

Removal of Cr(III) through Bread Mold Fungus

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Abstract

Cr(III) removal ability of indigenous soil fungus of Pakistan (i.e. *Rhizopus arrhizus* Fisher) was checked through batch trials. Experiments were performed by taking 0.1 g of powdered fungal biomass in 100 mL of metal solution kept at 150 rpm for 3 hours. Results of FTIR spectroscopy revealed that amine (-NH₂) and hydroxyl (-OH) groups participated in Cr(III) binding with the fungal biomass. There was an inversely proportional relationship between adsorption efficiency and capacity on increasing initial metal concentration in the range of 20-100 mg/L. The adsorption capacity of fungal biomass was 43.47 mg/g as determined by Langmuir isotherm. High correlation coefficient (R²: 0.99) acquired through Langmuir and Freundlich models adequately described the adsorption mechanism on fungal biomass. pH optimization trials in the range of 2-10 exhibited significantly greater adsorption efficiency of 75% at pH 4, while metal removal rate declined with increasing pH. Adsorption/adsorption trials with four acids indicated that maximum desorption of Cr(III) was found with HCl, followed by HNO₃, CH₃COOH, and H₂SO₄. Adsorption-based trials summarized that *R. arrhizus* is a potential and inexpensive biomaterial with viable application in the removal of Cr(III) from the aqueous solution.

Keywords: adsorption, bread mold fungus, metal, Cr(III), desorption

Introduction

Chromium (Cr) is classified in Group A of human carcinogens by the U.S. Environmental Protection Agency [1] the second most common contaminants in groundwater, soil, and sediments [2]. It is widely found in municipal sewage as well as in the effluents of steel, electro-plating, leather tanning and textile industries. Moreover, it is released as a result of chromate preparation, wood preservation, fungicides, nuclear power, and weapons production [3]. Discharge of Cr along with other heavy metals into surface and ground water has become a serious problem in Pakistan over the last two decades [4]. Thus the concentration of chromium in wastewater, drinking water, and soil of

Pakistan was found to be in the range of 16.12-36.83 mg/L, 1.0483-3.1824 mg/L, and 3.45-11.43 mg/kg, respectively [5]. According to the Pakistan standards, the maximum discharge limits for Cr in wastewater is 1.0 mg/L, and the utmost permissible level in drinking water is 0.05 mg/L [6].

The usual methods to remove Cr from aqueous effluents include nanofiltration [7], chemical reduction [8], ion exchange [9], adsorption on activated carbon [10], and silica composites [11]. Yet major drawbacks with these conventional methods are incalculable, like high reagent requirements, formation of sludge, and its disposal in addition to high installation and operational costs, particularly in developing countries [12]. Therefore, integrated technology, namely 'Biosorption,' "based on binding capability of various biological materials" is a preferred prospective option because of its high efficiency, easy handling, and simple accessibility to the biosorbent materials [4].

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Various biosorbents such as algae, fungi, bacteria, and industrial waste biomass have been utilized for Cr removal [13, 14]. Utilization of the fungal biomass as a biosorbent could fulfill requirement of economic and efficient adsorbents for treatment of metal-bearing effluents. Fungal biomass can easily be acquired from cheaper sources (e.g. fermentation waste of many industries) required in small amounts to efficiently adsorb metal along with reutilization and desorption capabilities [15]. However, information available on interaction of the fungi with Cr ions is still limited [16]. Literature showed better chromium removal ability of many fungal species of *Rhizopus*, *Aspergillus*, and *Saccharomyces* [17]. Sepehr et al. [18] findings demonstrated that *Aspergillus oryzae* can remove 90-97% Cr(III) from tanning house effluent. The experimental work of Ahmad et al. [19] revealed significant uptake capacity of 6.20-9.5 mg/g and 2.3-8.21 mg/g for Cr(III) and Cd(II), respectively, by pretreated dead biomass of *Aspergillus* and *Rhizopus* spp. Pretreated biomass of *Aspergillus niger* was found to be the most promising for 95% removal of Cr(VI) within 288 minutes from an aqueous solution of 400 mg/L [20]. Prigione et al. [21] findings demonstrated that *Fusarium solani*, *Mucor circinelloide*, *Cunninghamella elegans*, *Rhizomucor pusillus*, and *Rhizopus stolonifer* can remove up to 40% of Cr(III) from tannery effluents. Lokeshwari and Joshi [22] have obtained 95% removal of Cr(III) with biomass of yeast.

