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Original Research Article

Biosorption of Hexavalent Chromium Using *Aspergillus niger* dead biomass and its optimization studies

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Keywords

Biosorption, heavy metal, hexavalent chromium

A B S T R A C T

The use of fungal biomass as a biosorbent for the removal of toxic and heavy metal ions from different industrial effluents has received much attention in the recent years. In the present study dried biomass of the fungal strain *Aspergillus niger* MTCC 281 was exploited as a biosorbent for the removal of hexavalent chromium ions Cr^{6+}. The metal tolerance ability (T_i) and the Minimum Inhibitory Concentration (MIC) of the test strain *Aspergillus niger* were determined by amending different concentrations of Cr^{+6} ions in the potato dextrose agar medium. From the present study, pH 3 was found to be optimum for the fungal biosorption of Cr (VI) with biosorption of 67.5 %. Maximum biosorption of 70.28% was observed at a temperature of 27°C and 71.94% with an incubation period of 24 hours. Experimental results also showed maximum biosorption percentage of 75.36 % and 72.2 % for a biomass concentration of 3 mg/ml and metal concentration of 1 mg/ml respectively. The findings of the present study revealed that fungi from metal polluted sites could show higher metal tolerance and biosorption efficiency and hence could be exploited for heavy metal biosorption.

Introduction

The increasing trend towards artificial high life standards are compelling the people towards misuse of resources which ultimately result in environmental pollution in a large scale. Incidentally, increased industrialization has also affected the ecosystem through waste disposal which contains toxic metal contaminants (Ahmad et al., 2005).

Degradation of such heavy metals is not possible as these are elements which cannot be converted to non-toxic end products like CO_2 and H_2O (Pandey and Banerjee, 2012). Since heavy metals are the major pollutants of both terrestrial and aquatic (even in treated waste water), novel technologies for effective metal removal are still managing to register the success (Nuhoglu et al., 2002). Some of the important sources of metal pollution include electroplating, painting, dying, tannery etc. These heavy metals are toxic and cause several health hazards that ultimately damage the vital organs (Sanyal et al., 2005; Volesky and Holan, 1995).

The traditional methods for removing heavy metals have several disadvantages. Chemical precipitation leads to the production of toxic sludge (Tomko et al., 2006). Biological methods of metal removal from aqueous solution known as biosorption have been recommended as cheaper and more effective process (Artola et al., 1997). This method is based on the use of the metal binding capacities of various biological materials, including algae, fungi and bacteria. It is expected that soil receiving long-term application of wastewater/industrial effluents containing toxic metals may result in the development of selection pressure on soil fungi which may cause increased level of metal tolerance as well as metal adsorption capacity (Ahmad et al., 2005).

Fungi are the most studied microbe for variety of fermentation processes from which a constant supply of biomass can be obtained for metal removal. Hence, fungal biomass could serve as an economical method for effective removal of toxic metals (Luo et al., 2010). Several types of fungi are capable of metal removal such as white rot fungus (Arica et al., 2001), filamentous fungus *Phanerochaete chrysosporium* (Say et al., 2001), *Aspergilus niger* (Mukhopadhyay et al., 2011), fungal biomass of *Mucor racemosus* (Liu et al., 2007), by products of brown rot fungus *Lentinus edodes* (Chen et al., 2008) and industrial fungus *Rhizopus cohni* (Luo et al., 2010).

In general, biosorption takes place by both living and non-living microbial biomass, but there are differences in the efficiency and mechanisms involved (Park et al., 2005). Since commercial biotechnological processes such as alginate extraction result in the production of large quantities of biomass and since this material is currently viewed as a low value by-product, these industries represent an ideal source of non-living material for use as metal biosorbents (Bustard and McHale, 1998). In addition, since metal biosorption by non-living biomass is a metabolism independent process. Therefore, it is not a rule by physiological restriction. This is due to the argument that dead cells do not have toxicity limitations, no requirement of growth and nutrient media, storage property for extended time period and easy desorption of adsorbed metal ions (Awofolu et al., 2006).
The main objective of the present study is to determine the metal tolerance ability of the selected test strain and to study the biosorption ability of dried fungal biomass against hexavalent chromium which are found in the most waste waters.

