch and the1042.2 cm^{-1} and 1039 cm^{-1} is strong band assigned to -CN stretch.

The spectral analysis after modification of functional groups that is methylation of amine groups indicated that the strong adsorption bands at 2931.9 cm^{-1} and 2866 cm^{-1} can be assigned to -CH stretch and the band at 1714.8 cm^{-1} attributed to C=O stretch. In earlier studies, it was reported that at wave number 1739.7 cm^{-1} a shoulder is observed which may be due to carbonyl stretch of unionized caboxylates. (Kapoor and Viraraghvan, 1997). The adsorption band at 1621.9 cm^{-1} could be attributed to C=C alkene stretch and the moderately strong band present at 1039 cm^{-1} -CF alkyl halide stretch and C-O and -CN stretch.

Estrification of carboxylic groups indicated that the bands present at 2931.9 cm^{-1} and 2864.6 cm^{-1} can be assigned to -CH stretch strong absorption bands and the band at 1737.2 cm^{-1} assigned to C=O stretch and the band present at 1634.7 cm^{-1} and 1407.3 cm^{-1} assigned to C=C stretch. The strong band present at 1039 cm^{-1} could be attributed to -CF and C-O stretch and -CN stretch.

Inverted microscopy studies:

Morphological studies of biomass surface were carried out by inverted microscopy of the biomass.

The sorption efficiency of the functional groups of the biomass was further confirmed by inverted microscopic observation of biomass after sorption studies. Morphology was found to change soon after exposure to lead. More particular, morphology of biomass showed region of quite smooth surfaces but after exposure to lead the biomass had rough appearance and appeared thicker than unexposed biomass (Plate 1 and Plate 2a, b).

Similar results were seen on *P. chrysogenum* biomass and live culture of *Aspergillus niger*. after their exposure to arsenic and chromium respectively (Loukidou *et al.,* 2003; Srivastav and Thakur, 2006). The present investigation indicated the involvement of ion exchange and surface adsorption mechanism in removal of lead ions. Many researchers have reported biosorption of heavy metals by fungi resulting from ionic interaction and complex formation between metal ions and functional groups present on fungal cell surfaces. Thus, it can be concluded that the mechanism of lead sorption involves a complex process involving multilayered interactive or multiple sites type binding or some combination of these phenomena which are taking place simultaneously. This study revealed that *Rhizopus stolonifer* biomass can be utilized to remove heavy metal like lead from aqueous dilute solutions. As opposed to the conventional

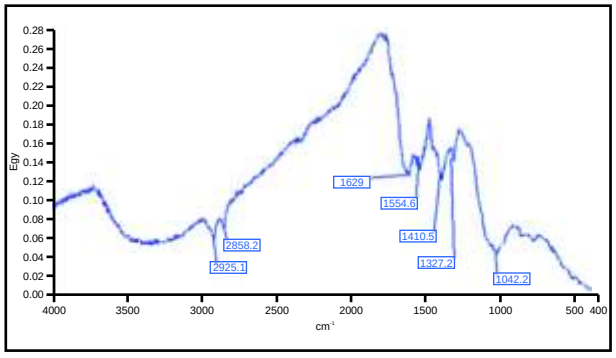

Fig. (a) Infrared spectra of raw biomass (After sorption).

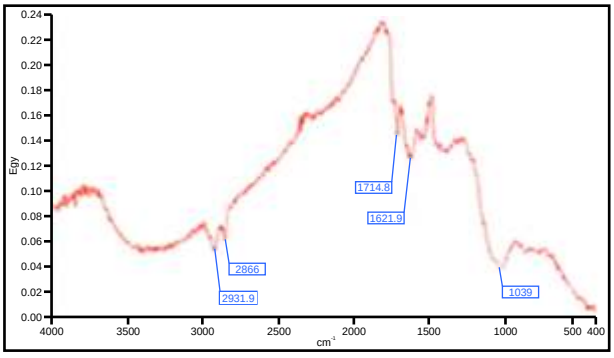

Fig. (b) Infrared spectra after methylation of amine groups.

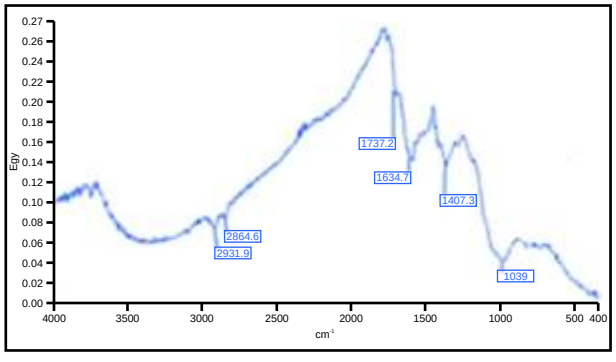

Fig. (c) Infrared spectra after Estrification of carboxylic groups.

