Mycoremediation of hexavalent chromium by macrofungi: A novel approach

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Abstract
In the present study the biosorption efficiency of macrofungal cultures (Pleurotus florida, Pleurotus eous, Hypsizygus ulmarius, Schizophyllum sp., Coprinus sp. and Ganoderma lucidum) in the removal of hexavalent chromium (Cr (VI)) from aqueous solution was being explored. The influence of parameters like initial Cr (VI) concentration, macrofungal culture and contact time were studied. From the results, it was evident that maximum biosorption of Cr (VI) was observed in Schizophyllum sp. (86.24%) and Ganoderma lucidum (83.97%) at the Cr (VI) concentration and contact time of 75 mg/L and 15 days respectively. The FTIR spectra of the fungal biomass before and after Cr (VI) biosorption confirmed the presence of functional groups and their involvement in the biosorption process. Hence, Schizophyllum sp. and Ganoderma lucidum can be utilized as a Mycoremediation tool in removing Cr (VI) from contaminated water.

Keywords: Cr (VI), biosorption, macrofungal cultures, mycoremediation

Introduction
Heavy metal pollution is an overarching environmental problem which we are facing today. The heavy metals include toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, Ru etc.) and radionuclides (such as U, Th, Ra, Am, etc.) [1]. Chromium is the seventh most abundant element on earth [2]. Chromium is widely used in industries such as metallurgy, electroplating, production of paints and pigments, tanning, wood preservation, chemical production and pulp and paper production. These industries play a major role in chromium pollution with an adverse effect on biological and ecological species [3]. Tanneries discharge numerous polluting heavy metals and compounds into the water streams [4]. The most commonly occurring forms of chromium in the tannery discharge are trivalent- Cr$^{3+}$ and hexavalent- Cr$^{6+}$, with both states being toxic to animals, humans and plants. Cr (III) is immobile in its reduced form and is insoluble in water whereas Cr (VI) in its oxidized state is highly soluble in water and thus mobile [5]. The leather industry is associated with the generation of huge amount of liquid wastes, for the production of leather from one tonne of raw hides around 15,000 to 40,000 litres of wastewater is produced [6]. There are around 2091 tanneries functioning in India with major concern in Tamil Nadu accounting for 939 of total number of tanneries [7]. The World Health Organization (WHO) recommended maximum allowable limit for hexavalent chromium and total chromium in drinking water include 0.05 and 2 mg/L, respectively [8]. The maximum tolerance limit recommended by Central Pollution Control Board, India on drinking water for Cr (VI) is 0.05 mg/L [9]. Physical method explores physicochemical properties of the substances for remediation. The techniques like adsorption, electro dialysis method, membrane filtration, capping, granular activated carbon, photocatalysis, and soil washing etc., is included among physical methods of remediation [1]. Chemical remediation involves the use of chemicals like sulfur dioxide, sodium metabisulfite, ferrous sulfate, sodium sulfite, barium sulfite, lime and limestone for reduction of Cr (VI) to Cr (III).

Living organisms including bacteria, fungi, yeast, algae, and plants have shown remediation capabilities, but primarily bacteria and fungi have proven to be more efficient in remediation [10]. Fungi are the widespread organisms on earth with high biodiversity and strong biochemical potentials. Mycoremediation is the removal of toxic metals from soil and water in the polluted site using fungi. Fungal biomass has received much attention as a biosorbent
because of the presence of a high percentage of cell wall material, which increases the variety of functional groups involved in metal binding and the biomass production is high within short duration\textsuperscript{[11]}. The process parameters like adsorbent, contact time and solute concentration were determined to arrive at the maximum biosorption of hexavalent chromium from metal solution. The present study was carried out with an objective to explore the potential of macrofungi in the biosorption of Cr (VI) from the aqueous solution.

**Materials and methods**

**Preparation of the biosorbent material**
The pure culture of macrofungi such as *Pleurotus florida*, *Pleurotus eous*, *Hypsizygus ulmarius*, *Schizophyllum* sp., *Coprinus* sp. and *Ganoderma lucidum* were obtained from the culture collection facility available at the Department of Plant Pathology, TNAU, Coimbatore. The cultures obtained were sub-cultured using Potato Dextrose Agar medium. The disks were taken using sterile cork borer and placed in sterile petridish containing 20 ml of PDA medium\textsuperscript{[12]}. The plates were incubated in the incubation chamber at 25 ± 2°C for the development of mycelia.