Materials and Methods

Microorganisms

The fungal strain *Aspergillus niger* MTCC 281 was obtained from Microbial Type Culture Collection (MTCC), Chandigarh, India and sub cultured using potato dextrose agar (PDA) slants stored at 27°C.

Metal solution preparation

The stock solution of Cr (VI) metal was prepared by dissolving 1000 mg of potassium dichromate ($K_2Cr_2O_7$) in 10 ml of double distilled water. Working concentrations of chromium solutions with different initial concentrations were made from the stock solution using metal free distilled water and used for batch experiments.

Heavy metal tolerance and Minimum Inhibitory Concentration test

To determine the metal tolerance ability of

the fungal strain, *Aspergillus niger* was studied on PDA medium amended with a concentration of 1mg $ml^{-1}$of Cr^{6+}. The pH of the medium was maintained at 5.2 ± 0.2 and the plates without metal solution was kept as a control. The fungal strain was spot inoculated (10^{-6} spores/ml) at the centre of the plate and incubated for 4-7 days at 27° C. The Metal Tolerance Index (Ti) was determined as the ratio of the extended radius of the treated colony to that of the untreated colony in the plates grown.

Ti = Dt/Du, where Dt is the radial extension (cm) of treated colony and Du is the radial extension (cm) of untreated colony.

The minimum inhibitory concentration (MIC) of the fungal strain, *Aspergillus niger* was also determined by amending the Cr^{+6} metal ions separately to PDA medium at concentration between 1 mg ml^{-1} to 5 mg ml^{-1}. The plates were then incubated at 27°C for 4-7 days and observed for the visible fungal growth. The minimum inhibitory concentration (MIC) was determined based on the lowest concentration of metal which have inhibited the fungal growth (Akhtar et al., 2013). All the experiments were performed in triplicates and the data were expressed as mean value ± SD.

Preparation of Biosorbent

Aspergillus niger MTCC 281 was inoculated in Potato dextrose broth (PDB) and incubated for 4-5 days. After sufficient growth, the fungal mat was treated with 0.5N NaOH and kept in a boiling water bath for 15 min to kill the fungal spores. Mat was washed twice with tap water and thereafter with double distilled water until pH reaches 7. The washed biomass was then air dried and kept in a hot air oven at 80°C for overnight. The dried biomass was then grounded using pestle and mortar and stored for further use.

Biosorption efficiency

Biosorption efficiency (%) was calculated using the following equation (Bajpai and Rai, 2010):

$E = (C_i – C_f / C_i) \times 100$

Where,

E = Percentage removal of hexavalent chromium

C_i = initial metal ion concentration, mg/L

C_f = final metal ion concentration, mg/L

Batch experiments

Different parameters such as pH, temperature, incubation time, initial biomass concentration and initial metal concentration were investigated for maximum biosorption. Batch equilibrium biosorption experiments were conducted in 125 mL Erlenmeyer flasks. Effect of initial pH on metal biosorption experiments were conducted by adjusting the pH in range of 1 to 10 using 0.1 N NaOH and 0.1 N HCl. Different incubation time such as 6, 12, 18, 24, 30 and 36 hours were investigated for maximum biosorption process. Further, effect of biosorption at different temperature was also studied by incubating the conical flasks at 23, 27, 30, 37 and 40°C. Effect of initial metal concentration and biomass concentration were also investigated by varying the concentrations from 1mg to 10 mg ml^{-1}during the batch experiments. At the end of each experiment the metal solutions were centrifuged at 9000 rpm for 15 min and the supernatant was analyzed for the residual concentrations of the metal ions using Diphenylcarbazide assay method (Goyal and Banerjee, 2003).

Scanning Electron Microscopic Analysis (Khambhaty et al., 2009)

The Scanning Electron Microscopic (SEM) analysis was performed to analyze the

surface texture of untreated and treated biosorbent against hexavlaent chromium using (SEM) S-3400. Both treated and untreated biosorbent were mounted on aluminum stab, followed by a thin layer of gold coating under vacuum to enhance the quality of the micrographs.

