Removal of Cr(III) Ions from Tannery Waste Water Through Fungi

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Abstract: Cr(III) removal potential of a wood-rotting fungus viz., *Ganoderma lucidum* (Curt. Fr.) P. Karst was studied from tannery wastewater. Preliminary laboratory assays indicate an optimum pH, 4.5, stirring intensity 150 rpm with increase in removal rate on increasing initial metal ion concentration (4-20 mg L^{-1}) in the medium. The maximum biosorption capacity of fungus biomass was 2.16 mg g^{-1} with suitability of Langmuir and Freundlich models on acquired experimental data. In tannery wastewater, fungus showed a maximum of 1.6 mg g^{-1} biosorption capacity and 43% efficiency. To make this technique practically applicable and economically feasible, the study was further extended by mass cultivated this fungus on agro-wastes followed by assessment of its biosorption potency for Cr(III) ions. Rice straw colonized with *G. lucidum* mycelia could be utilized as an excellent biosorbent thus exhibited 73-76% efficiency for Cr(III) adsorption from tannery wastewater at low concentration of the metal (4-20 mg L^{-1}).

Keywords: Removal of Cr(III), Waste water, Fungi.

Introduction

Biosorption, metal removal ability of the certain biomass e.g. algae, fungi and bacteria is well-recognized, attractive and cost effective biotechnology for treatment of metal-loaded water. Fungi have been well known for metal removal ability from aqueous phase. Gadd (2001) stated filamentous network of hyphae help them to grow and survive in variety of ecosystems thus provides additional metal binding potential from the contaminated environment. The multilaminate and microfibrillar structure of fungal cell wall along with distinctive aspects of high percentage of cell wall material attributes excellent metal-binding properties. Generally on the basis of sizes, fungi are recognized as micromycetes and macromycetes. So far, numbers of micomycetes like species of *Aspergillus, Rhizopus*, *Penicillium*, *Mucor* and *Absidia* have been studied for their metal sorption capability (Dursun *et al.,* 2006; Congeevaram *et al*., 2007; Subudhi and Kar, 2008). The role of wood rotting fungi or macromycetes in removing heavy metals from the metal-loaded medium could not be negated. Number of macromycetes like *Pleurotus ostreatus*, *Ganoderma lucidum*, *Schizophyllum commune*, *Phanerocheate chrysogemum, Coriolus versicolor, Agaricus bisporus* and *Pycnoporus cinnabarinus* has been investigated for their metal uptake capability with very promising results (Arica et al., 2003, 2004; Javaid et al., 2008, 2010, 2011).

Macromycetes are commonly famous as mushrooms are found in world humid environment. Mushrooms are a appetizing and nutritious food group of fungi. Resihi mushrrom or *Ganoderma lucidum* is well-known oldest medicinal mushroom and is very important economically. Its fruit bodies typically grow in a fanlike or hoof like form on the trunks of living or dead trees. Spores have double-walled, truncate with yellow to brown ornamented inner layers. *G. lucidum* produces a group of triterpenes, called ganoderic acids, having similarity in molecular structure to steroid hormones. Other common compounds of this mushroom are variety of polysaccharides (beta-glucan, coumarin, mannitol) and alkaloids (Paterson, 2006). The potential of both fruiting body and mycelium of *G. lucidum* in bioremediation of heavy metal is well known.

The most compelling reasons for using biosorption technology are utilization of renewable or waste raw materials in economic way. The application of biosorption technology could become more effective keeping economic feasibility through utilization some cheap source for mycelial production of fungi. By doing this practice we not only get economic substrate for mycelial mass cultivation but there would be addition adsorbent having plant cell wall properties. The plant by products e.g. wheat straw and husk, rice straw and husk and cotton waste etc are easily available substrates for raising fungal mycelium in bulk. However, the biosorption potential of macromycetes preparation on agro-waste especially for industrial heavy metal ions removal has not been extensively attempted.

