

Biosorption of *Aspergillus flavus* NCBT 102 Biomass in Hexavalent Chromium

M. N. Abubacker^{1*} and B. Kirthiga²

¹Department of Biotechnology, National College, Tiruchirappalli, India.

²Department of Microbiology, D.G.Vishnav College, Chennai, India.

DOI: <http://dx.doi.org/10.13005/bbra/1194>

(Received: 28 September 2013; accepted: 19 November 2013)

Biosorption, using microbes is regarded as cost-effective technology for the treatment of metal-bearing waste waters. In recent years, several biosorbents have been investigated. In the present study, *Aspergillus flavus*, Chromium resistant fungal strains was isolated and morphologically characterized from the soil samples textile effluent discharged unit is used as biosorption organism. The efficacy of isolates for the biosorption of hexavalent chromium was analyzed. Effect of various parameters was assayed, which include temperature (optimized at 27 °C), pH (optimized at 1), different carbon (effective with sucrose), initial metal (decreased with increasing concentration) and different biomass concentration (increases with increasing concentration). In chromium treated biosorbent, adsorption bands observed at 3430.72 cm⁻¹, 2932.84 cm⁻¹, 1638.72 cm⁻¹, 1542.89 cm⁻¹, 1155.47 cm⁻¹ and 1047.63 cm⁻¹. SEM image concludes over the biosorption period, the morphology of the fungus had undergone remarkable physical disintegration. The isolate has 58% biosorption capacity and released 40% biosorbed Cr(VI) ions on NaOH desorption studies.

Key words: *Aspergillus flavus*, Biosorption, Hexavalent chromium

The natural capacity of microorganisms, algae, fungi and plants to take up heavy metal ions and radionuclides and in some cases, to promote their conversion to less toxic forms has sparked the interest of micro biologists, biotechnologists and environmental engineers for several decades. Consequently, various concepts for 'bio-removal' of metals from waste streams and bioremediation of contaminated environment are being proposed, some of which were brought to pilot or industrial scale (Bargar *et al.*, 2008; Tomas Macek *et al.*, 2009).

Heavy metal ions are used in various industries due to their technological importance. Waste waters from these industries include metal

ions having permanent toxic effect. Algae, fungi, yeast and bacteria remove heavy metals from waste waters through functional groups such as ketones, aldehydes, carboxyls present on their cell walls. Arsenic, chromium, lead, mercury, nickel are some of the metal contaminants, of which biosorption of chromium is studied in this report.

Chromium (Cr) is one of the world's most strategic and critical materials having a wide range of uses among the metals and in chemical industries. The most common forms of chromium are Cr(II), Cr(III) and Cr(VI). Cr(VI) is the form of chromium that is mostly found at contaminated sites. Hexavalent chromium is toxic and mutagenic to most organisms (Wong and Trevors, 1988) and is known to cause irritation, corrosion of the skin and respiratory tract and lung carcinoma in humans (Ajmal Rehman, 2001). Hexavalent chromium is generated in wastewaters by several industrial processes, like leather tanning, electroplating,

* To whom all correspondence should be addressed.
E-mail: abubacker_nct@yahoo.com

metal cleaning and processing, alloy preparation and wood preservation.

Recently microbial systems like fungi, bacteria and algae have been successfully used as adsorbing agents for removal of heavy metals. Microbial populations in metal environments adapt to toxic concentrations of heavy metals and become metal resistant. Different species of *Aspergillus*, *Pseudomonas* and *Bacillus* have been reported as efficient chromium reducers (Guihong Yan *et al.*, 2003). One important and widely studied method of chromium reduction is biosorption, in which certain types of biomass are able to bind and concentrate metals from even very dilute aqueous solutions (Gadd, 1993). The present study reveals the biosorption ability of hexavalent chromium by *A. flavus* (NCBT 102).

MATERIALS AND METHODS

Chromium resistant fungal strains was isolated from the soil samples using fungal medium potato dextrose broth (PDB). To isolate metal resistant fungal strains the medium was amended with 100 mg l⁻¹ Cr(VI) by standard spread plate method. The inoculated plates were incubated at room temperature for 3 days. After incubation the isolates were morphologically characterized and identified as *Aspergillus flavus* (NCBT 102). The fungi were purified on the same medium. The cultures were routinely maintained at 4 °C on potato dextrose agar slants. The isolates were further employed for heavy metal removal and tolerance studies. Stock solution (0.1 g in 10 ml) of Cr⁶⁺ was prepared by dissolving analytical grade of K₂Cr₂O₇ in double distilled water. Before mixing with the biosorbents, the stock solution was diluted to the required concentration.