Results and Discussion

In the present study, dead biomass of fungal strain *Aspergillus niger* MTCC 281 was investigated for the biosorption ability of hexavalent chromium. Exposure of microbes to toxic heavy metals may lead to physiological adaptation and increased metal tolerance ability [28] which may lead to an increased metal biosorption capacity. In our study, exposing the test strain to varying concentrations of Cr^{6+} reveals the metal tolerance ability (T_i) of upto 0.75 and MIC value of 2 mg/mL. Fungal resistance against heavy metals results from different mechanisms such as active transport of metal ions from inside to the outside of the cell (Balamarugan and Schaffner, 2006), metal chelating ability, enzymatic transformation (Hastrup et al., 2005;) and ability to produce specific compounds for metal binding within the cell (Gonzalez-Chavez et al., 2002).

Batch experiments

When the pH of the medium varied from 1 to 10, biosorption capacity showed increased results in increasing order until pH 6. Figure 1 shows the biosorption capacity of the dried biomass obtained from *Aspergillus niger* MTCC 281 and a maximum biosorption efficiency of 67.5% was observed for pH 3. Thereafter, decreased biosorption efficiency was observed with increase in pH. Previous studies on biosorption by Donmez and co-workers (1999) elaborated that pH is one of the most important parameters to be considered for biosorption process. Increased binding of the chromium ions at lower pH was demonstrated by the electrostatic binding of ions to that of amino groups present in the cell wall (Bajpai et al., 2004; Gupta and Keegan, 1998).

Temperature plays a vital role in the biosorption of metal ions. Therefore, experiments were performed to examine the temperature dependency of Cr (VI) biosorption by the dead biomass. Based on the results, maximum biosorption of Cr (VI) was observed at 27°C with biosorption efficiency of 70.28% (Figure 2). At higher temperature the energy of the system facilitates Cr (VI) attachment on the surface and also when temperature is very high. There is a decrease in the metal sorption due to distortion of some sites of the cell surface available for metal biosorption (Puranik and Paknikar, 1995). Due to the exothermic nature of some adsorption processes, an increase in temperature has been found to reduce the biosorption capacity of the biomass (Mameri et al., 1999). It is always desirable to evaluate the biosorption at room temperature, as this condition is easy to duplicate.

Biosorption capacity of dried fungal biomass was studied at different incubation periods of 6, 12, 18, 24, 30 and 36 hrs. From the investigated incubation time, maximum biosorption efficiency of 72.32 % was observed at 36 hrs. But, 24 hours was chosen as the best incubation time for further experiments concerned with processing time and biosorption ability percentage (Figure 3). Rate of biosorption was high at the beginning due to larger surface area of the fungal biosorbent. Once the adsorbent capability gets exhausted, the uptake rate is controlled by the transportation of biosorbent from exterior site to interior (Verma et al., 2006).

