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## Bioaccumulation and Biosorption of Chromium VI by different Fungal Species

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### Abstract

Biosorption of the hexavalent chromium ion (Cr (VI)) onto the cell surface of different sized fungal species in aerobic condition was investigated. The assessment of the metal-binding capacity of new biosorbents has been discussed. Batch experiments were conducted with various initial concentrations of chromium ions to obtain the sorption capacity and isotherms. The results obtained at pH 5.5 of chromium solution were 97.39% reduction by *Trichoderma* and 100% reduction by *Agaricus*. It was found that the sorption isotherms of fungi for Chromium (VI) appeared to fit Freundlich and Langmuir's models. The results of FT-IR analysis suggested that the chromium binding sites on the fungal cell surface were most likely carboxyl and amine groups. The fungal surfaces showed efficient biosorption for Chromium in Cr<sup>+6</sup> oxidation state. Biosorption isotherm curves, derived from equilibrium batch sorption experiments, were used in the evaluation of metal uptake by these fungal biosorbents.

*Keywords: Biosorption, Trichoderma and Agaricus, Chromium VI, sorption isotherms, FT-IR spectroscopy.*

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### 1 Introduction

Rapid industrialization and technological advancements in this century have resulted in severe environmental pollution. Heavy metals are among the worst group of pollutants of the environment and among all the heavy metals Chromium(VI) is considered to be highly toxic, which is often present in industrial wastewaters as chromate and dichromate ion (Ahalya, 2003). The discharge of Chromium (VI) into aquatic ecosystems has become a matter of concern in all the tannery area in India over the last few decades. This pollutant is introduced into the aquatic systems significantly from the effluents of leather processing units as a result of chrome tanning of leather. Chromium can replace other metals in biological systems with toxic effects and its accumulation throughout the food chain leads to serious ecological and health problems as Chromium(VI) is a known human carcinogen. In this context, most studies on prokaryotes and chromium involve reduction of Chromium(VI) to less toxic and less mobile forms such as Chromium(III). In this regard, the increasing awareness of

accumulation of heavy metals in the environment has also led to a quest for new and improved cleaner technologies to overcome metal pollution. An innovative heavy metal removal process called bioremediation composed of phytoremediation and biosorption has gained enormous impetus. In the process of phytoremediation, certain plants have been found to remove metals from contaminated water. Heavy-metal ions can be entrapped in the cellular structure and subsequently biosorbed onto the binding sites present in the cellular structure. This method of uptake is independent of the biological metabolic cycle and is known as biosorption or passive uptake. The heavy metal can also pass into the cell across the cell membrane through the cell metabolic cycle. This mode of metal uptake is referred to as active uptake. The metal uptake by both active and passive modes can be termed as bioaccumulation. The disadvantages include that it takes a long time for removal of metals and the regeneration of the plant for further biosorption is difficult. The removal of toxic metals from wastewaters by biosorption is based on metal binding capacities of various biological materials. Biosorption is the binding and concentration of heavy metals from aqueous solutions (even very dilute ones) by certain types of inactive, dead, microbial biomass or it can also be defined as the ability of biological materials to accumulate heavy metals from wastewater through metabolically mediated or physico-chemical pathways of uptake (Fourest and Roux, 1992). Algae, bacteria and fungi and yeasts have proved to be potential metal biosorbents (Volesky, 1986). The major advantages of biosorption over conventional treatment methods include: Low cost; good efficiency; minimization of chemical and or biological sludge; regeneration of biosorbent; and possibility of metal recovery. The biosorption process involves a solid phase (sorber or biosorbent; biological material) and a liquid phase (solvent, normally water) containing a dissolved species to be sorbed (sorbate, metal ions). Due to higher affinity of the sorber for the sorbate species, the latter is attracted and bound there by different mechanisms. The process continues till equilibrium is established between the amount of solid-bound sorbate species and its portion remaining in the solution. The degree of sorber affinity for the sorbate determines its distribution between the solid and liquid phases. Mechanisms of metal biosorption are essential to be understood first in order to successfully exploit the phenomenon and for regeneration of biosorbent materials in multiple reuse cycles. The complex nature of biosorbent materials makes this task particularly challenging.

### **1.1 Biosorbent material**

Strong biosorbent behaviour of certain micro-organisms towards metallic ions is a function of the chemical make-up of the microbial cells. This type of biosorbent consists of dead and metabolically inactive cells. Some types of biosorbents would be broad range, binding and collecting the majority of heavy metals with no specific activity, while others are specific for certain metals. Some laboratories have used easily available biomass whereas others have isolated specific strains of microorganisms and some have also processed the existing raw biomass to a certain degree to improve their biosorption properties.

