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Biosorption of Cadmium (II) using Discarded Biomass of *Aspergillus aculeatus* DBF9

Akhil Pandey • Debdulal Banerjee*

Microbiology Laboratory, Department of Botany, Vidyasagar University, Midnapore- 721 102, West Bengal, India

Corresponding author: * **debu33@yahoo.com; db@vidyasagar.ac.in**

ABSTRACT

The cadmium (Cd) biosorption potential of *Aspergillus aculeatus* DBF9 biomass was investigated. Among different forms of biomass, air-dried one was found most suitable in Cd removal. Maximum Cd (II) biosorption takes place at initial solution pH 4.5 after 90 min. Air-dried 150 mg of cell mass of *A. aculeatus* can remove about 78% of Cd (II) from 10 ml of 300 mg/L Cd (II) solution. The adsorption kinetics of Cd was modeled with a pseudo-second order equation to correlate the experimental data. The equilibrium data fitted very well to a Langmuir isotherm model more than to the Freundlich isotherm model. Metal accumulation was confirmed with FTIR, EDAX, and SEM analysis. This indicates that biosorption of Cd in *A. aculeatus* mainly occurs through ion exchange. Metal absorption properties of *A. aculeatus* can be used in Cd removal from industrial effluents.

Keywords: adsorption kinetics, *Aspergillus aculeatus*, biosorption, biosorption isotherms, cadmium, heavy metal, ion-exchange

INTRODUCTION

Environmental pollution as a result of rapid technological development is a serious concern for ecology. Heavy metal (HM) ion contamination represents a significant threat to the ecosystem (Hajiaghababaei *et al*. 2011).

Biodegradation of HMs is not possible because unlike organic pollutants metals as elements cannot be mineralized to non-toxic compounds such as H_2O and CO_2. However, biomobilization is a valid concept in the management of metal pollution (Ramanathan and Muthukkaruppan 2007). It is known that HMs can be extremely toxic as they damage nerves, the liver, bones and also block functional groups of vital enzymes (Sanyal *et al*. 2005). HMs are major pollutants in marine, ground, industrial and even treated wastewater. Stringent regulations have increased the demand for new technologies for metal removal from wastewater to obtain facts about today's toxicity- driven limits (Nuhoglu *et al*. 2002). The main sources of HM pollution include electroplating, painting, dying, surface treatment industry, etc. These pollutants are toxic and non biodegradable and probably cause adverse effect on health (Volesky and Holan 1995). Biosorption of HMs is one of the most promising technologies involved in removal of toxic metals from industrial waste fluid and natural waters (Pagnanelli *et al*. 2000).

Cadmium (Cd) is known for its high toxicity to almost all biota including humans and its high mobility in the terrestrial environment. In municipal solid waste compost Cd is encountered in a variety of forms including a substantial fraction that is associated with organic matter (He *et al*. 1992). Sources of Cd in waste water and environment are pigment and stabilizer for PVC, plastics, tyres, rechargeable cells, electroplating, coal, oil and phosphate rocks (Ramanathan and Muthukkaruppan 2007; Hajiaghababaei *et al*. 2011). Cd is a wide spread HM and is released into the environment by power stations, heating systems, metal working industries, waste incinerations, urban traffic, cement factories and as a by-product of phosphate fertilizers. Cd has been considered as an extremely significant pollutant affecting all live forms because of its high toxicity and grate solubility in soil and water. It has been demonstrated that the level of Cd in the soil appears to be increasing overtime. Cd accumulation in soil and water now poses a major environmental and human health problem. Studies on Cd toxicity in plants are well documented, Cd can induce low mitotic index and pycnosis, inhibit cell division and cell proliferation and has toxic effects on chromosome morphology including cytoplasm mitosis, anaphase bridges, chromosome stickiness (Liu and Kottke 2004). Cd has toxic effect on nucleoli in the root tip cells of *Allium cepa* L. with subcellular localization of Cd in the root cells (Liu *et al*. 2004).

