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# Isolation and process parameter optimization of *Aspergillus* sp. for removal of chromium from tannery effluent

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**Abstract**

Five morphologically different fungi were isolated from leather tanning effluent in which *Aspergillus* sp. and *Hirsutella* sp. had higher potential to remove chromium. The potential of *Aspergillus* sp. for removal of chromium was evaluated in shake flask culture in different pH, temperature, inoculums size, carbon and nitrogen source. The maximum chromium was removed at pH 6, temperature 30 °C, sodium acetate (0.2%) and yeast extract (0.1%). *Aspergillus* sp. was applied in 2 l bioreactor for removal of chromium, and it was observed that 70% chromium was removed after 3 days.


*Keywords:* *Aspergillus* sp.; Bioreactor; Chromium; *Hirsutella* sp.; Biosorption; Fungi

## 1. Introduction

Chromium is considerable environmental concern as it is widely used in leather tanning, electroplating, metal finishing and chromate preparation. Chromium occurs in aqueous system in trivalent and hexavalent forms (Horitsu et al., 1987). Chromium(III) is used in tannery as chromium sulphate, which may be converted into chromium(VI) in the effluent. Hexavalent chromium may be converted to Cr(III) under reduced environment, which is much less toxic and less soluble by several microorganism possess chromate reductase, and thus reduction by these enzymes affords a means of chromate bioremediation. Chromium(III) proved to be biologically essential to mammals as it maintains effective glucose, lipid and protein metabolism. Chromium(VI) is taken up via sulphate or thiosulphate transporter, and oxidize biological molecules resulting in toxicity (Krishna et al., 2004). The hexavalent chromium has higher toxicity, and leads to liver damage, pulmonary congestion, skin irritation resulting in ulcer formation, and carcinogenic (Park et al., 2000). The maximum permissible levels of Cr(VI) in potable and industrial wastewater are 0.05 and 0.1 mg/l, respectively (Goyal et al., 2003).

Physicochemical methods employed for removal of heavy metals from the effluent such as precipitation with hydroxide, carbonates and sulphides, adsorption on the activated carbon, use of ion exchange resins and membrane separation processes are responsible for generation of pollution, and the processes related to removal of chromium at large scale are expensive (Kratochvil et al., 1998; Brown, 1991; Volesky and Holzen, 1995).

Biotransformation and biosorption are emerging technologies, which utilize the potential of microorganisms to transform or to adsorb metal (Chen and Hao, 1998). Intact microbial cell, live or dead and their products can be highly efficient bioaccumulator of both

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soluble and particulate forms of metals (Kratochvil et al., 1998). The cell surfaces of microorganisms are negatively charged owing to the presence of various anionic structures. This gives microorganisms an ability to bind metal cation (Chen and Hao, 1998). Microbial viability is essential for biotransformation as these reactions are enzyme mediated. Generally metal ions are converted into insoluble form by specific enzyme mediated reactions and are removed form the aqueous phase (Brierly et al., 1986). There are reports of live microbial systems for the purpose of remediation of contaminated soils and waters (Kratochvil et al., 1998). Higher fungi (mushrooms), seaweed and plant bark materials are abundantly available in nature and can be a source of low cost biosorbents (Pagnanelli et al., 2000; Brady et al., 1994; Nada et al., 1995). There are potent biosorbents easily available in algae, fungi and bacteria. A source of low cost biomass produced in great quantities, are marine macroalgae. The use of microbial cells as biosorbents of heavy metals is a potential alternative to conventional methods used to decontaminate liquid wastes.

Tanneries are mainly responsible for the release of huge amount of chromium in the environment. Pentachlorophenol and related biocides used in the leather tanning processes are refractive for the growth of microorganism and it also reduces removal of chromium in tannery effluent. Physicochemical methods have been practiced for several decades for the removal of toxic heavy metals from the effluent have not been successful (Brierley, 1991; Nada et al., 1995). Bacterial strain, *Acinetobacter* sp., has been used for removal of chromium from tannery effluent in sequential bioreactor indicated 80% reduction in chromium after 15 days (Micera and Dessi, 1988; Shrivastava and Thakur, 2003). But such a long time for removal of chromium may leads to generation and accumulation of metal and toxic compounds in the environment. Effective and efficient removal of chromium in short time is basic necessity of present time. Therefore, in the present investigation fungal strains have been isolated from tannery effluent and process parameters are optimized in presence of toxic form of chromium [Cr(VI)] with biotechnological methods for removal of chromium from tannery effluent and soil.

## 2. Methods

### 2.1. Isolation of fungal strains and fungal inoculums

The soil sample was collected from the sediment core of main channel of tannery effluent located at Jazmau, Kanpur, UP. The soil was serially diluted in 10-fold, and diluted sample (0.1 ml) was spread on the potato dextrose agar (PDA) plate. The plates were incubated at 30 °C for 4 days. The microbial colonies (fungi) appeared on the PDA plates were isolated, purified and characterized based on their morphological structures as colour, texture, and diameter of the mycelia and microscopic observation of spore formation. Fungal inoculum was prepared in the form of pellets. Erlenmeyer flasks (250 ml) containing potato dextrose broth and streptopenicillin (100 ppm) was taken and inoculated by mycelial discs. These flasks were incubated at 30 °C for 4 days with shaking in orbital shaker. The mycelium was filtered by cheesecloth and placed on petriplates. Water was evaporated and fungal disc was prepared by cutting in approximately 1.5–2.0 mm size.

### 2.2. Screening of potential strain

The fungal isolates were screened for their chromium removal potentiality under minimal salt medium containing (gm/l): $Na_2HPO_4 \cdot 2H_2O$, 7.8; $KH_2PO_4$, 6.8; $MgSO_4$, 0.2; $Fe(CH_3COO)_3NH_4$, 0.01; $Ca(NO_3)_2 \cdot 4H_2O$, 0.05, and pH adjusted to 5.5 as described by Thakur (1995). The salt of potassium chromate (500 ppm) was used as source of hexavalent chromium. The effluent was inoculated in an Erlenmeyer flask with individual fungal isolates and incubated at 30 °C in a rotary shaker for 7 days. Chromium was measured at an interval of 0, 1, 3, 5 and 7 days. On the basis of commiserative analysis percentage reduction in parameter was studied by the individual isolates along with the control and the most potential strains were selected for further analysis.

### 2.3. Optimization of process parameters

Sucrose, dextrose, sodium acetate and sodium citrate were used for optimization of carbon source. Batch study was conducted in the Erlenmeyer flasks containing potassium chromate (500 mg/l) supplemented with MSM, different carbon sources i.e. sodium acetate, dextrose, sodium citrate and sucrose (0.2%) and pH adjusted to 5.5. It was inoculated with 10% (w/v) of the fungal isolate for 7 days at 30 °C with shaking in rotary shaker (150 rpm). Sample was removed on 0, 1, 3, 5 and 7 days and chromium removal was analysed by flame atomic absorption spectroscopy. Sodium nitrate, urea and yeast extract powder were used for the optimization of nitrogen source. The rate of nitrogen source (0.1%), inoculum size, incubation period and temperatures, pH and sampling interval was similar. For the optimization of pH, the MSM-Potassium chromate solution was adjusted at pH 2, 3, 4, 5, 6, 7 and 8, respectively, potassium chromate (500 ppm) and C:N (0.2:0.1%, sodium acetate:sodium nitrate). It was inoculated with 10% (w/v) fungal (FK1) inoculum and incubated as described above. Erlenmeyer flask containing potassium chromate (500 mg/l) supplemented with MSM, carbon and nitrogen sources (0.2:0.1%, sodium acetate:yeast extract)