### **1.2 Biosorption Mechanisms**

The complex structure of microorganisms implies that there are many ways for the metal to be taken up by the microbial cell. The biosorption mechanisms are various and are not fully understood. They may be classified according to various criteria. According to the dependence on the cell's metabolism, biosorption mechanisms can be divided into: Metabolism dependent and Non -metabolism dependent. According to the location where the metal removed from solution is found, biosorption can be classified as 1.

Extra cellular accumulation/precipitation 2. Cell surface sorption/precipitation and 3. Intracellular accumulation.

Transport of the metal across the cell membrane yields intracellular accumulation, which is dependent on the cell's metabolism. This means that this kind of biosorption may take place only with viable cells. It is often associated with an active defense system of the microorganism, which reacts in the presence of toxic metal.

During non-metabolism dependent biosorption, metal uptake is by physico-chemical interaction between the metal and the functional groups present on the microbial cell surface. This is based on physical adsorption, ion exchange and chemical sorption, which is not dependent on the cells' metabolism. Cell walls of microbial biomass, mainly composed of polysaccharides, proteins and lipids have abundant metal binding groups such as carboxyl, sulphate, phosphate and amino groups. This type of biosorption, i.e., non-metabolism dependent is relatively rapid and can be reversible (Kuyucak and Volesky, 1988).

Traditional taxonomy of *Trichoderma* was based upon differences in morphology, primarily of the asexual sporulation apparatus. Most strains are highly adapted to an asexual life cycle. Thus, this fungus is highly adaptable and evolves rapidly. The organism grows and ramifies as typical fungal hyphae, 5 to 10  $\mu\text{m}$  in diameter.

### 1.3 Factors affecting Biosorption

The investigation of the efficacy of the metal uptake by the microbial biomass is essential for the industrial application of biosorption, as it gives information about the equilibrium of the process which is necessary for the design of the equipment.

The metal uptake is usually measured by the parameter 'q' which indicates the milligrams of metal accumulated per gram of biosorbent material and 'qH' is reported as a function of metal accumulated, sorbent material used and operating conditions.

The following factors affect the biosorption process: 1. Temperature seems not to influence the biosorption performances in the range of 20-35<sup>o</sup>C. 2. pH seems to be the most important parameter in the biosorptive process: it affects the solution chemistry of the metals, the activity of the functional groups in the biomass and the competition of metallic ions 3. Biomass concentration in solution seems to influence the specific uptake: for lower values of biomass concentrations there is an increase in the specific uptake. 4. Biosorption is mainly used to treat wastewater where more than one type of metal ions would be present; the removal of one metal ion may be influenced by the presence of other metal ions.

### 1.4 Biosorption equilibrium models - Assessment of sorption performance

Examination and preliminary testing of solid-liquid sorption system are based on two types of investigations: (a) equilibrium batch sorption tests and (b) dynamic continuous flow sorption studies (Gadd, 1988).

The equilibrium of the biosorption process is often described by fitting the experimental points with models usually used for the representation of isotherm adsorption equilibrium. The two widely accepted and linearized equilibrium adsorption isotherm models for single solute system are given by the following:

**$q = q_{\text{max}} \frac{b C_{\text{eq}}}{1 + b C_{\text{eq}}}$  Langmuir equation**

where q is milligrams of metal accumulated per gram of the biosorbent material;  $C_{\text{eq}}$  is the metal residual concentration in solution;  $q_{\text{max}}$  is the maximum specific uptake corresponding to the site saturation and b is the ratio of adsorption and desorption rates. This is a theoretical model for monolayer adsorption.

Another empirical model for monolayer adsorption is

**$q = K_F C_{\text{eq}}^{1/n}$  Freundlich equation**

**where  $K_F$  and  $n$  are not constants**

These models can be applied at a constant pH. These models are used in literature for modeling of biosorption equilibrium in the presence of one metal. These values are plotted in a 2D line where the specific uptake  $q$  is reported as a function of the metal concentration  $C_{eq}$ .

But the above said adsorption isotherms may exhibit an irregular pattern due to the complex nature of both the sorbent material and its varied multiple active sites, as well as the complex solution chemistry of some metallic compounds. Various applications are available for biomass immobilization. The principal techniques that are available in literature for the application of biosorption are based on adsorption on inert supports, on entrapment in polymeric matrix, on covalent bonds in vector compounds, or on cell cross-linking.