The aim of current research work was to explore the role of *G. lucidum* in removing Cr(III) from tannery wastewater. For large scale industrial application mycelium of *G. lucidum* was mass cultivated on rice straw and colonized straw was used for removal of Cr(III) from tannery water.

Methodology

Fungal biomass was prepared in liquid phase in 250 ml Erlenmeyer flasks filled with 100 mL of a culture medium i.e. 2% Malt Extract (ME) composed of 20 g L^{-1} ME powder (DIFCO). The prepared fungal mat after 10 days of incubation was collected, washed with generous amounts of distilled water as long as the pH of the washing solution was in the near-neutral range (7.0-7.2). Dried at 60 ± 1 °C was utilized in metal sorption assays.

Batch experiments were performed by taking 0.2 g of oven dried biomass of test fungus in 250 mL flask containing 100 mL of 14.35 mg L^{-1} (particular concentration chosen on the basis of quantitative measurement of Cr(III) present in tannery water) of Cr(III) solution at 150 rpm and 25°C for 3 hours. To select the optimum pH, this parameter was varied over the range of 2-6.

The rotation speed was monitored at low (50 rpm), medium (100 rpm) and high (150 and 200 rpm) taking non-agitated system as the control. Effect of initial metal ion concentration was investigated by changing the initial concentration of Cr(III) ions within the range of 4-20 mg L^{-1} at constant pH and temperature. At the end of experiment, remaining Cr(III) in the supernatant was measured on Atomic absorption spectrophotometer (Model, Varian AA 1275 series).

Cr(III) removal ability of fungus was checked with water of tannery by repeating batch biosorption experiment in same way mentioned above. Acid digestion of the samples was carried out with aquaregia for measurement of residual metal ion concentrations.

Rice straw was used for mycelial cultivation *G. lucidum*. The pre autoclaved inoculated plastic bags (20 x 30 cm) were kept in an incubator at 25°C for 12-15 days. The prepared straw colonized with fungus mycelia was dried in oven and sun. Oven drying was carried out at 60°C for 24 hours and for sun drying prepared material was exposed directly to sun light (40-42°C) for 12-14 hours at 40-50 °C. The biosorption experiments were performed with tannery water under pre optimized biosorption conditions as mentioned in section batch biosorption experiments.

Results and Discussion

Effect of pH

The biosorption capacity/efficiency of the candidate fungus for Cr(III) was recorded to increase significantly at appreciable amount on increasing pH from 4.5 (2.0 mg g^{-1}/28%) to 5.5 (2.0 mg g^{-1}/27%) with slight reduction at pH 6.0 (1.63 mg g^{-1}/23%). However, at high acidic pH range i.e., 2.0-4.0 the test fungus exhibited only up to 7-18% efficiency. Consequently, predilection of pH by the test fungus for the highest adsorption of metal ion exhibited following sequence 4.5 > 5.0 ≥ 5.5 > 6.0 > 4.0 > 3.5 (Fig.1). Similar to present records, pH range 4.5-6.0 has been reported as optimal for biosorption of Cr(III), Cu(II), Ni(II) and Zn(II) ions both by micro and macro fungi in several previous studies (El-Syed and El-Morsey, 2004; Javaid et al., 2010; Javaid et al, 2011). These authors attribute that low pH (4.0 and below) limits the biosorption metal ions on to fungal biomass surfaces, probably due to the ion exchange between metallic species and competition effects with oxonium (hydronium) ion to some extent in the biosorption mechanism.

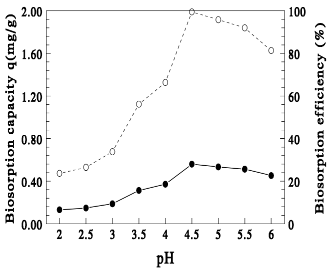

Figure 1: Effect of pH on biosorption potential of the *G. lucidum* for Cr(III) ions. Initial concentration of Cr(III) ion in the reaction mixture: 14.35 mg L^{-1}. Biosorption conditions: biosorbent concentration, 0.2 g 100 mL^{-1}; pH range (2.0-6.0); 150 rpm and 25°C for 5 hours.