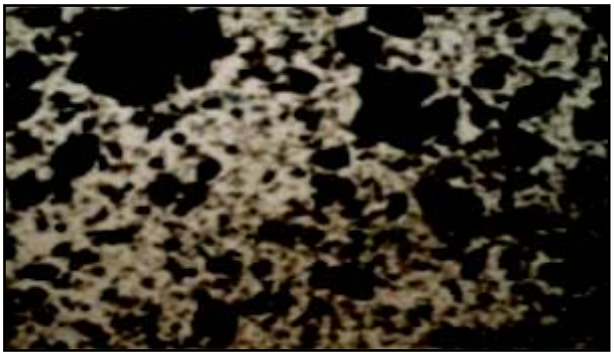

Plate 1 Biomass after adsorption of lead.

Plate 2 (a) Methylation of amine groups.

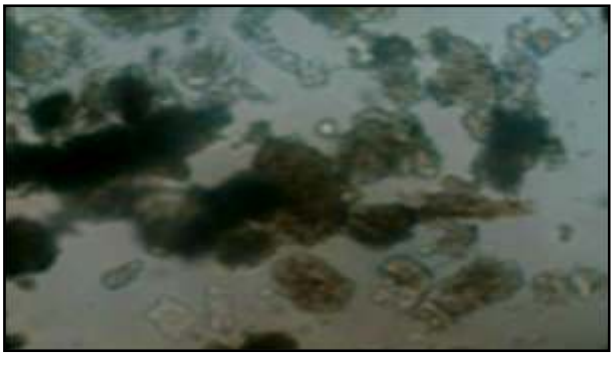

Plate 2 (b) Esterification of carboxylic groups.

techniques that lack specificity and are ineffective at low metal ions concentration. Pretreatment of biomass resulted in the reduction of biosorption of lead indicating active role played by the functional groups present on the biomass. As regard the cost of *R. stolonifer* biomass, it can be obtained free of charge or at low cost from fermentation industries, since it already presents disposal problems/cost to them. Large scale studies will help fine tune the biosorption studies for industrial applications.

Acknowledgements

The investigator gratefully acknowledges the help of Dr. M.A. Wahid, Principal, and Dr. Abrar Ahmed, Assistant Prof. Department of Botany, Maharashtra College, Mumbai for their help and support.

References

Awofolu, O.R., Okonkwo, J.O., Merwe, R.R., Badenhorst, J. and Jordan (2006) A new approach to chemical modification protocols of Aspergillus niger and sorption of lead ion by fungal species, *Electronic J. Biotech.,* **9**, 341-348.

Bai, R.S. and Abraham, T.E. (2002) Studies of enhancement of Cr(VI) biosorption by Chemically modified biomass of *Rhizopus nigricance*. *Water Res.,* **36**,1224-1236.

Davis, B.D., Dulbecco, H.N., Eisern, H.S. and Ginsberg, W.B. (1973) Wood Micrology, Harper and Row New York.

Fein, J.B., Daughney, C.J., Yee, N. and Davis, T.A.A., (1997) Chemical equilibrium model for metal adsorption onto bacterial surfaces. *Geochim, cosmochim. Acta,* **61**, 3319-3328.

Kapoor, A. and Viraraghvan, T. (1997) Heavy metal biosorption sites in Aspergillus niger. *Biores. Technol.,* **61**, 221-227.

Loukidou, M.X., Matisa, K.A., Zouboulisa, A.L. and Kyriakidoub, M.L. (2003) Removal of As (V) from wastewaters by chemically modified fungal biomass. *Water Res.,* **37**, 4544-4552.

Narvekar and Vaidya Varsha, K. (2009) Role of functional groups on *Aspergillus niger* biomass in the detoxification of hexavalent chromium. *J. Environ. Sci. Sci. Eng.,* **51**, 233-238.

Parvathi, K., Naendran, R. and Nareshkumar, R. (2007) Lead biosorption onto waste beer yeast by-product, a means to decontaminate effluent generated from battery manufacturing industry. *Electronic J. Biotech.,* **10**, 1-14.

Park, D., Yun, Y. and Park, J.M. (2005) Studies on hexavalent chromium biosorption by chemically treated biomass of Ecklonia sp. *Chemosphere,* **60**, 1356-1364.

Srivastava, S. and Thakur, I.S. (2006) Biosorption potency of *Aspergillus niger* for removal of chromium (VI). *Curr. Microbiol.,* **53**, 232-237.

Tsekova, K., Christova, D. and Lanis, M. (2006) Heavy matal biosorption sites in *Penicillium cyclopium*. *J. Appl. Sci. Environ. Management,* **10**, 117-121.