Our current study was designed to explore the Cr(III) removal potential of dried biomass of *R. arrhizus* and its FTIR spectroscopy, followed by optimization of pH and the influence of initial concentrations of metal ions on the biosorption process. To make this technique economically feasible, desorption capacity of fungal biomass for Cr(III) were checked with different acids.

Experimental Procedure

The stock solutions of Cr(III) were prepared by dissolving 7.70 g Cr(NO₃)₂·9H₂O (Merck Germany) in one liter of distilled water. Further dilutions (20, 40, 60, and 100 mg/L) were made from the stock solution with double distilled water.

R. arrhizus (Accession number: FCBP 800) was procured from First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan. Fungal biomass was prepared on 2% Malt Extract and incubated for 5 days at 25±2°C. Prepared biomass was washed with distilled water, dried at 60°C, and powdered for utilization in experiments.

Adsorption trials were conducted by taking 0.1 g of fungal biomass in 100 mL of 25 mg/L of metal solution. The mixture pH was kept at 4 in an orbital shaker at 150 rpm for 3 hours. After desired contact time biomass was filtered and filtrate was analyzed for remaining metal concentration on an atomic absorption spectrophotometer. pH optimization experiments were performed at pH values of 2, 4, 6, 8, and 10, while other parameter were as described above.

Influence of initial concentration of metal ions was investigated over the range of 20-100 mg/L at constant adsorbent dose, pH and temperature.

After adsorption the fungal biomass was checked for its metal desorption capability under four desorption media (HCl, HNO₃, H₂SO₄, and CH₃COOH) with two working concentrations (0.1N and 0.5N) for each acid. Pre-used metal-loaded fungal biomass was kept in 100 mL of acid solution stirred at 400 rpm for 60 minutes at 25±2°C in 250 mL Erlenmeyer flasks. Residual metal ion concentrations in the desorption medium were determined on an atomic absorption spectrophotometer.

For the analysis of IR spectra of control and metal-loaded fungal biomass, the sample preparations were carried out according to the method of Patty and Feist [23]. Mulls (a two phase mixture) were prepared by grinding the sample (2-5 mg) in a smooth mortar. Spectra of the sorbents before and after Cr(III) sorption were studied by using hexachlorobutadiene and chlorofluorocarbon oils as complementary mulling agents.

The amounts of chromium accumulated by biomass and efficiency of biosorbents were calculated by the following formulas [24]:

$$q = \left(\frac{C_i - C_f}{m} \right) V$$

$$E = \left(\frac{C_i - C_f}{C_i} \right) * 100$$

...where C_i is the initial concentration of the metallic ion (mg/L), C_f is the final concentration of metallic ion (mg/L), m – dried mass of the biosorbent in the reaction mixture (g), V – volume of reaction mixture (mL).

Langmuir [25] and Freundlich [26], adsorption isotherm models were applied on experimental data acquired through biosorption experiments at different initial concentrations of Cr(III) in aqueous solution.

$$\text{Langmuir model: } q_{eq} = q_{max} b C_{eq} / (1 + b C_{eq})$$

$$\text{Freundlich model } q_e = K_F (C_e)^{1/n}$$

...where: q_{eq} – metallic ions adsorbed per unit of weight of adsorbents at equilibrium (mg/g), q_{max} – maximum adsorption capacity of metal (forming monolayer) per unit weight of the adsorbent (mg/g), b – constant related to the affinity of binding sites for metal ions (L/mg), C_{eq} – equilibrium concentration (mg/L), K_F (L/g), and n – Freundlich characteristic constants.