Fungi are widely used in a variety of industrial fermentation processes which could serve as an economical and constant supply source of biomass to remove metal ions from wastewater. Fungi can also easily grow in substantial amounts using unsophisticated fermentation techniques and inexpensive growth media. Therefore, a fungal biomass could serve as an economical method for removal or recovery of HM ions from aqueous solutions (Kapoor *et al*. 1999; Luo *et al*. 2010). The literature reports that many types of fungi are capable of removing HMs during sewage treatment, such as white-rot fungus (Arica *et al*. 2001), filamentous fungus *Phanerochaete chrysosporium* (Say *et al*. 2001), *Aspergillus niger* (Kapoor *et al*. 1999; Srivastava and Thakur 2006; Mukhopadhyay *et al*. 2011), fungal biomass of *Mucor racemosus* (Liu *et al*. 2007) HM-resistant fungi (Congeevaram *et al*. 2007), by-products of brown-rot fungus *Lentinus edodes* (Chen *et al*. 2008), the endophytic fungus, *Microsphaeropsis* sp. (Xiao *et al*. 2010) and the industrial fungus *Rhizopus cohnii* (Luo *et al*. 2010).

Cd was selected in this study because it is a toxic metal commonly present in wastewater effluent from a variety of industries, especially electroplating and paint industries. The present work was undertaken to study the effectiveness of discarded biomass of *Aspergillus aculeatus* DBF9 as a biosorbant substrate of Cd.

MATERIALS AND METHODS

Microorganism and growth conditions

A. aculeatus DBF9, a potent tannase and amylase producer was previously isolated from forest soil, was used in present study

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(Banerjee *et al*. 2001, 2007). A pure culture of *A. aculeatus* was routinely maintained through sub-culture on PDA slants. Spores from PDA slants were collected and cultured in a 250-ml Erlenmeyer flask with 50 ml of sterilized modified Czapex-Dox medium composed of (g/L) 30, sucrose; 10, soluble starch; 3.0, $NaNO_3$; 1.0, K_2HPO_4; 0.5, $MgSO_4$; 0.00001, KCl; pH 5.0; de-ionized water as solvent at 28°C for 24 h on a rotary shaker (200 rpm).

200 µl of germinating spores (6×10^6/ml) were transferred to a 250-ml Erlenmeyer flask containing 50 ml of previously mentioned sterilized media and incubated for 72 h.

Harvesting and preparation of mycelia

Cells were harvested using zero mess sieves and then washed three times with de-ionized water. One part of cells were dried in a hot air oven at 60°C for 12 h while another part was dried in air. Some mycelia were killed by autoclaving at 15 lb pressure for 15 min, and these were used as dead cells. From a part of the mycelia, water was soaked by blotting paper and was used as living cells. The remaining parts were lyophilized (Eyela FD-5N) for 75 min. All types of treated cells were used as HM accumulators or absorbents (Ilhan *et al*. 2004; Akar *et al*. 2007).

Preparation of metal solution

Stock solution of 1000 mg/L Cd was prepared by a reaction with a sufficient amount of HCl with an accurate quantity of anhydrous CdO in de-ionized water. All other concentrations were prepared by diluting the stock solution separately with de-ionized water. The pH values of the experimental solution were adjusted to a desired value in the range of 2.0 to 6.0 using 0.1 M HCl and 0.1 M NaOH. All the chemicals (Merck, India) used were of analytical grade.

Biosorption studies

Biosorption of Cd ion on the air-dried biomass of *A. aculeatus* was investigated in the batch mode at room temperature. The effects of initial pH, contact time were studied to elucidate the optimum operating conditions. The effects of state of cell mass (as air dried, blot dried, lyophilized, oven dried and killed 'autoclaved') were studied to elucidate the optimum operating state of cell.

To study the effect of the state of cell mass as mentioned above, 150 mg biomass of each type and 300 mg/L Cd solution were added separately by maintaining all other conditions constant. After completion of the biosorption processes, the experimental mixtures were centrifuged at 2000 rpm for 10 min and the residual HM ion concentrations in the supernatant liquid were analyzed (Ilhan *et al*. 2004; Akar *et al*. 2007).

The effect of initial pH on the biosorption capacity of *A. aculeatus* was determined by using 150 mg of air dried biomass and 300 mg/L of Cd in 10 ml solution separately at different pH values for 150 min.