Preparation of Biosorbents

The fungal strain was grown in the potato dextrose broth (PDB) medium for 5 to 7 days. 0.5 N NaOH was taken in a conical flask containing the fungi mat was kept in water bath for 15 minutes. The mat was washed with distilled water for about 6-7 times in order to get rid of alkalinity. The pH was maintained at 7. The mat was transferred to a clean petriplate and placed in hot air oven for 24 hours. The dried dead fungus was powdered using mortar and pestle and stored in eppendroff tubes (Bhavana Jha *et al.*, 2009).

Analytical estimation of chromium

A 0.25% w/v solution of diphenyl carbazide was prepared in 50% acetone. 15 ml each of the sample solutions containing various concentrations of Cr(VI) were pipetted out into 25 ml standard flasks. To this 2 ml of 3M H₂SO₄ was added followed by 1 ml of diphenylcarbazine and the total volume was made upto 25 ml using deionised, double distilled water. Chromium concentration estimated by the intensity of the colour complex formed was measured using a UV visible spectrophotometer. The absorbance was measured against a reagent blank at 540 nm. A linear plot was obtained indicating adherence to the Beer Lambert's law in the concentration range studied. The amount of metal ion adsorbed per gram of biomass and the sorption efficiency (%) were calculated (Bajpai and Leena Rai, 2010).

Biosorption studies

Biosorption studies were done using biomass as a function of various parameters such as pH (range 1 to 10), Temperature (25 °C, 27 °C, 29 °C, 31 °C and 35 °C), the residual concentrations of the metal ions for pH, temperature was analyzed using Diphenylcarbazine assay method (Goyal Jain and Banerjee, 2003).

Effect of incubation time and biomass concentration

To determine the incubation time on biosorption of Cr(VI) ions, the experiment was conducted with 1 mg/ml fungal biomass concentration dispersed in 50 ml of the solution containing 1 mg/ml of metal concentration of pH-1. An individual flask for each incubation time was maintained (6, 12, 18, 24, 30, 36, 42 and 48 hours) at 27 °C. Effect of biomass concentration on biosorption experiment was carried out using different weights of the biomass ranging from 1 to 10 mg/ml were dispersed in solutions containing the 1 mg/ml metal concentration. Samples were centrifuged after and the supernatant was analyzed for the residual concentrations of the chromium ions using Diphenylcarbazine assay method (Sethuraman and Balasubramanian, 2010).

Initial metal concentration

To investigate the effect of initial metal concentration, biosorption experiments were conducted using conical flasks containing initial metal concentrations of 1 to 10 mg/ml working

biomass concentration of 1 mg/ml (pH-1) incubated at 27 °C.

Different carbon sources

Effect of different sugar source on biosorption experiments were conducted using conical flasks containing different sugars (1 % conc.), glucose, sucrose, lactose, maltose, mannitol and fructose with an initial metal concentration of 1 mg/ml. The biomass concentration maintained was 1 mg/ml and the flasks were maintained at pH-2 and temperature at 27 °C for 24 hours residues were analyzed using Diphenylcarbazide assay method (Goyal Jain and Banerjee, 2003).

Infrared Spectroscopy (FTIR)

A raw sample of fungal biomass and biomass loaded with Cr⁶⁺ were analyzed using an Infrared Spectrophotometer (IR) Model 470 Shimadzu Corporation adopting KBr disk technique (Umesh Jadhav *et al.*, 2008).

Scanning Electron Microscopy Analysis

The surface structure of biosorbent was analyzed by scanning electron microscopy (SEM) S-3400. Unloaded and metal-loaded fungal biomass samples were mounted on aluminum stub sequenced by coating with a thin layer of gold under vacuum to increase the electron conduction and to improve the quality of the micrographs.

Desorption Studies

The fungal biomass loaded with Cr(VI) was treated with 0.1 M NaOH at room temperature (27°C) with constant shaking for 2 hours (Goyal Jain and Banerjee, 2003).

Immobilization of Biomass

A 2% (w/v) slurry of sodium alginate was prepared in hot (60 °C) distilled water. After cooling 0.1% of fungal biomass was added and stirred. The alginate biomass slurry was then extruded into 50 mM CaCl₂.2H₂O for polymerization and bead formation (Sudha Bai and Abraham Emilia, 2003). Biosorption studies were performed by dispersing 0.1% beads in 50 ml of the solution containing 1 mg/ml of metal concentration. The flask was maintained at pH-2 at 27 °C for 24 hours. Solutions were centrifuged and the supernatant was analyzed for the residual concentrations of the chromium ions using Diphenylcarbazide assay method (Goyal Jain and Banerjee, 2003).