Fig.1 Effect of pH on fungal biosorption

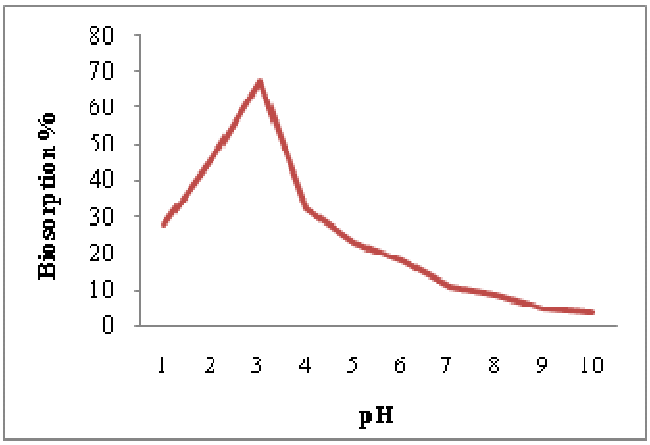


Fig.2 Effect of temperature on fungal biosorption

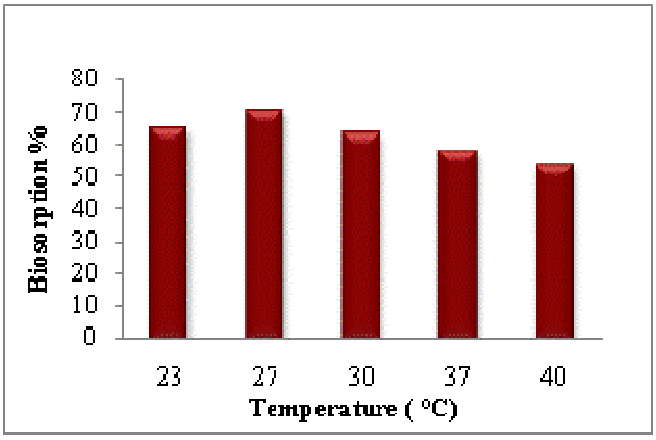


Fig.3 Effect of incubation period on fungal biosorption

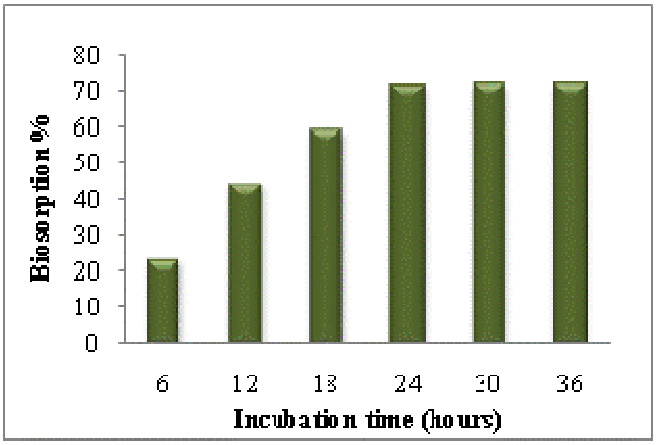


Fig.4 Effect of biomass concentration on fungal biosorption

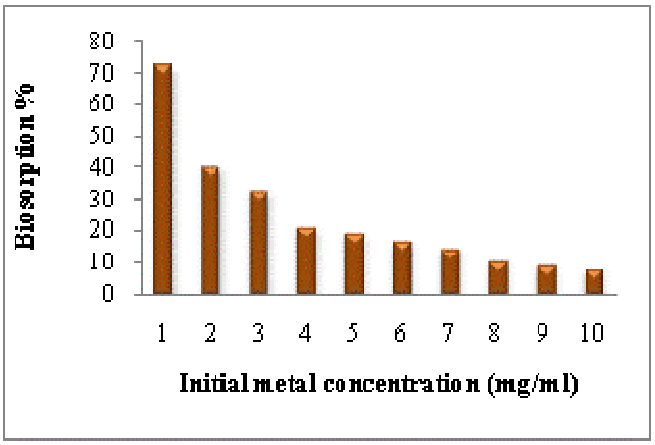


Fig.5 Effect of initial Cr (VI) metal concentration on fungal biosorption

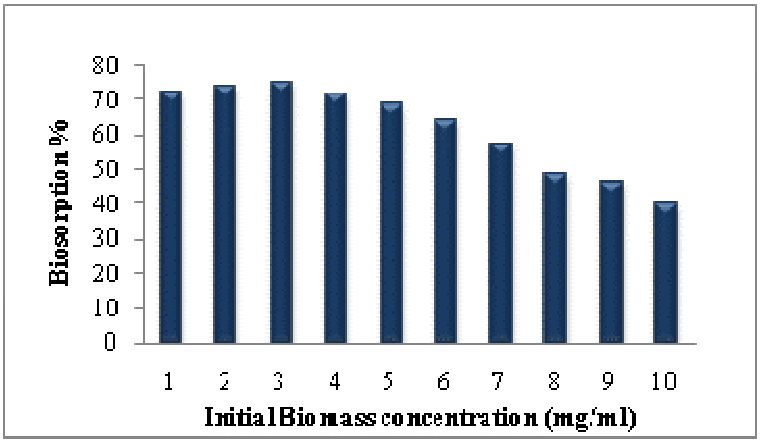


Fig.6a SEM analysis of untreated biosorbent

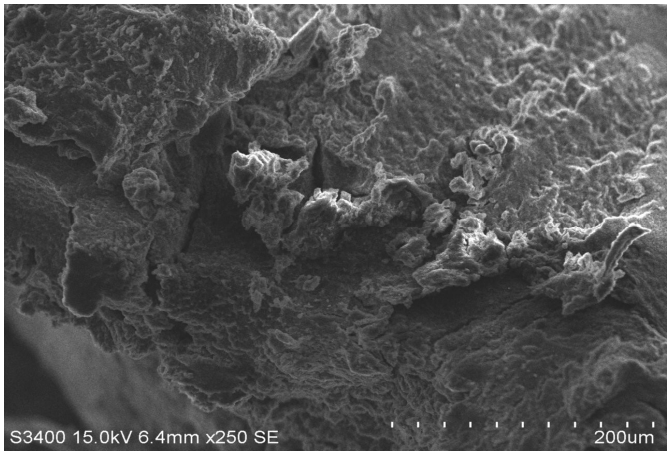
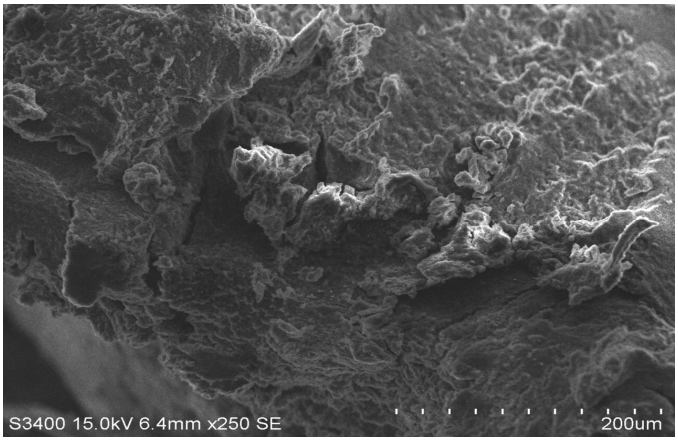


Fig.6b SEM analysis of treated biosorbent



The differences in biosorption capacity may be due to the intrinsic ability of organism as well as its cell wall composition leading to difference in interaction of metals with fungi (Gadd and White, 1993). Several authors have reported the biosorption ability of dead/living biomass of *Aspergillus* sp. (Gadd, 1990; Fourest et al., 1996; Sudha Bai and Abraham, 2001 Teskova and Petrov, 2002).

The effect of biomass concentration on Cr (VI) ion removal was examined by varying biomass dosage from 1mg to 10 mg/ml. The present study shows that removal efficiency increased with increase in biomass concentration. Maximum efficiency of biosorption of 75.36% was observed with 3 mg/mL of biosorbent (Figure 4). Biosorbent dosage strongly influences the extent of biosorption. In many instances, lower biosorbent dosages yield higher uptake and lower percentage removal efficiencies (Vijayaraghavan et al., 2006). As the biomass concentration increases, the uptake of heavy metal by the biosorbent also gets increased, because of the increased surface area (Esposito et al., 2001).