Effect of Stirring Intensity

The effect of the agitation of the sorbent/sorbate system indicated that all agitation speeds exerted a remarkable increase in biosorption efficiency of 21-27% over the non-agitated system that holds only 3.48% efficiency. Therefore, metal removal capacity reached its maximum level at 150 rpm (1.92 mg g^{-1}) and then decreased slightly at 200 rpm (1.68 mg g^{-1}) followed by 50 and 100 rpm (1.50 mg g^{-1}) in comparison to control (0.25 mg g^{-1}) (Fig. 2). Agitation facilitates proper contact between the metal ion in solution and the biomass binding sites and thereby, promotes effective transfer of sorbate ion to the sorbent sites (Chergui *et al*., 2007).

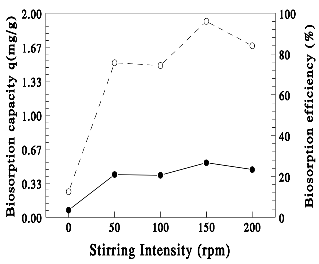

Figure 2: Effect of stirring intensity on sorption potential of *G. lucidum* for Cr(III) ions. Initial concentration of Cr(III) ion in the reaction mixture: 14.35 mg L^{-1}. Biosorption conditions: biosorbent concentration, 0.2 g 100 mL^{-1}; pH, 5.0 at 25°C for 5 hours.

Effect of Initial Concentration of Metal ions

The fungus subjected to varied concentrations of Cr(III) from 4-20 mg L^{-1}, exhibited an increase in sorption capacity of 1.02-2.40 mg g^{-1} on increasing metal ion concentrations and maximum uptake was evident at the highest applied concentration (Fig. 3). There is evidence that at high metal ion concentration the number of ions sorbed is more than at low metal concentration, where more binding sites were free for interaction (Cossich *et al*., 2002; Loukidou *et al*., 2004).

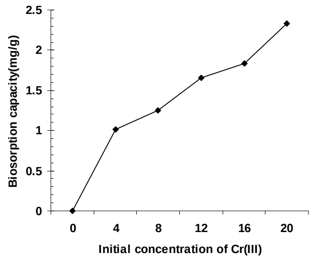

Figure 3: Biosorption capacity of *G. lucidum* for Cr(III) ions at various initial concentrations.

Isotherm Assessment

Through the scatter diagram the analysis of Langmuir (1906) and Freundlich (1916) isotherm models were obtained for the test fungal biomass (Table 2, Fig. 4 A, B). The distribution of q_{eq} point calculated by the model in function of the experimental values of q_{eq} shows a linear tendency among the observed and predicted values. This indicated that the experimental data were very well adjusted to the two models.

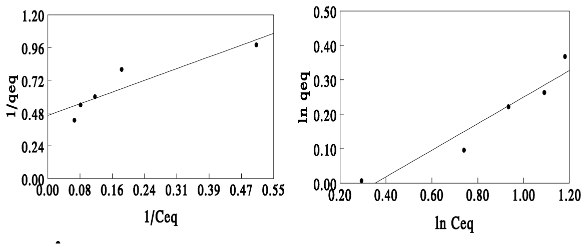

Figure 4 A & B: The linearized Langmuir (A) and Freundlich (B) adsorption isotherm for Cr(III) ion biosorption by *G. lucidum*..

Physicochemical Analyses of Tannery Wastewater

The results obtained on physicochemical analyses of the tannery wastewater are shown in Table 1. All analytical techniques employed were item standard methods (APHA, 1995) and chemicals used in the experiment were of analytical grade (MERCK).

Table 1: Physico-Chemical Characterization of Tannery Treatment Plant.