RESULTS AND DISCUSSION

The environmental parameters show great influence on the biosorption process. The important parameters like temperature, pH, initial metal concentration and initial biomass concentration are considered essential for the biosorption of Cr(VI).

Table 1. Optimization Parameters for Biosorption Efficiency

S. No.	Parameters Range	Optimum	% Biosorption
1.	pH (1 to 10)	1	48.0
2.	Temperature (°C)	27	54.8
3.	Incubation Time (h)	24	60.8
4.	Biomass concentration (mg/ml)	10	82.5
5.	Metal concentration (mg/ml)	1	62.6
6.	Carbon Source	Sucrose	65.4

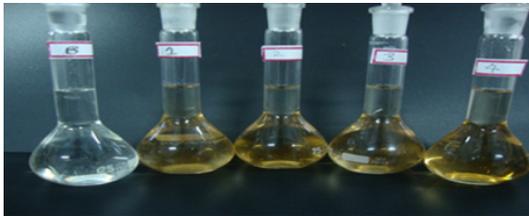
The pH of solution usually plays a major role in biosorption and seems to affect the solution chemistry of metals/dyes and the activity of the functional groups of the biomass. For metals, the pH strongly influences the speciation and biosorption availability of the metal ions (Chen *et al.*, 2003; Done *et al.*, 2003). Based on the results it was observed that optimal pH for biosorption of Cr(VI) ions is 1.0.

Temperature seems to affect biosorption only to a lesser extent within the range from 20° to 35 °C (Veglio and Beolchini, 1997). 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 conduct/evaluate biosorption at room temperature as this condition is easy to replicate.

Potato dextrose broth showing biomass



Analytical estimation of chromium

Immobilization of Biomass
Beads without Biosorbent

Beads with Biosorbent



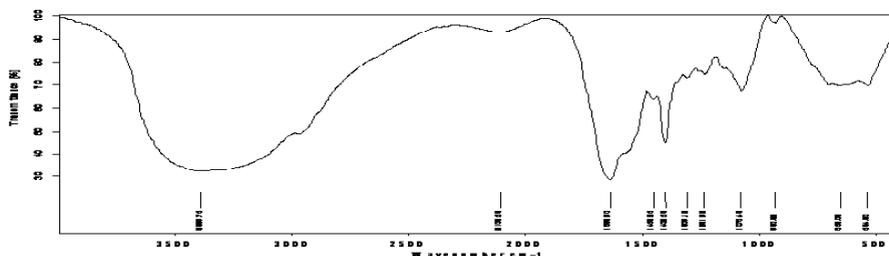
The maximum biosorption was observed at 27 °C with a biosorption efficiency of 54.5%. This study noticed that biosorption efficiency increased with increase in biomass concentration. Based on the results, it was observed that maximum biosorption was observed at 42 hours, since absorption rate against timing is concerned, 24 hours was chosen as the best incubation period in this experiment. An increase in the biomass concentration generally increases the amount of solute biosorbed, due to the increased surface area of the biosorbent, which in turn increases the number of binding sites (Esposito *et al.*, 2001), also associated with present study in which removal efficiency increased with increase in biomass concentration whereas the biosorption percentage decreases with increasing concentration of initial metal concentration.

The biosorption percentage decreases with increasing concentration of initial metal concentration due to increase in electrostatic interaction present on the site affinity for metal ions gradually moves downwards (Saleh *et al.*, 2010). The amount of Cr(VI) adsorbed with cultures grown in glucose, fructose and sucrose with lower concentration of Cr(VI) (50 mg/l) was about 4.3, 5.2 and 3.8 mg/g cell mass (Goyal Jain and Banerjee, 2003) and it has been reported that the ability of the yeast to take up metal ions apparently is influenced by the carbon sources (Stoll and Duncan, 1996).

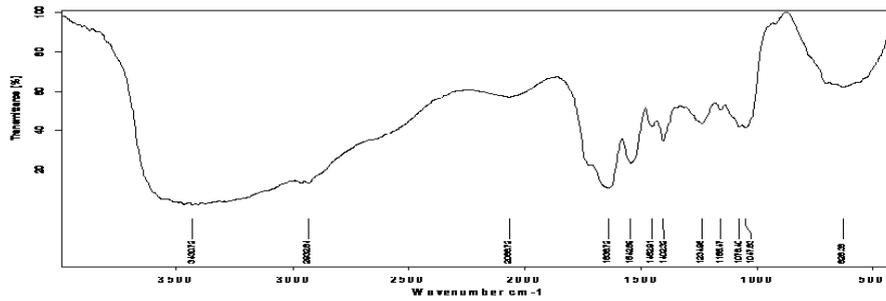
Characterization of Biosorbent Material FTIR