The period of contact time was studied up to 150 min by using the procedure described earlier. Samples were collected at 1, 30, 60, 90, 120 and 150 min at an optimum pH of 4.5.

Metal analysis

The concentration of Cd in experimental solutions was analyzed with an atomic absorption spectrophotometer (Perkin Elmer 2380) at 228.80 nm (Cihangir and Saglam 1999; Ilhan *et al*. 2004).

1. Measurement of ζ-Potential

The zeta (ζ)-potential of the cell biomass was measured in different pH values 2-6 by a zeta potential analyzer (Malvern Zetasizer nano ZS) (Akar *et al*. 2007).

2. FTIR analysis

The functional groups of dried *A. aculeatus* biomass that might be involved in Cd sorption were determined and interpreted before and after metal sorption, by FTIR spectroscopy, wave number 799-3950 cm^{-1}. The spectra were obtained with a Jasco FT/IR-6200 (Luo *et al*. 2010).

3. SEM and EDAX analysis

SEM analysis was carried out to observe cell surface variations during Cd accumulation process. A scanning electron microscope (JEOL JSM-5800, Jeol Ltd, Japan) equipped with an energy-dispersive X- Ray microanalyzer (Oxford ISIS-300 EDS system, Oxford Instruments, Japan) was used to take microscopic surface pictures and EDAX spectra of the biosorbent. EDAX analysis was done for a comparative examination Cd binding sites on the surface of *A. aculeatus* (Srivastava and Thakur 2006; Yuan *et al*. 2007).

RESULTS AND DISCUSSION

A. aculeatus is a well-known fungus, which is used for tannase and amylase production (Banerjee *et al*. 2001, 2007). Generally, the cell mass of *A. aculeatus* is discarded after enzyme production. Use of discarded biomass of *A. aculeatus* to remove HMs is cost effective and environmental friendly. Cd ion biosorption was investigated as a function of the state of cell mass, initial pH, surface charge, temperature, and contact time. Metal accumulation was confirmed by FTIR, SEM and EDAX analysis and the adsorption isotherms were used for mathematical description of biosorption of Cd ions.

Effect of state of cell mass on Cd (II) biosorpion

Among various forms of cell mass in use, the air-dried cell mass was found to be most suitable for Cd biosorpion, removing about 78% of the metal ion from aqueous solution. Cd accumulation potency of different types of cell masses were: air dried > dead (autoclaved) > lyophilized > oven-dried > living (**Fig. 1**).

Effect of pH on Cd (II) biosorpion

Removal of Cd (II) is strongly pH dependent. The metal uptake capacity of microorganism depends on various functional groups on it. pH is an important variable governing the uptake of HMs through biosorption as it not only affects metal species in solution, but also influences the surface properties of biosorbents in terms of dissociation of binding sites and surface charge. The effect of solution pH on the biosorption process can vary with the type of biomass and the type of metal ion being studied (Akar *et al*. 2007). The pH values studied were less than 7.0, because insoluble cadmium hydroxide starts precipitating from the solution at higher pH values, making true sorption studies impossible (Xiao *et al*. 2010). In this study optimum pH was found to be 4.5 for Cd accumulation, and at that pH 150 mg air-dried cell mass removes about 77% of Cd from 10 ml of a 120.8 mg/L Cd solution (**Fig. 2**). A nearly similar pH value had also been observed when *Aspergillus fumigatus* was employed as biosorbents, removing 99% Cd at pH 5.0 (Al-Garni *et al*. 2009).

The ζ-potential curve of the *A. aculeatus* cell biomass in different solutions (pH 2−6) is given in **Fig. 3**. Zeta potentials of cell biomass at pH 2, 3, 4, 5 and 6 are -2.66, -6.45, -7.09, -7.96 and -11.6 mV, respectively. The results demonstrated that the surface charge of a cell biomass is negatively charged at all pHs (2-6).