**2 Materials and Method****2.1 Microorganisms, Medium and Culture conditions**

There are several fungal strains which are used for chromium remediation and among them a newly Chromium(VI) reducing fungal strain identified as *Trichoderma sp.* was studied for its capability to remove Chromium(VI).

Two different fungal sp. were used in this study, one is *Trichoderma sp.*, a filamentous fungi and the other is *Agaricus sp.*, one with a fruiting body. *Trichoderma sp.* was obtained from MTCC Section of Indian Institute of Pulse Research, Kanpur. The fungal strain was routinely maintained and propagates on readymade Potato Dextrose Agar (PDA) of Titan make. The culture was grown at room temperature for obtaining fungal biomass.

**2.2 White Button Mushroom**

White button mushroom or *Agaricus bisporus* was obtained from local horticulture site (Fig. 2). This mushroom belongs to Agaricaceae family which includes the best known mushroom "the white buttons" sold generally as edible mushrooms in every supermarket.

**2.3 Sterilization**

Media and other glassware like pipette, Petri plates and Test tubes etc. were sterilized every time before use at 121<sup>0</sup>C at 15psig for one hour in an autoclave. Prior to sterilization, glasswares and plasticwares were washed in detergent, rinsed with distilled water and then used.

**2.4 Reagents**

Metal stock solution was prepared by Potassium dichromate salt of Merck AR grade and deionized water. It was then diluted to required concentration by deionized water. The standard calibration was also carried out by this stock so as to use the values for reference.

**2.5 Plating Potato Dextrose Agar**

PDA was accurately weighed and dissolved in distilled water, kept in conical flask and plugged with cotton before sterilization. After sterilization about 20 ml. agar was poured in each sterilized Petri plates then these plates were allowed to cool so that agar solidifies and then inoculation was given. The full grown fungal colonies of *Trichoderma* (Fig. 1) appeared in 5 days of time and then these colonies were taken from the plates to be poured in the chromium solution.

## 2.6 Bioaccumulation and Biosorption Methodology

In this study, batch biosorption experiments were carried out for determining the biosorption capacity of both the fungal species in aerobic conditions. Aqueous Chromium solution with constant initial concentrations (4,6,8 and 10 ppm) were used and then weighed amount of dry as well as wet fungal biomass, thallus in case of *Agaricus sp.* and hyphae in the case of *Trichoderma sp.* were poured in fix volume for different time period. Before putting the thallus of *Agaricus sp.* in chromium solution it was cut into small pieces whereas *Trichoderma* was taken from the plates as such and weighed. The adsorption was also measured by dried fungal biomass in which weighed quantity of fungal biomass was placed in chromium solution of constant concentration. The mixture was then kept at room temperature, sample were withdrawn at different time intervals from it for photometric determination of chromium by spectrophotometer of Thermo Helios a model. Simultaneous control/standard readings were taken to compare and calculate percentage reduction.

## 2.7 Estimation of Chromium VI by DPC Method

Diphenyl carbazide reagent combines with hexavalent Chromium to form a complex which give a beautiful pink/magenta color in acidic medium by the method ISO 11083 (1994) Water quality, Determination of Chromium(VI) – Spectrometric method using 1,5-diphenylcarbazine.

## 2.8 Micrographs of Fungi

In the study micrographs of the fungi were also taken from optical microscope of Leica DFC 280 model. Micrographs were magnified about 50 times. These micrographs were taken before and after biosorption so as to compare change in both stages of fungal species on cellular level.

## 3 Result and Discussion

The main purpose of this work was to evaluate the Chromium(VI) removal by two different fungi *Trichoderma* and *Agaricus*. *Trichoderma sp.* is fungi that are present in nearly all soils and other diverse habitats. In soil, they frequently are the most prevalent culturable fungi. The *Agaricus* or Meadow Mushroom (Fig. 2) is a close relative that really does grow wild, although it too is somewhat domesticated - often to be found on cultivated lawns. The adsorption phenomenon was called biosorption as fungal species were used for the adsorption. The fungus can have remarkable ability to remove high concentrations of Chromium(VI) when grown in plates containing glucose as the sole carbon and energy source, under aerobic conditions. In this respect the remediation of chromium containing water has been carried out by two different kind of fungal sp., i.e., *Agaricus sp.* and *Trichoderma sp.* The purpose of selecting two different kinds of fungi