The observations indicate the involvement of the functional groups in biosorption. The

FTIR analysis



Biomass without Chromium



Biomass with Chromium

band around 3,425 cm^{-1} is indicative of the existence of -OH groups. The band at 2926 cm^{-1} is representative of -CH . The band located at 1633 confirms the presence of N-H stretching while, the band at 1557 could be attributed to Amide-I and II (mostly N-H bending). The band around 1150 cm^{-1} signifies the presence of SO_2 , whereas band at 1036 cm^{-1} indicates the presence of P-O alkyl (phosphorous compounds) (Williams and Fleming, 1991).

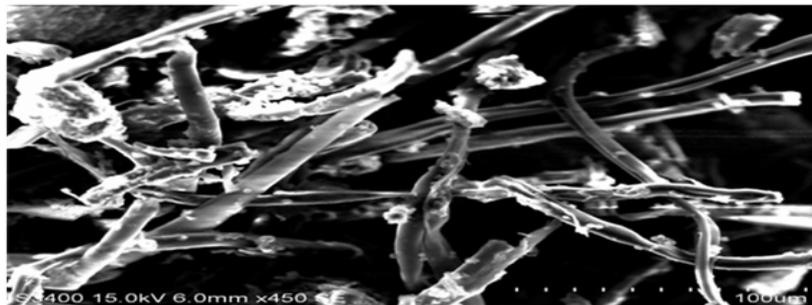
SEM

Over the biosorption period, the morphology of the fungus had undergone

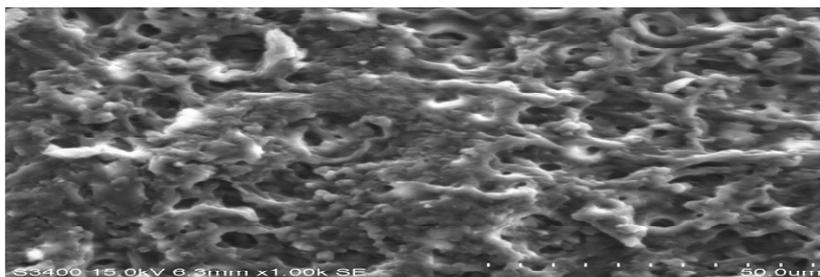
remarkable physical disintegration. The integrated and cluster arrangement that occurs before and after biosorption which clearly shows that metal particles adhere on the surface of the *A. flavus* cell wall.

Biosorption is a process of treating pollutant-bearing solutions to make it contaminant free. However, it is also necessary to be able to regenerate the biosorbent. This is possible only with the aid of appropriate elutants, which usually results in a concentrated pollutant solution. Therefore, the overall achievement of a biosorption process is to concentrate the solute, *i.e.*, sorption

SEM analysis



Biosorbent untreated (Intact Mycelia)



Biosorbent treated with Chromium (Degenerated Mycelia)

followed by desorption. Electrostatic attraction was found to be the main mechanism responsible for the biosorption of negatively charged dye anions to a positively charged cell surface (O'Mahony *et al.*, 2002; Sethuraman and Balasubramanian, 2010). Therefore, it would be logical to make the cell surface negative using alkaline solutions to repel the negatively charged reactive dyes (Won and Yun, 2008). The test isolate showed 58% biosorption capacity. 40% of biosorbed Cr(VI) ions was released in desorption studies using NaOH.

CONCLUSION

The concept of biosorption includes concentrating a sorbent in the biomass. The biological material laden with sorbate is then regenerated and reused, and sorbate is recovered by eluent. It is important to choose desorbing agent very carefully, so all sorbate would be removed from the biomass by low volume of solution and that the biosorbent would not be destroyed and would sustain its sorptive properties, so it can be reused in the subsequent biosorption cycle. The present studies also show that *Aspergillus flavus*, shows a maximum biosorption ability towards the reduction of Chromium (VI) ions.

ACKNOWLEDGMENTS

Author (MNA) wish to thank DST-FIST, Government of India, New Delhi for providing the infrastructure facilities to the Department of Botany, National College, Tiruchirappalli, Tamil Nadu. Authors also expresses thanks to Padmavibhushan Dr. V. Krishnamurthy, President, Sri. K. Ragunathan, Secretary and Dr. K. Anburasu, Principal, National College, Tiruchirappalli for all the supports and encouragement given to PG and Research Department of Biotechnology to carry over the research work.

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