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RESEARCH PAPER

Biosorption of Chromium by Fungal Strains Isolated from Industrial Effluent Contaminated Area

Swati Narolkar[1*], Nitesh Jain[1] and Arvnabh Mishra[2]

1. Department of Integrated Biotechnology, Ashok & Rita Patel Institute of Integrated Study and Research in Biotechnology and Allied Sciences (ARIBAS), New Vallabh Vidyanagar 388 121, India
2. Department of Industrial Chemistry, Institute of Science and Technology for Advanced Studies and Research (ISTAR), Vallabh Vidyanagar 388 120, India

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ABSTRACT

The ability of fungi to act as bio-sorbent had been extensively evaluated and has shown excellent metal sequestering ability for heavy metals such as cadmium, copper, zinc, lead, iron, nickel, radium, thorium, and uranium from aqueous solution. In the present study, tolerance, removal efficiency and adsorption capacity of hexavalent chromium using isolated fungal strains were analysed. Total nine fungal isolates were obtained from organic pollutants and metals contaminated Gujarat Industrial Development Cooperation sites. Filamentous fungi isolated belonged to *Aspergillus* spp., *Rhizopus* spp., *Trichoderma* spp., and *Penicillium* spp. Chromium sorption experiments using isolated fungal strains were carried out to check adsorption capacity and adsorption intensity. At higher chromium concentration, removal efficiency and adsorption capacity were observed in the order of *Aspergills candidus* > *Aspergillus ochraceus* > *Aspergillus flavus* > *Rhizopus* spp. > *Trichoderma* spp. *A. candidus* showed higher adsorption capacity, 5.49mg/g with 98.75% chromium removal efficiency at 150ppm of hexavalent chromium. The observed R_L value for Langmuir isotherm for all the three concentrations was less than 1, depicting favourable sorption and in Freundlich isotherm, the value of 1/n exceeds more than 1 showing co-operative or similar type of adsorption.

Key words: Chromium; Heavy metal; Isotherm; Adsorption kinetic

INTRODUCTION

Rapid industrialization has led to increased disposal of heavy metals and radionuclides into the environment. Therefore, the removal of toxic heavy metals from environment by eco-friendly method is inevitable along with keeping cost in mind. Chromium is a transition metal of broad application, with multiple oxidation states ranging from Cr (II) to Cr (VI), but the trivalent Cr (III) and the hexavalent states Cr (VI) are the most stable (Yadav et al., 2005).

Chromium naturally found in rocks, soil, plants, volcanic filth, and animals. Chromium compounds are largely used in many industries like metallurgical, textile, metal cleaning, dye refractory, wood processing, leather, and chemical manufacture (Dhal et al., 2013). Several physicochemical processes such as solvent extraction (Mitra et al., 2017), membrane filtration (Jamshidifard et al., 2019), adsorption (Peng et al., 2020), ion-exchange (Meshram et al., 2018), and chemical precipitation (Ramakrishnaiah & Prathima, 2012) are used for

* Corresponding author Email: swatizalanarolkar@gmail.com

bioremediation of Cr (VI). Conventional methods used for heavy metal removal have several disadvantages like incomplete metal removal, energy requirements, production of toxic sludge (Ahalya, et al., 2003; Prigione et al., 2009). Chromium is a transition metal of broad application, with multiple oxidation states ranging from Cr (II) to Cr (VI), but the trivalent Cr (III) and the hexavalent states Cr (VI) are the most stable (Yadav et al., 2005). Hexavalent chromium Cr (VI) is extremely soluble, and solubility accelerate active transport of chromate across the biological membranes and occupational exposure may damage cellular components by generating free radicals. Chromate reductase producing microbial cells enable to reduce Cr (VI) to Cr (III) by direct or indirect means both outside and inside cells (Tang et al., 2021). The biological reduction of Cr (VI) to Cr (III) has provoked scientists and engineers as the process has capability not only to diminish chromium toxicity in living organisms but may also aid by precipitating chromium at unbiased pH in the form of Cr (OH).