Effect of contact time on Cd (II) biosorption

The sorption (removal) rate of Cd (II) is dependent on time. Cd accumulation takes place at its optimum level within 90 min and increases gradually and reaches its optimum level of 78% at 90 min (**Fig. 4**). Yuan *et al.* (2007) reported that the Cd (II) adsorption ability of the lyophilized biomass of *Phoma* sp. F_2 was 91%; i.e., 15.7 mg Cd(II) was removed in 60 min from 51.6 mg/L Cd solution by 1 g biomass. Earlier,

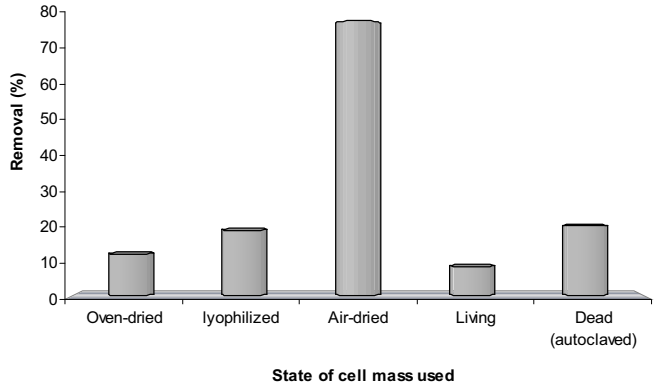

Fig. 1 Effect of state of cell mass on cadmium (II) accumulation.

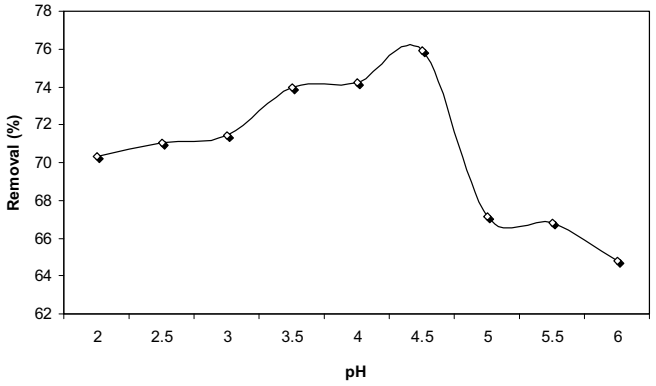

Fig. 2 Effect of pH on cadmium (II) accumulation.

time for attaining equilibrium between the adsorbing surfaces and Cd ions was found 90 min at pH 5.0 (Al-Garni *et al*. 2009).

Adsorption kinetics of Cd

Understanding about the kinetics of a metal biosorption process is essential for selecting optimum operating conditions for the removal of HM ions from the solution or waste water. Adsorption kinetics gives indispensable information on the solute uptake rate and reaction pathways. A kinetics study with different time course having fixed temperature (27°C), metal concentration (220 mg/L), biosorbent amount (150 mg) and in other optimum conditions, was carried out. The investigation was done to determine the moment of equilibrium for biosorption of Cd. In the first 30 min, biosorption was rapid and the rate of Cd ions uptake reached equilibrium after approximately 90 min of contact time.

Adsorption kinetics of Cd was modeled with pseudo - second order equation as shown below:

$$dq/dt = k(q_e-q_t)^2 \quad (1)$$

where k (g/mg min) is the rate constant of second-order adsorption; q_e the amount of Cd ion biosorbed at equilibrium (mg/g) and q_t (mg/g) is the amount of Cd ion biosorbed at any given time t (Ucun *et al*. 2009). The linearlized form of the above equation is given below:

$$t/q_t = 1/kq_e^2 + t/q_e \quad (2)$$

Experimental results obtained from the biosorption of Cd ions by biomass of *A. aculeatus* are shown in **Fig. 5**. We have calculated the t/q_t value at different time intervals and plotted them over time. Then we fitted the plot using Equ. 2 to get the value of k (rate constant), q_e (biosorbed Cd at equilibrium) and the correlation between the observed and predicted values (R^2).

The values of K, theoretical q_e ($q_{e,cal}$), experimental q_e ($q_{e,exp}$) and R^2 are 0.047815 g/mg min, 9.81065, 8 and 0.998, respectively.