Biosorption experiment was conducted with all the optimized parameter supplemented to the medium containing different concentrations of initial Cr (VI) metal. Figure 5 depicted the biosorption of Cr (VI) by dried biomass of *Aspergillus niger* MTCC 281 showing an increased biosorption efficiency with maximum result of 72.2% recorded at 1 mg/mL of Cr (VI) metal concentration. However, increase in C_i value showed no increase in biosorption but decreased results was obtained. These results clearly indicate that at low Cr(VI) concentrations, the ratio of the biosorptive surface of the biomass to the total Cr(VI) metal was high. Therefore, Cr(VI) may be effectively interacted with the fungal biosorbent (Ucun et al., 2002).

The scanning electron microscopic analysis reveals changes that occurred in the surface texture of the treated and untreated biosorbent against the hexavalent chromium ions. The SEM micrograph of treated biosorbent shows significant changes compared with the untreated biosorbent, with slight morphological disintegration and few pores with irregular cases over the treated biosorbent (Fig 6a & b). The present results support the earlier observations of Sethuraman et al., (2010) who have also

studied the removal efficiency of Cr (VI) ions using *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Enterobacter cloacae*; Pandey and Banerjee (2012) who have investigated the biosorption ability of cadmium (II) using *Aspergillus aculeatus* DBF9 biomass.