was to see the effect of fungal size on chromium adsorption. The pH of Chromium solution was 5.5-5.8. The percentage reduction in the chromium concentration by *Trichoderma* on different time periods was given in table 1. The standard solutions of chromium were of 4, 6, 8 and 10 ppm concentration. The readings were taken after 1, 1.5, 2 hours and keeping the solution overnight. All the Chromium(VI) solution showed decrease in concentration after one hour onwards. *Trichoderma* found to be very good in decreasing the chromium content; it started showing decreasing trend in all the concentration as the reduction was 75.13, 83.72, 80.35 and 75.54% in 4, 6, 8, 10 ppm in one hour's time. The reduction was rapid in 1.5 hour time maximum in 4 ppm solution to about 96.98% and moderate 89.89% in 6 ppm, 81.81% in 8 ppm, 77.27% in 10 ppm solution. In 2 hours time it has been seen that the percentage reduction didn't increase whereas it was remarkably good in overnight started from 76.08% in 4 ppm to maximum in 97.39% in 10 ppm solution. The results indicated that from 1.5 hours to 2 hours the reduction did not increase due to shorter time gap and little time for fungal mycelia to adsorb further metal or to bind with metal species. Micrographs of *Trichoderma* also showed structural difference of dark portions as can be seen from Fig. 3 A, B. Before biosorption cellular structure of *Trichoderma* filaments showed regular gaps or spaces in between, prominently visible in the micrographs whereas these spaces appeared to be blocked or filled in somehow after biosorption (fig. 4 A, B).

Another experiment for chromium biosorption was carried out with *Agaricus*'s thallus in the same concentration, i.e., 4, 6, 8 and 10 ppm. The purpose of this study was to evaluate a fruiting fungi's ability to adsorb metal in its aqueous form. Actually the thallus of *Agaricus* is large and that's why it has been cut into small pieces before putting it in chromium solution for different time intervals. The results of the experiment were given in table 2. The thallus gave outstanding reduction in chromium concentration right from the one hour, i.e., 96.28% for 4 ppm, 99.65% for 6 ppm, 99.82% for 8 ppm and 99.91% for 10 ppm. In 1.5 hours and 2 hours time period reduction was remarkably good, only in 2 hours time fungi didn't show much difference from the earlier reduction, the reason may be the saturation or small time gap between the readings. In the same trend, the thallus of *Agaricus* gave 100% reduction for 10 ppm solution from 1.5 hours onwards which was really very encouraging. Micrographs of the thallus were found to be supportive of the fact that the thallus quickly adsorbs metal species from the solution. The micrographs taken before (fig. 5 A, B) the biosorption clearly shown the surface of thallus uninterrupted and smooth whereas after biosorption surface (fig. 6 A, B) of the thallus show some dotted pattern over it suggesting some chemical bonding on cellular level. Thus it can be assume that the biosorption may be due to rapid bonding between metal and surface of thallus. The time period selected was 1, 1.5 and 2 hours as *Agaricus* and *Trichoderma* both showed very good reduction in just one hour. In both the species initial rapid phase of biosorption followed by slower, metabolism-dependent uptake of metals, which may be responsible for somewhat stagnant reduction percentage.

The biosorption capacity of *Agaricus* was also evaluated in the dry form (table 3- A, B). In this experiment dried thallus was kept overnight in 100 ml and 200 ml volumes of 10 ppm solution. Then reduction in Chromium (VI) concentration was 94.84% and 96.63%, respectively for 100 ml and 200 ml solution which was very encouraging as well. When 15gm. of this thallus was used (wet wt. basis) in 200 ml chromium solution of 10 ppm concentration the reduction was 100% in overnight. This was 7.5% (w/v) of solution on wet wt. basis whereas it was 2.77 gm on dry wt. basis and 1.385% (w/v) of solution as moisture content of the thallus was 81.54%. This experiment was proved very useful as it gave the idea of using dried biomass for metal remediation/biosorption. It proved that

nature, physical status (dried, wet) as well as quantity of fungus largely effect bioaccumulation of Chromium (VI) in thallus.