All experimental data acquired in triplicate were used to compare data. The results obtained in various biosorption experiments were subjected to Duncan Multiple Range Test [27].

The eluted metal per gram of biomass (q_{des}) from the concentration of metal desorbents (C_{des}) in the solution was calculated by the following equation:

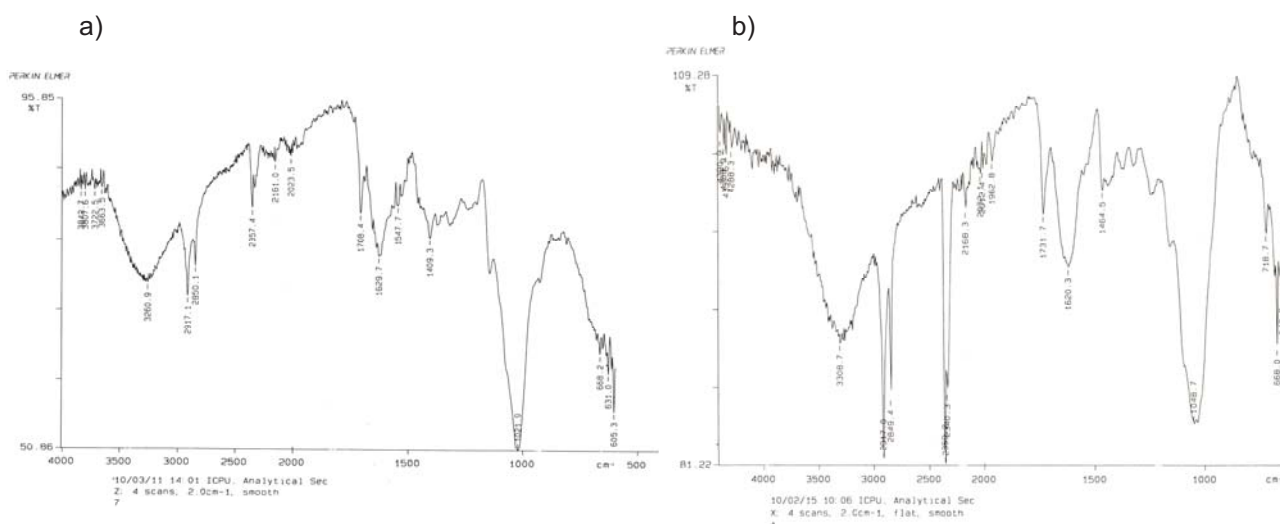


Fig. 1. Comparison and characterization of infrared spectra of raw (a) and Cr(III)-loaded (b) mycelial biomass of *Rhizopus arrhizus*.

$$q_{des} = C_{des} \frac{V}{W}$$

...where: V – volume of solution, W – weight of biosorbent

Results

The results from FTIR spectra of raw biomass showed peaks between the 3,300 and 3,700 cm^{-1} regions due to hydroxyl linkage, a peak at 1,708 cm^{-1} corresponds to C=O of ketones, two peaks at 1,629 cm^{-1} and 1547 cm^{-1} indicates NH of secondary amines, and the peak at 1,021 cm^{-1} is due to C-C, C=C, C-O-C, C-O-P of saccharides. After treatment with Cr(III) all peaks either disappeared or shifted their position, indicating involvement of functional groups on these peaks in metal bindings (Fig. 1).

When concentration of Cr(III) was increased from 20 mg/L to 100 mg/L, the recorded efficiency significantly decreased from 75% to 50% and adsorption capacity increased from 15 mg/g to 41 mg/g (Fig. 2).