The R^2 value is very high ($R^2 > 0.998$) and theoretical $q_{e,\,cal}$ values were closer with the experimental $q_{e,\,exp}$ values. From the data of the fitted pseudo second-order kinetics model, it can be said that this model presents a good relation for the biosorption of Cd onto *A. aculeatus* biomass. Earlier, Luo *et al*. (2010) reported a 0.99 R^2 value during biosorption of Cd with *Rhizopus cohnii*.

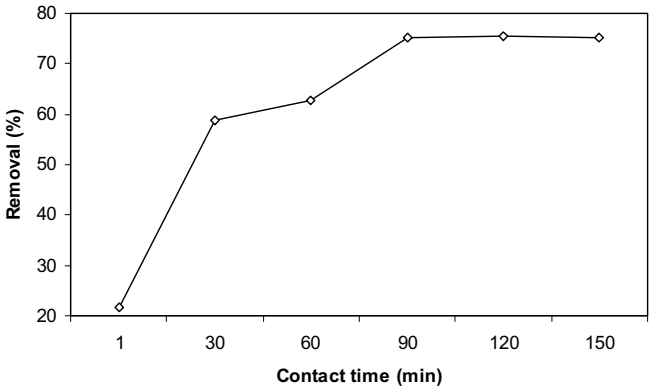

Fig. 3 Surface charge of *Aspergillus aculeatus* biomass at different pH.

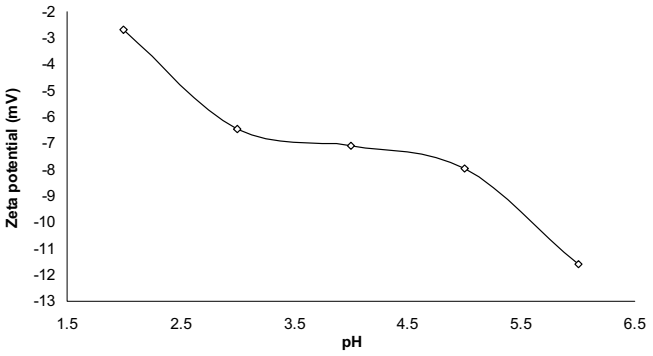

Fig. 4 Effect of contact time on cadmium (II) accumulation.

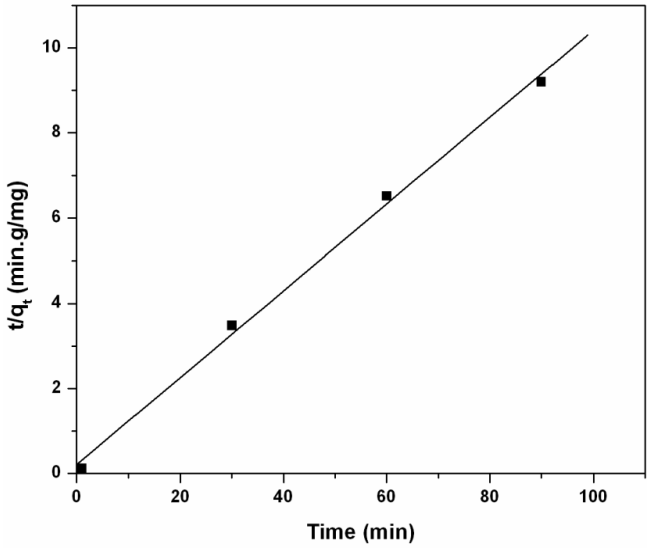

Fig. 5 Plot of the pseudo second-order adsorption kinetics of cadmium by *Aspergillus aculeatus.* Black solid squares represent the actual variation obtained from the experiment.

Biosorption isotherms models

Langmuir and Freundlich isotherm models were used for the analysis of fit of data and to understand the mechanistic