Parameters	Tannery Treatment Plant, Current status	NEQS Acceptable limits	WHO Acceptable limits
Copper (II), mg L^{-1}	1.21	1.00	0.20
Chromium (III), mg L^{-1}	14.35	1.00	0.10
Nickel (II), mg L^{-1}	0.05	1.00	0.20
Zinc (II), mg L^{-1}	0.54	5.00	2.00
pH Value (acidicity/basicity)	8.5-9.5	6.0-10	No guideline
Temperature ($^{\circ}C$)	27-29	40	No guideline
Biochemical Oxygen Demand (BOD) at 20°C, mg L^{-1}	500	80	No guideline
Chemical Oxygen Demand (COD), mg L^{-1}	4000	150	No guideline
Total Dissolved Solids (TDS), mg L^{-1}	1510	3500	No guideline

National Environmental Quality Standards (NEQS) for liquid Industrial Effluents (2001); World Health organization Standards (WHO) for drinking water (2006).

Table 2: Isotherm parameters for the biosorption of Cr(III) ion onto fungal biomass.

Test fungus	q_{exp} (mg g^{-1})	Langmuir			Freundlich		
		q_m (mg g^{-1})	b (mg L^{-1})	R^2	K_F	n	R^2
G. lucidum	1.92	2.16	0.42	0.91	1.37	2.59	0.96

Biosorption Assays with Tannery Water

The optimized conditions determined from preliminary biosorption assays were used to conduct biosorption experiment with tannery water. For comparison parallel batch experiment was conducted with synthetic solution. Results acquired indicate only 1% reduction in biosorption efficiency of the biosorbent in tannery wastewater in comparison to control (synthetic solution) (Table 3). Similar findings were recorded by Matheickal and Yu (1999) while investigating the effect of light metal ions Na^+, K^+, Mg^{2+} and Ca^{2+} on the biosorption of Pb(II) by *Durvillaea potatorum* and *Ecklonia radiata*. They found that biosorbents had much higher relative affinity for heavy metals than for light metals.

Table 3: Comparison of biosorption capacity and efficiency of the test fungus for Cr(III) ions in synthetic solution and Tannery Treatment Plant Water.

Treatments	Rice straw (OD)	Rice straw (SD)
Control (uncolonized agro-waste)	67.73c	69.82b
Colonized agro-waste with mycelia of *G. lucidum*	73.98ab	75.84a

Initial concentrations of Cr(III) ion in both systems: 14.35 mg L^{-1}. Biosorption conditions: pH 4.5, 150 rpm for 3 hours.

Table 4: Comparison of biosorbents biosorption efficiency (%) for uptake of Cr(III) ions from Tannery wastewater

Parameters	Synthetic solution of Cr(III)	Actual wastewater of Tannery treatment plant
Biosorption capacity (mg/g)	1.57	1.51
Biosorption efficiency (%)	43.87	42.51

Values with the different letters showed significant difference among treatments at $p < 0.01$ according to Duncan's multiple comparison tests.

Biosorption Assays with Colonized and Uncolonized Agro-waste

Both uncolonized and colonized straw with *G. lucidum* mycelia exhibited significantly greater metal removal potential from tannery water (Table 4). However, colonized agro-wastes exhibited greater biosorption potential (70-76%) as compared to un-colonized (60-70%). Enhancement in adsorption ability of colonized rice straw could be owing to fungal lignin and cellulose degradation of agro-wastes. As results of this degradation, the polymer core of straw turn into soluble form and more binding sites on straw are available for metal binding in addition to fungal cell wall molecular binding sites on mucelium (Lee and Rowel, 2004). Results showed that sun dried rice straw exhibited significantly greater adsorption capacity/efficiency (1.36 mg g^{-1}/75.84%) in comparison to oven dried straw (1.33 mg g^{-1}/73.98%). There might be possibility that oven drying cause denaturtaion of some important chemical groups on the cell wall of biosorbents which remain intact in case of natural drying under sun (Huang and Hunag, 1996).

Conclusion

Due to low cost involved and simplicity of the technique, rice straw colonized with *G. lucidum* mycelia could be utilized as an excellent biosorbent exhibited efficiency of 73-76% for removal of Cr(III) ions from tannery water containing diluted concentration of the metal (4-20 mg L^{-1}).

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