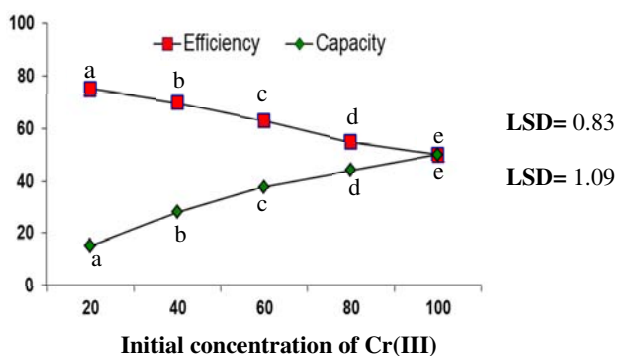


Fig. 2. Influence of different concentrations of Cr(III) ions on adsorption efficiency and capacity of the *Rhizopus arrhizus*. Different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

The data for the adsorption of Cr(III) onto *R. arrhizus* was analyzed in light of the Langmuir and Freundlich mode of adsorptions. The Langmuir equation is based on the concept of preponderance of a distinct binding site. A theoretical maximum biosorption capacity (i.e. q_{max} : 43.47 mg/g) obtained through Langmuir equation is very close to q_{exp} 41 mg/g. Other parameters, like high correlation coefficient (R^2 : 0.99) and small stability complex (b : 0.008 mg/L) also confirmed application of the Langmuir model on the adsorption of Cr(III) by *R. arrhizus* over the concentration range (20-100 mg/L) (Fig. 3a).

The Freundlich equation serves as a practical tool for simulating adsorption processes. Data evaluation according to the Freundlich constant along with correlation coefficient (R^2) were calculated from the corresponding plot for the biosorption of Cr(III). The high correlation coefficient (R^2 : 0.99) calculated through Freundlich model adequately described the multilayered adsorption mechanism on to fungal biomass (Fig. 3b).

The effect of pH on fungal metal removal ability was a critical factor. In present findings, both metal adsorption efficiency and capacity of the fungus significantly increased on increasing pH from 2 to 4, and on further increasing pH from 4 to 10, both efficiency and capacity declined (Fig. 4).

Adsorption/desorption trials were done with four acids i.e., HCl, HNO₃, CH₃COOH, and H₂SO₄. Among these, HCl showed maximum desorption of Cr(III) ions and desorption capacity of the fungal biomass was found maximum with HCl (13.2 mg/g), followed by HNO₃ (9 mg/g), CH₃COOH (8.25 mg/g), and H₂SO₄ (7.3 mg/g) (Fig. 5).

Discussion

Current results showed that biomass of *R. arrhizus* exhibited the potential to remove Cr(III) from the aqueous solution. FTIR spectra of both crude and Cr(III)-laden bio-