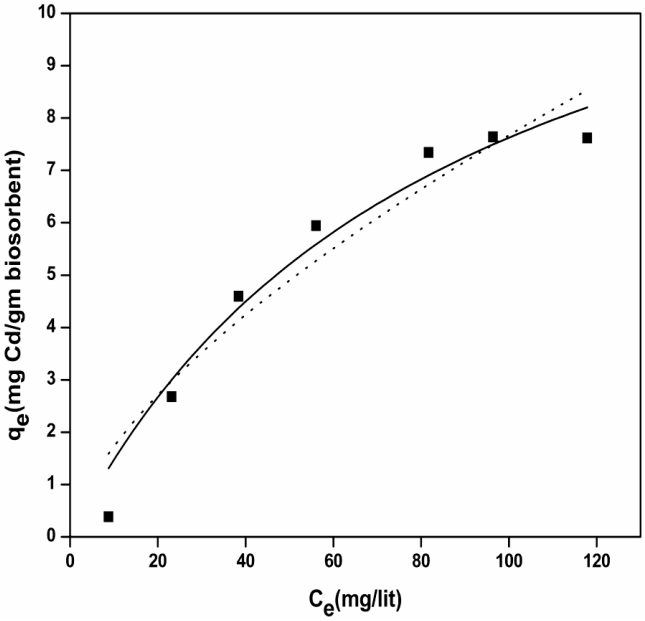

Fig. 6 Plots of the isotherms models by *Aspergillus aculeatus* biomass for cadmium ions. Solid line represents Langmuir and dotted line represents Freundlich isotherm fit.

Table 1 Langmuir and Freundlich parameters for the biosorption of cadmium ions by biomass of *Aspergillus aculeatus* (50 mg/10 ml) at 27ºC.

Langmuir isotherm	Q_{max}	K_2	R^2
	14.24	0.011	0.96
Freundlich isotherm	K_f	n	R^2
	0.389	1.54	0.92

parameters of Cd biosorption. These two isotherms are the most widely used models for studying the biosorption equilibrium between the metal solution and the solid biomass phase (Ucun *et al*. 2009). Data for Cd biosorption isotherms were obtained at constant biosorbent dosage and other optimum condition such as time, pH, etc.

The Langmuir isotherm model assumes monolayer biosorption on to homogeneous sites of biosorbent as shown below:

$$q_e = (Q_{max}\, bC_e)/(1+bC_e) \quad (3)$$

where Langmuir constants Q_{max} is denoting maximum adsorption capacity, b is denoting the affinity of the binding sites (Ucun *et al*. 2009).

The Freundlich isotherm is an empirical model describing the adsorption of the solutes from a liquid to heterogeneous surface of solid.

$$q_e = K_f C_e^{1/n} \quad (4)$$

In the above equation, Freundlich constants K_f and n denote adsorption capacity and intensity, respectively.

The batch biosorption data of different concentrations were fitted with the above two isotherms models by nonliner regression (**Fig. 6**).

The Langmuir and Freundlich parameters for the biosorption of Cd are represented in **Table 1**. **Fig. 6** and **Table 1** show that both models are suitable for describing the Cd biosorption equilibrium by *A. aculeatus* but the Langmuir isotherm model is more fitting for the biomass of that organism.

Evidence of Cd adsorption in *A. aculeatus* mycelium through FTIR and SEM analysis

The SEM pictures of *A. aculeatus* biomass are given in **Fig. 7**. Panel **A** in **Fig. 7** shows a SEM picture of pure biomass having no metal adsorption. From this photograph it is clearly evident that biomass surface is covered with so many pores and irregular cages, which are considered to be responsible for the HM adsorption. Other SEM pictures panels (**Fig. 7B**) confirm the existence of Cd on the biomass surface. For further confirmation of the HM adsorption on the biomass surface we carried out an FT-IR experiment. Details of the IR spectra and their comparisons are given in **Fig. 8**. For proper comparison we plotted the IR spectra of MH-adsorbed biomass (**Fig. 8B**) against normal biomass (**Fig. 8A**) having no HM adsorption. The spectra shown in **Fig. 8** confirm appreciable differences from the reference spectrum (**Fig. 8A**). Although the peak maximum of each frequency did not change too much, there was certain deviation in the intensity and shape. This confirms that HMs are bio-adsorbed and interact strongly with some functional groups. Main changes were observed in the hydroxyl (-OH) and