For the removal of Cr (VI) from wastewaters techniques used from prolong time are ion-exchange resins, filtration, chemical precipitation, reverse osmosis, electro dialysis, photo catalysis and adsorption. These Cr (VI) reduction and Cr (III) precipitation steps not only devour considerable number of acids and bases but also produce large volumes of sludge and possess tendency to release obnoxious gases such as H_2S, which is toxic and detrimental to health (Zakaria et al., 2009). In current era, detoxification by biosorption of heavy metals by biomaterials has been the efficient physicochemical technologies. Many biomaterials such as seaweed, micro-algae, fungi, and a variety of other plant resources have been studied for chromium binding ability. The properties of some microorganisms to equally tolerate and reduce Cr (VI) make possible their application in biotechnological processes for detoxification of hexavalent chromium. The exceptional capabilities of certain types of biomasses to accumulate and immobilize particularly heavy metals can be selective (Volesky, 2001).

Fungi are known for tolerating and detoxifying heavy metals by various mechanisms and are considered as possible biosorbents for heavy metal removal (Igiri et al., 2018). Fungal phenolic polymers and melanins have many possible metal-binding sites with oxygen-containing groups like carboxyl, hydroxyl, carbonyl and methoxyl groups important in biosorption (Crini et al., 2010). Filamentous fungi can be beneficially used in processes for heavy metals removal from wastewater due to their low cost and high ion exchange capacity of their cell walls (Abubacker et al., 2013).

Sorption kinetics offers information on the rate of metal uptake and hydrodynamic parameter which is significant for successful biosorption process. The kinetics explain about the solute uptake that lastly controls the dwelling time of a sorbate at the solid–solution boundary, which in turn provides insight into the reaction pathways and method of the sorption reaction (Dhankhar & Hooda, 2011; Padmavathy, 2008). Langmuir and Freundlich adsorption isotherms express the relation between the amount of metal adsorbed by the adsorbent and unabsorbed component in solution at a stable temperature and provide the information to envisage the adsorption model.

The main purpose of the study was to ensure the efficiency of fungal species isolated from various sources to remove chromium from the wastewater and to study the kinetics of the biosorption efficiency of sorbents (fungi) and adsorbate (metal). The objective of the sorption isotherms is to reveal the specific relation between the balance concentration of adsorbate in the mass and the adsorbed quantity at the surface.

MATERIALS AND METHODS

For isolation of different fungal species three different sources were used. Industrial

wastewater and hydrocarbon contaminated wastewater were collected from Vatva GIDC of Ahmedabad. Different soil samples were collected from Nadiad industrial area. Rotten fruit (apples) were collected from vegetable market of Anand.

Two different hydrocarbon and heavy metal contaminated wastewater sites near Vatva, Ahmedabad and Nadiad, GIDC were selected for isolation fungi. Soil suspension of both samples were prepared using sterile distilled water which were directly streaked on Glucose Yeast Extract (GYE) and Potato Dextrose Agar (PDA) media and plates were incubated at 27ºC. After incubation isolated fungal colonies transferred to GYE slants and morphological characteristics were recorded.

Stock solution of chromium was prepared by dissolving 0.283g $K_2Cr_2O_7$ in 100 ml distilled water which gives 1000 ppm of Cr (VI). 5 ppm of standard solution was prepared by diluting stock solution. Modified APHA (2005) method was used to determine Cr concentration. To prepare standard curve different aliquots in the range from 0.2 to 2 ml were taken from standard solution of Cr. 0.25 ml of H_3PO_4 was added. Using 0.2 N H_2SO_4 pH was adjusted to 2 ± 0.5. Final volume of the system was made to 10 ml with distilled water. 0.2 ml diphenlycarbazide solution was added and tubes were incubated for 10 minutes. Using system blank as reference absorbance was check at 540nm.

To check tolerance of Cr (VI), 50 ppm, 100 ppm and 150 ppm of Cr solutions were prepared from stock solution of Cr (1000ppm) using glucose yeast extract medium. After sterilization of medium, spores of nine isolated fungal strains were inoculated in separate flask and incubated on shaker. Tolerance of chromium was determined by monitoring of growth and beads forming capabilities of fungal isolates was also verified.