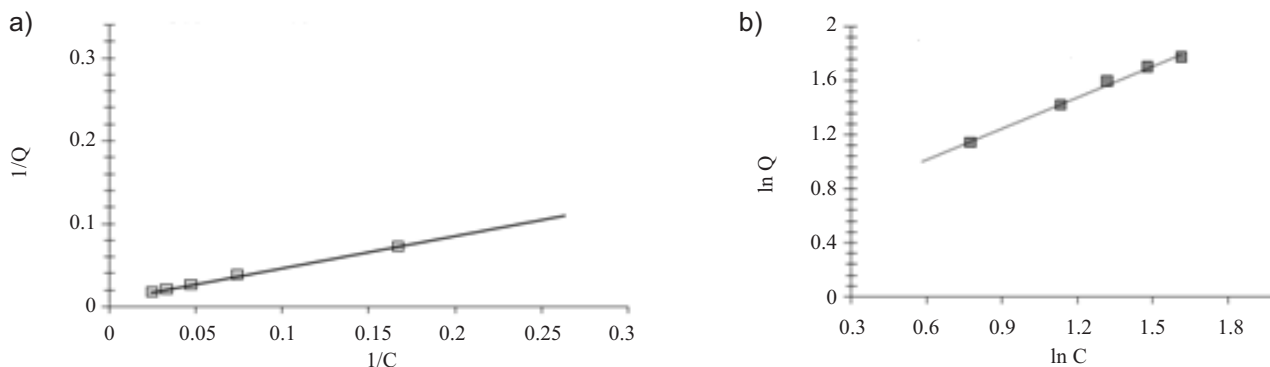


Fig. 3. Langmuir (a) and Freundlich (b) adsorption isotherms for biosorption of Cr(III) ions onto biomass *Rhizopus arrhizus*.

mass showed involvement of NH, H-bonded, and OH groups in metal binding. *R. arrhizus* belongs to zygomycota, being rich in chitin and chitosan content of cell wall, which are well-known for their metal-binding potential [28]. N-containing groups of chitin and chitosan are accountable for the immobilization of heavy metals depending on the metal and pH [29]. Thus, presently the change in wave number of 1,515-1,595 cm^{-1} is due to N-containing groups of secondary amines and amides (def) in carboxylate and hydroxylate of protein (amides I and II) of

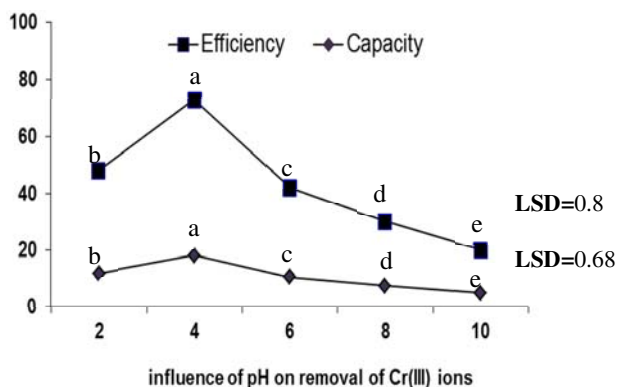


Fig. 4. Influence of different pH values on adsorption efficiency and capacity of the *Rhizopus arrhizus*. Different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

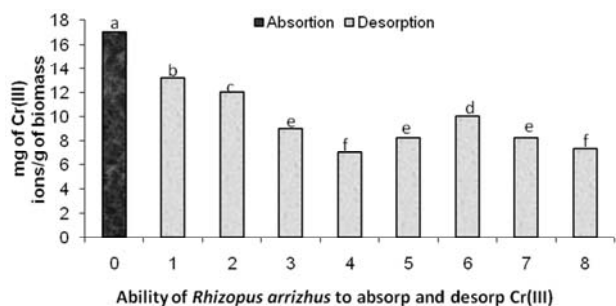


Fig. 5. Ability of different acids to desorb Cr(III) ions from the biomass of fungus. 1: HCl (0.1M), 2: HCl (0.5M), 3: HNO₃ (0.1M), 4: HNO₃ (0.5M), 5: CH₃COOH (0.1M), 6: CH₃COOH (0.5M), 7: H₂SO₄ (0.1M), 8: H₂SO₄ (0.5M). Different letters show significant difference ($P \leq 0.05$) as determined by Duncan's Multiple Range Test.

the biomass after association with metal cations [30]. However, the phenomena can also occur due to conversion of unbound N-H into the bonded NH group that resulted in a downshift of the stretching as well as the bending absorption bands. Alteration in the intensity between the 3,300 and 3,700 cm^{-1} regions is mainly due to free O-H in the control spectrum transformed into bonded O-H, when fungal biomass uptake metal. These hydroxyl groups are an integral part of polysaccharides and become negatively charged, thereby possibly having a high affinity for divalent cations [30]. Vibrational shifting of various functional groups such as C-O, C-C, C-O-C and C-O-P stretching vibrations of polysaccharides to various modes of higher and low stretching frequencies signify their involvement in Cr(III) bonding onto treated biomass [31].