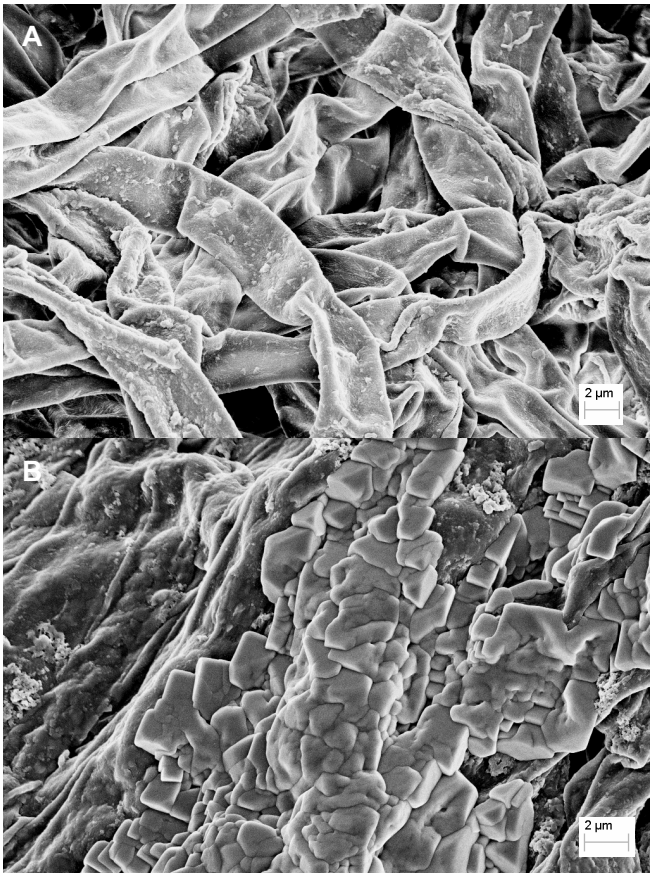

Fig. 7 Scanning electron micrograph of *A. aculeatus* (A) without metals (×7500), (B) after Cd^{+2} (×10000) adsorption.

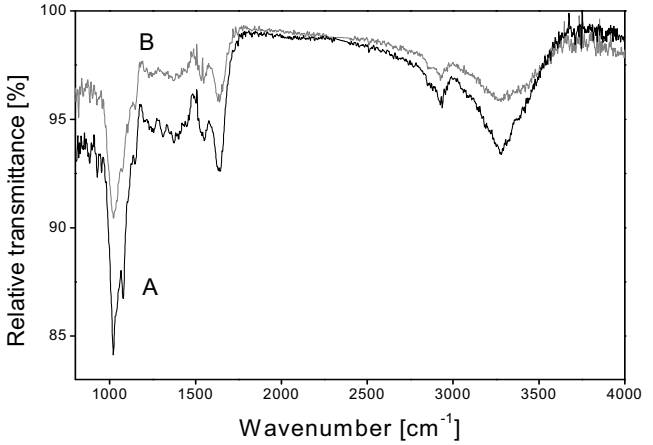

Fig. 8 FTIR spectra of *Aspergillus aculeatus* before (A) and after (B) Cd^{+2} biosorption.

Table 2 List of infrared frequency observed in the biosample *A. aculeatus* and their assignment.

Chemical functional group	Structural formula	Frequency in cm^{-1} (present in the sample)
Hydroxyl and Amine	-OH and -NH	3274
Alkyl	CH_3-CH_2-	2928 (symmetric and Asymmetric)
Amide	-$CONH_2$	1642-1550 (-C=O frequency)
-------	-CH_3 (symmetric bending)	1374
	-CH_2 (scissoring)	
-------	-CH_2 (bending)	1910
-------	-CH_2 (wagging)	1077
	-CH_2 (twisting)	
-------	-C─O	1254
-------	-C─N	1021