References

Ahmad, I., S. Zafar and Ahmed, F.2005. Heavy Metal Biosorption potential of *Aspergillus* and *Rhizopus* sp. isolated from Wastewater treated soil *J. Appl. Sci. Environ. Man*., 9(1): 123-126.

Pandey, A., and Banerjee, D. 2012. Biosorption of cadmium (II) using discarded biomass of Aspergillus aculeatus DBF9. *Terres. Aqua. Envir. Toxic.*, 6(1): 8-13.

Nuhoglu, Y., E. Malkoe, A. Gurses and Canpolat, N. 2002. The removal of Cu (II) from aqueous solutions by *Ulothrix zonata*. 2002, *Biores. Technol*., 85: 331-333.

Sanyal, A., D. Rautaray, V. Bansal, A. Ahmad and Sastry, M. 2005. Heavy-metal remediation by a fungus as a means of production of lead and cadmium carbonate crystals. *Langmuir*. 21, 7220-7224.

Volesky, B., and Holan, Z.R. 1995. Biosorption of Heavy Metals. *Biotech. Prog.*, 11, 235-250.

Tomko, J., M. Baèkor and Štofko, M. 2006. Biosorption of heavy metals by dry fungi biomass. *Acta Metallurgica Slovaca*. 12, 447-451.

Artola, A., M. D. Balaguer and Rigola, M. 1997. Heavy metal binding to anaerobic sludge. *Water Res*., 31(5): 997-1004.

Luo, J., X. Xiao and Luo, S.L. 2010. Biosorption of cadmium (II) from aqueous solutions by industrial fungus *Rhizopus cohnii*, *Transactions of Non ferrous metals society of china.* 20, 1104-1111.

Arica, M.Y., Y. Kacar and Genc, O. 2001. Entrapment of white-rot fungus *Trametes versicolor* in Ca-alginate beads: preparation and biosorption kinetic analysis for cadmium removal from an aqueous solution, *Biores. Technol.*, 80 (2): 121-129.

Say, R., A. Denizii and Yakup, A.M. 2001. Biosorption of cadmium (II), lead (II) and copper (II) with the filamentous fungus *Phanerochaete chrysosporium*, *Biores. Technol.*, 76(1): 67-70.

Mukhopadhyay, M., S. B. Noronha and Suraishkumar, G.K. 2011. A review on experimental studies of biosorption of heavy metals by *Aspergillus niger*, *Canad. J. Chem. Engi.*, 89(4): 889-900.

Liu, T., H.D. Li and Deng, L. 2007. Removal of hexavalent chromium by fungal biomass of *Mucor racemosus*: influencing factors and removal mechanism. *World J. Microbiol. Biotechnol.*, 23(12): 1685-1693.

Chen, G., G. Zeng, L. Tang, C. Du, X. Jiang, G. Huang, H. Liu and Shen, G. 2008. Cadmium removal from simulated waste water to biomass byproduct of *Lentinus edodes*, *Biores. Technol.*, 99(15): 7034-7040.

Park, J.M., D. Park, Y.S. Yun and Jo, J.H. 2005. Mechanism of hexavalent chromium removal by dead fungal biomass of *Aspergillus niger*, *Water. Res.*, 39, 533-540.

Bustard, M., and McHale, A.P. 1998. Biosorption of heavy metals by distillery-derived biomass, *Bioproc. Eng*., 19(5): 351-353.

Awofolu, O.R., O.J. Okonkwo and Merwe, R.V.D. 2006. A new approach to chemical modification protocols of *Aspergillus niger* and

sorption of lead ion by fungal species, *Elec. J. Biotechnol*., 9, 341-348.

Goyal, N., S.C. Jian, and Banerjee, U.C. 2003. Comparative studies on the microbial adsorption of heavy metals, *Adv. Environ. Res.,* 7, 311–319.

Clesceri, L.S., A.E. Greenberg and Eaton, A.D. 1998. Standard methods for the examination of water and wastewater. 20th edition. Washington: American Public Health Association, 1325.

Donmez, G.Z., Z. Aksu and Ozturk, A. 1999. A comparative study on heavy metal biosorption characteristics of some algae, *Proc. Biochem*., 34, 885-892.