There was significant reduction in uptake efficiency and considerable increase in adsorption capacity with increasing metal concentration from 20-100 mg/L. However, the biomass of *R. arrhizus* exhibited a maximum efficiency of 75% and minimum capacity of 15 mg/g at a concentration of 20 mg/L. Overall, a net reduction in efficiency was 1-30% and capacity increased 3-fold on elevating metal concentration. These results are similar to the observations obtained with different biomaterials [16, 22, 32]. The decrease in removal efficiency can be explained by the fact that all the adsorbents had a limited number of active sites, which would have become saturated above a certain concentration. The increase in adsorption capacity with increase in metal concentration may be due to the higher adsorption rate and utilization of all active sites available for adsorption at higher concentration [33].

The applicability of Langmuir isotherm to the adsorption of Cr(III) ions was proved by the high correlation coefficients ($R^2 > 0.99$), indicating strong binding of metal ions to the adsorbent surface over the concentration range (20-100 mg/L) studied. The Langmuir model is based on the assumption that maximum adsorption occurs when a saturated monolayer of solute molecule is present on the adsorption surface, and the energy of adsorption is constant and there is no migration of adsorbate molecule in the surface plane [25]. The same findings were observed in our experiment. The value of n ($0 < n < 1$) related to the distribution of bonded ions on the sorbent surface specify beneficial adsorption even at high metal concentrations.

Presently for Cr(III), the calculated value of n (i.e. 1.30 for *R. arrhizus*) is greater than one indicating favorable adsorption. The magnitudes (K_f) of Freundlich constant express easy separation of metal ions from aqueous medium [34].

pH optimization trials in the range of 2-10 exhibited significantly greater adsorption efficiency of 75% at pH 4 [11, 35]. At low pH (2-3) adsorption sites are more protonated and are less attractive against various cationic forms of metals, i.e. electrostatic repulsion will be high. Electrostatic repulsion decreases with increasing pH (4-5) due to reduction of positive charge density on the sorption edges, thus resulting in increased metal biosorption [36]. At pH values 6-10, the amount of metallic ions taken up was decreased due to the formation of soluble complexes of the metal ions and their competition with the active sites [37].

The acids were used as the desorbing agents because in acidic medium the carboxyl, carbonyl, or hydroxyl groups in the biomass become protonated and do not attract the positively charged metal ions, and so the protons replace the bound metal ions [21]. The result indicated high desorption efficiency of 0.1M HCL during the adsorption/desorption operation for Cr(III) ions followed by HCL (0.5M) > CH₃COOH (0.5M) > HNO₃ (0.1M) > CH₃COOH (0.1M) > H₂SO₄ (0.1M) > H₂SO₄ (0.5M) > HNO₃ (0.5M). Diniz and Volesky [38] confirmed in their studies that mineral acids like hydrochloric acid and nitric acid seem to be the most practicably applicable eluting agents. They also revealed that a new low-pH equilibrium attained in the desorption system during weak acidity prevents arrant elution and discharge of the adsorbate metal ion into the solution. It is also stated that the comparatively small individual metal affinity differences did not play any role when the concentration of protons is very high: the protons actually just embroiled the metals away from the sorbent [31, 38].

Conclusions

- FTIR technique proves to be an efficient tool for detecting changes in functional groups e.g. NH₂ and OH of fungal biomass after loading with Cr(III) from aqueous solution.
- The well-fitted isotherms viz., Langumir and Freundlich explain that chromium adsorption is favorable for *R. arrhizus* biomass.
- The solution pH was found to play a critical role in the biosorption process and adsorbent displayed higher efficiency (75%) at pH 4.
- Desorption possibility of metal ions from the fungal biomass was maximum with HCl, which suggest it as a potential and economic adsorbent for treatment of chromium-loaded wastewater.

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