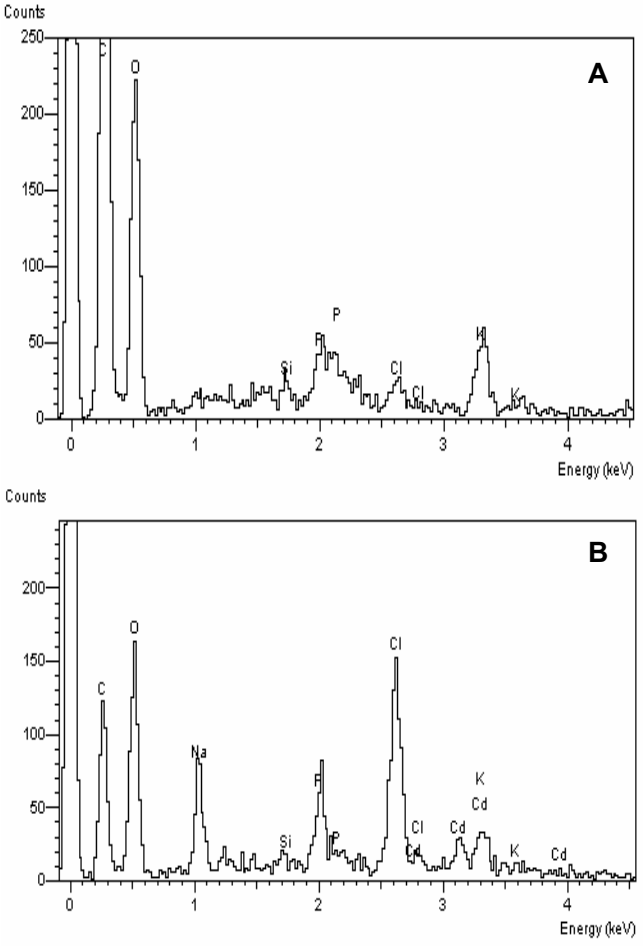

Fig. 9 EDAX spectra of *A. aculeatus* (A) before and after (B) Cd^{+2} biosorption.

amine (-NH) frequency region (3274 cm^{-1}) and in the amide (-$CONH_2$) frequency region (1642-1550 cm^{-1}). We also observed little dependence on CH_3-CH_2 symmetric and asymmetric frequency with biosorption. Thus, HMs are bio-adsorbed on the biomass surface and the chemical functional groups -OH, -NH and -$CONH_2$ are affected. Details and particulars of the IR spectral region that we observed during our IR analysis are summarized in **Table 2**. Akar *et al.* (2007) reported that -NH, -OH, -CH_3, -CH_2, -C─O, and P═O groups are involved in HM biosorption.

We confirmed Cd ion biosorption with the help of EDAX (energy-dispersive analysis of X-ray) analysis (**Fig. 9**). When we compared the typical EDAX spectra of pure biomass (**Fig. 9A**) with the HM-adsorbed sample (**Fig. 9B**), it was evident that HM ions were adsorbed mainly by the direct replacement of K^+ ion (peak position is 3.3 keV). The element percentage of K^+ ion present in the pure sample was 54% which decreased after bio-adsorption to 8%. The individual binding percentage of Cd in the HM bio-adsorbed sample was 17%.

This result also supports the findings from Tay *et al.* (2011) who suggest the possibility of ion exchange mechanism in biosorption process, in which ion exchange between potassium and cadmium ions occurs in the fungi *Pleurotus ostreatus*. EDAX analysis of the biosorbent of a macrofungus *Pleurotus platypus* before and after metal uptake revealed that the main mechanism of adsorption was ion-exchange where Ca^{2+} was replaced by Cd (II) (Vimala and Das 2011).

CONCLUSION

In this experiment biomass from a tannase and amylase producing *A. aculeatus* was used. Among different types of biomass used, air dried one was found most suitable for Cd biosorpion as it removes about 78% metal ion from aqueous solution. In this study optimum pH was found 4.5 and at that pH 150 mg air dried cell mass removes about 77% Cd from 10 ml of 120.8 mg/L Cd solution. Cd accumulation takes place at its optimum level within 90 minutes. Biomass surface is covered with so many pores and irregular cages, which are considered to be responsible for the metal adsorption. Metal is getting bio-adsorbed and interacting strongly with some functional groups. Metal ion was adsorbed mainly by the direct replacement of K^+ ion. The adsorption isotherms were used for a mathematical description of biosorption of Cd ions on to *A. aculeatus* biomass. It was seen that the adsorption equilibrium data fitted well with the adsorption models. The results of this study indicate the possibilities that exist in the clean-up of the environment with the use of byproduct after enzyme production.

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