Bajpai, J., R. Shrivastava and Bajpai, A.K. 2004. Dynamic and equilibrium studies on adsorption of Cr (VI) ions onto binary bio-polymeric beads of cross linked alginate and gelatin, *Colloids and Surfaces A: Physico chem. Eng. Asp.,* 236, 81-90.

Gupta, G., and Keegan, B. 1998. Bioaccumulation and biosorption of lead by poultry litter microorganisms, *Poult. Sci. Mar.,* 77(3): 400-404.

Puranik, P.R., and Paknikar, K.M. 1999. Biosorption of lead, cadmium, and zinc by *Citrobacter* strain MCM B-181: characterization studies, *Biotechnol. Prog.,* 15(2): 228-237.

Mameri, N., N. Boudries, L. Addour, D. Belhocine, H. Lounici, H. Grib and Pauss, A. 1999. Batch zinc biosorption by a bacterial nonliving *Streptomyces rimosus* biomass, *Water Res*., 33 (6): 1347-1354.

Verma, A., S. Chakraborty and Basu, J.K. 2006. Adsorption study of hexavalent chromium using tamarind hull-based adsorbents, *Separ. Purif. Tech*., 50(3): 336–341.

Gadd, G.M., and White, C. 1993. Microbial treatment of metal pollution--a working biotechnology? *Trends in Biotechnol*., 11, 353–359.

Gadd, G. M., 1990. Heavy metal accumulation by bacteria and other microorganisms. *Cell. Mol. Life Sci*., 46, 834–840.

Fourest, E., A. Serre and Roux, J.C. 1996. Contribution of carboxyl groups to heavy metal binding sites in fungal wall. *Toxicol. Environ. Chem*., 54(1-4): 1-10.

Sudha Bai R and Abraham T.E., 2001. Biosorption of Cr (VI) from aqueous solution by *Rhizopus nigricans*. *Biores. Technol*., 79 (1): 73-81.

Teskova, K., and Petrov. G. 2002. Removal of heavy metals from aqueous solution using *Rhizopus delemar* mycelia in free and polyurethane-bound form. *Natur. sch*., 57, 629.

Vijayaraghavan, K., K. Palanivelu and Velan, M. 2006. Biosorption of copper (II) and cobalt (II) from aqueous solutions by crab shell particles. *Bioresour. Technol.*, 97(12): 1411–1419.

Esposito, A., F. Pagnanelli, A. Lodi, C. Solicio and Veglio. F. 2001. Biosorption of heavy metals by *Sphaerotilus natans*: an equilibrium study at different pH and biomass concentrations. *Hydrometallurgy*, 60, 129-141.

Ucun, H., B. Y. Kemal, K. Yusuf, C. Avni and Faruk, A.O. 2002. Biosorption of chromium (VI) from aqueous solution by cone biomass of *Pinus sylvestris*. *Biores. Technol.*, 85(2), 155-158.

Akhtar, S., M. Mahmood-ul-Hassan, R. Ahmad, V. Suthor, and Yasin, M. 2013. Metal tolerance potential of filamentous fungi isolated from soils irrigated with untreated municipal effluent. *Soil Environ*., 32(1): 55-62.

Balamurugan, K., and Schaffner, W. 2006. Copper homeostasis in eukaryotes: Teetering on a tight rope. *Biochemistry Biophysic Acta*, 1763(7): 737–746.

Gonzalez-Chavez, C.D., J. Haen, J.J. Vangronsveld and Dodd, D.C. 2002. Copper sorption and accumulation by the extra radical mycelium of different *Glomus* sp. (arbuscular mycorrhizal fungi) isolated from the same polluted soil. *Plant and Soil*, 240:287–297.

Hastrup, S.C.F., I.I.I Green, C.A. Clausen and Jensen. B. 2005. Tolerance of Serpulalacrymans to copper-based wood preservatives. *International Biodeterioration and Biodegradation*, 56: 173–177.

Pandey, A., and Banerjee, D. 2012. Biosorption of cadmium (II) using discarded biomass of *Aspergillus aculeatus* DBF9. *Terrestrial and Aquatic environmental toxicology*, 6(1): 8-13.