**Batch biosorption experiment**
The experiment was carried in a completely randomized design with three replications. Batch experiments were performed at 25 ± 2°C in 250 ml Erlenmeyer flask containing 100 ml of potato dextrose broth and the desirable metal concentration (0, 25, 50, 75 mg/L) were prepared by adding suitable quantities of potassium chromate salt. The broth was autoclaved and initial concentration of chromium was determined by the methodology outlined\textsuperscript{[13]}. The fungal disk was inoculated under laminar condition and incubated for mycelial growth and chromium reduction. The influence of macro fungi, adsorbate concentration and contact time (0, 5, 10 and 15 days after incubation (DAI)) on the biosorption of Cr (VI) was studied.

The samples were collected (both the adsorbent and adsorbate) at 5, 10 and 15 DAI and were estimated for the concentration of total metal ion (Cr). The residual concentration of total metal ion (Cr) was determined by atomic absorption spectrophotometer (Perkin Elmer, AAnalyst 400). The concentration of hexavalent Cr in the samples were determined in UV spectrophotometer (UV-1800, Shimadzu) using Diphenyl carbazide method\textsuperscript{[14]}. The dry weight of the mycelial biomass at the end of incubation period was determined. Data presented are the mean values of three replications. All statistical analysis was done using SPSS 16.0 for windows by which it is possible to evaluate whether the interaction between the factors included are significant. The amount of Cr biosorbed per gram of biomass (q) and the efficiency of biosorption (E) were calculated using the equations 1 and 2.

\[
q = \left(\frac{C_i - C_f}{m}\right) V \quad (1)
\]

\[
E = \left(\frac{C_i - C_f}{C_i}\right) \times 100 \quad (2)
\]

Where q = mg of Cr uptake per gram of biomass (mg g\textsuperscript{-1}); C\textsubscript{i} and C\textsubscript{f} are initial and final concentration of Cr (mg L\textsuperscript{-1}); m is the dried mass of the fungal biomass (g) and V= volume of the reaction mixture\textsuperscript{[4]}.

**FTIR analysis**
To find out the possible functional groups involved in the biosorption of chromium from aqueous solution, FTIR spectra of the biomass before and after Cr biosorption was recorded with FT/IR-6800type A\textsuperscript{[15]}.

**Result and discussion**
The batch experiment was conducted with the macro fungi viz., *Schizophyllum* sp., *Ganoderma lucidum*, *Pleurotus florida*, *Pleurotus eous*, *Hypsizygus ulmarius* and *Coprinus* sp. for 15 days. The influence of different biosorption factors like initial hexavalent chromium concentration, macro fungal culture and contact time on the removal efficiency of chromium are concised in this section. The hexavalent chromium biosorption on macro fungus was obviously influenced by the initial chromium concentration in the aqueous solutions. The biosorption study was conducted with the chromium concentrations of 25, 50 and 75 mg/L. The results showed that with the increase in metal concentration the chromium biosorbed in the biomass increased from 20.73 to 63.58 mg/ g of the biomass as shown in Fig. 1. The concentration of hexavalent chromium decreased to a greater extent in the Potato dextrose broth inoculated with *Schizophyllum* sp., and *Ganoderma lucidum* as depicted in Fig. 2. The chromium biosorbed per unit mass of the biomass increased with increase in the chromium concentration in the solution as reported by Umesh K. Garga\textsuperscript{[16]} in the adsorption of Cr (VI) by agricultural waste biomass.

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**Fig 1:** Chromium biosorbed on macro fungal mass (mg / wt. of the culture)
The macro fungal cultures (biosorbent) used in the study plays an important role, where the *Schizophyllum* sp. recorded the highest biosorption efficiency of hexavalent chromium followed by *Ganoderma lucidum*. The biomass was also high for *Schizophyllum* sp. and *Ganoderma lucidum* compared to other cultures (Fig. 3). The higher the biomass produced is directly proportional to the higher biosorption of hexavalent chromium. The increase in the biomass can provide more area on the surface and availability of sites for binding \[17\]. The contact time for the biosorption was also comparatively an important parameter, the biosorption efficiency increased with increase in the contact time. The maximum biosorption efficiency of 86.24% for *Schizophyllum* sp. and 83.97 % for *Ganoderma lucidum* was achieved on 15th day (Fig. 4). It may be due to the increase in the available sites for biosorption as Cr (VI) has biosorbed into the intercellular of the biomass \[18\].