### 3.1 FT-IR Spectra of Chromium-loaded fungal species

Another investigation related to the fungal biosorption phenomenon carried out was FT-IR using chromium-loaded different fungal species. The fungal biomass was dried to almost nil moisture before and after biosorption and send for FT IR analysis. The spectra were given in fig. 7 and fig. 8. This analysis had eventually confirmed the difference between functional groups in relation to biosorption of Chromium(VI). The absorption spectrum of chromium-loaded dried fungal biomass was compared with that of initial biomass. A change of absorption bands can be seen when comparing the FT-IR spectra of pristine and chromium-loaded biomass. Fig. 7 showed the changes in the spectrum of the biomass after sorption of Cr -VI. An interesting phenomenon was the sharp decrease in the band intensity at  $1456\text{ cm}^{-1}$  corresponding to  $\text{C}=\text{O}$  stretching after metal binding. On the basis of the change of the band, it was reasonable to assume that the peak value suggested the metal chelating. The structure of the metal bound to carboxyl ligands on the fungus is likely to take place.

FT-IR spectrum of *Agaricus* thallus before and after biosorption (fig. 8) shown the difference in peak at  $1547\text{ cm}^{-1}$  corresponding to  $\text{C}=\text{N}$  bond and little change in the fingerprint region correspond to  $\text{C}-\text{H}$  bond. There may be shifting of functional group and the change in this region suggested metal bonding during the adsorption in *Agaricus* also.

The adsorption data were fitted to linearly transformed Langmuir adsorption isotherm. The linear form of the Langmuir adsorption isotherm is represented as

$$C_e/q_e = 1/Q_0b + C_e/Q_0$$

Where  $C_e$  is equilibrium concentration (mg/l) and  $q_e$  is the amount of chromium(VI) adsorbed per gram at equilibrium (mg/g).  $Q_0$  (mg/l) and  $b$  (1/mg i.e. 1 of adsorbent per mg of adsorbate) are Langmuir constants related to adsorption capacity and energy of adsorption.

Linear plots were obtained for all the concentration between  $C_e/Q_e$  versus  $C_e$  (only 6 ppm concentration plots shown as representatives graphs) fig 9 and 10. The isotherm parameters for both the biosorbents i.e. *Trichoderma* and *Agaricus* sp. were given in table 4 along with regression coefficient.

## 4. Conclusion

The study of biosorption showed significant difference of chromium uptake by *Trichoderma* filamentous fungi and *Agaricus* fruiting thallus in tune of 100% by both the species in as high concentration as 10 ppm on wet weight of the fungi. Since metal biosorption from solution was predominantly due to physico/chemical interactions between the biomass and metal in solution, morphological differences existing within biomass can greatly influence the biosorption process. The cellular structure of *Trichoderma* mainly contains polysaccharide which can make significant difference in metal uptake by it. In the thallus of *Agaricus*, metal uptake may be due to amino acids present in the proteins of the thallus. Thus these two fungal species can be applied to develop cost effective potential biosorbent for removing chromium from industrial effluents as well. Another very important aspect of this study is the future use of these two fungal strains in biosorption of other heavy metal species generally present in various industrial effluents.

**Table 1: Percentage Reduction of Chrome VI by *Trichoderma sp.***

Concentration (ppm)	1 hour	1.5 hours	2 hours	Overnight
4	75.13	96.98	96.38	76.08
6	83.72	89.89	89.24	94.19
8	80.35	81.81	82.80	95.91
10	75.54	77.27	77.52	97.39

**Table 2: Percentage Reduction of Chrome VI by *Agaricus* (15 g/225 ml)**

Concentration (ppm)	1 hour	1.5 hours	2 hours	Overnight
4	96.28	98.0	97.3	99.56
6	99.65	98.93	99.44	99.87
8	99.82	98.87	97.64	99.30
10	99.91	100.00	100.00	99.95

**Table 4: Estimated Langmuir isotherm parameters and relevant statistical information**

pH of solution - 5.5-5.8

Biosorbent	Initial conc.(ppm)	1/Q <sub>0</sub>	1/bQ <sub>0</sub>	R
<i>Trichoderma sp.</i> Dose 0.0501g/l	4	0.2785	8.89	0.4211
	6	-0.2212	16.15	0.9920
	8	-0.2610	16.59	0.9933
	10	0.2080	4.753	0.9992
<i>Agaricus sp.</i> Dose 0.666g/l	4	0.0072	0.5506	0.9998
	6	0.0061	0.6009	0.9652
	8	0.0053	0.8064	0.8723
	10	-0.0025	-1.095	0.9346

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**Please see supplementary sheet for figures-1-6, FT-IR spectrum and table-3 A and B**