![Fig 2: Hexavalent chromium in the potato dextrose broth after 15 days at 75 mg/L.](image)

![Fig 3: Effect of hexavalent chromium on the fungal biomass](image)

![Fig 4: Biosorption Efficiency of the macro fungi at 75 mg/L (Cr (VI))](image)
For interpretation of functional groups involved in the binding of Cr (VI), FTIR analysis was carried out with the spectra of the adsorbents measured in the range of 4000-400 cm\(^{-1}\) wavenumber. FTIR absorption peaks of native and Cr (VI) loaded biomass (Schizophyllum sp, and Ganoderma lucidum) confirmed the existence of several functional groups on the surface of biosorbent.

The FTIR spectra of *Schizophyllum* sp. with and without hexavalent chromium adsorption is picturized in Fig. 5a and 5b. There is conspicuous changes (appearance or disappearance) in the FTIR spectrum. Figure 5b. showed fluctuation in the spectrum of the biomass after sorption of Cr (VI). The decrease in width and intensity of the band around 3295 cm\(^{-1}\) and the weakness of the band at 508 cm\(^{-1}\) with respect to control spectrum collectively illustrated that hydroxyl and nitro compounds, disulfide compounds contributed to adsorption. The FTIR spectra shows prominent weakness at the wave number of 2920 cm\(^{-1}\), 2360 cm\(^{-1}\), 1630 cm\(^{-1}\), 1548 cm\(^{-1}\), 1367 cm\(^{-1}\), 1021 cm\(^{-1}\) which corresponds to the functional group \(\text{CH group, NH group, stretching carboxyl amide group, amide II group, CN group, phosphate}

Similarly the FTIR spectra of *Ganoderma lucidum* biomass before (Fig. 6a.) and after Cr (VI) biosorption (Fig. 6b.), showed the spectra ranging from 3274 cm\(^{-1}\) \(-\text{OH to 554 cm}\(^{-1}\) \(-\text{nitro compounds and disulfide groups. FTIR spectral bands show decrease at different wave numbers such as 3272 cm}\(^{-1}\), 2924 cm\(^{-1}\), 1622 cm\(^{-1}\), 1022 cm\(^{-1}\) which corresponds to the functional groups include \(-\text{OH group, -CH group, amide I group and phosphate group respectively. The spectra analysis showed that there were several remarkable decrease in the absorption intensity of }\text{–NH and –CN after hexavalent chromium adsorption, which indicated that –NH and –CN group of protein were involved in Cr (VI) binding. Similar weakness occurred at band 1021 cm}\(^{-1}\) suggesting that \(-\text{PO}_4\) which consisted in polysaccharide, participated in Cr (VI) binding. As major composition of fungal cell membrane, phospholipid, protein and polysaccharide played a significant role in Cr (VI) biosorption. The peak intensity at 1630 cm\(^{-1}\), 1548 cm\(^{-1}\), moderately shrunk, showed that amide I and amide II were involved in Cr (VI) binding in some way. The peak intensity greatly lowers at 1021 cm\(^{-1}\) indicated that phosphate groups were fully loaded with hexavalent chromium. In present study, functional groups such as carboxylate, amine, phosphate, hydroxyl, sulphohydryl and other functional groups were responsible for cm\(^{-1}\) Cr (VI) binding ligands. The identification of these functional groups in the FTIR spectrum of the biomass before and after Cr (VI) sorption is indicative of their contribution to the biosorption of Cr (VI) by the *Schizophyllum* sp.

**Fig 5:** FTIR spectra of *Schizophyllum* sp, a) before and b) after Cr (VI) biosorption

**Fig 6:** FTIR spectra of *Ganoderma lucidum* c) before and d) after Cr (VI) biosorption
Amanita ∗. grows well, producing –. Vigna hafid W, and ardous materials. International Journal of Chemical Studies

References