Various concentration of Cr, 50ppm, 100ppm and 150ppm were prepared from stock solution of Cr. Small fungal beads of efficient isolated fungi were prepared in GYE broth. Known quantities of hydrated fungal beads were inoculated in chromium solution and flasks were incubated on shaker at 110 rpm. After regular time interval samples were withdrawn and chromium removal efficiency was calculated. Removal efficiency of each fungal strain was calculated by using given formula: Removal efficiency (%) = $(C_i\text{-}C_f) \times 100 / C_i$ where C_i is initial concentration of chromium ion and C_f is final concentration of chromium ion after incubation (Bajpai & Rai, 2010).

The Langmuir model assumes that maximum adsorption occurs when a drenched monolayer of solute particle is present on the adsorbent outer surface and the energy of adsorption is steady and there occurs no relocation of adsorbate particles in the surface plane (Kumar & Kirthika, 2009). The Cr^{+6} concentration retained in the adsorbent phase was calculated according to the given formula.

$$q_e = \frac{(C_i - C_e)V}{W}$$

Where q_e is the uptake of metal per unit weight of the adsorbent (mg/g), C_i and C_e are the initial and equilibrium concentrations (mg/L) of Cr^{+6} solution respectively; V is the volume (L); and W is the mass (g) of the adsorbent.

The Langmuir adsorption isotherm is possibly the best known of all isotherms describing adsorption and it is often expressed as

$$q_e = \frac{q_m K_L C_e}{1 + K_L C_e}$$

The constants in the Langmuir isotherm can be determined by plotting ($1/q_e$) versus ($1/ C_e$) and the above equation rewritten as:

$$\frac{1}{q_e} = \frac{1}{q_m} + \frac{1}{q_m K_L}\frac{1}{C_e}$$

Where q_m and K_L are the Langmuir constants, representing the maximum adsorption capacity for the absorbent and the energy constant related to the heat of adsorption, respectively.

One of the essential characteristics of Langmuir isotherm modelling is the term of equilibrium dimensionless parameter (R_L), which can be calculated using below equation:

$$R_L = \frac{1}{1 + K_L C_0}$$

The R_L value determines whether the adsorption is unfavourable ($R_L > 1$), favourable ($0 < R_L < 1$), linear ($R_L = 1$), and irreversible ($R_L = 0$) (Isam et al., 2019)

The Freundlich isotherm model is an empirical relation that describes the adsorption of solutes from a liquid to a solid surface and assumes that diverse sites with multiple adsorption energies are occupied.

Freundlich formula is given as:

$$q_e = K_f C_e^{1/n}$$

The logarithmic form of the equation becomes,

$$\log q_e = \log K_f + \frac{1}{n}\log C_e$$

Where, K_f and n are the Freundlich constants, the characteristics of the system. K_f and n are the indicators of the adsorption capacity and adsorption intensity, respectively. To determine the values of Freundlich constants the plot of log C_e versus log q_e was employed.

RESULTS AND DISCUSSION

To identify probable fungus in the culture plate, colony characteristics are first assessed to determine the broad group of isolates. Once the initial observations were made, following microscopic criteria were used to make a genus/species identification of fungal isolates: pigmented or non-pigmented, size and shape of spores, presence of special structures, hyphae, and pseudo- hyphae, septate or aseptate, and branching.

Table 1. Characteristics of isolated fungal species

Sr. No	Source	Colony Characteristics			Isolated fungal strains
		Colony shape	Colony color	Spore shape	
1.	Nadiad-GIDC	Circular	White yellow	Spherical	*A. ochraceus*
2.	Nadiad-GIDC	Circular	Green	Globose	*A. flavus*
4.	Nadiad-GIDC	Circular	Black	Globose	*A. niger*
5.	Vatva-GIDC	Circular	Brown	Spherical	*A. tamarii*
6.	Vatva-GIDC	Cottony	White	Spherical	*Rhizopus* spp.
7.	Vatva-GIDC	Circular	White, green	Spherical	*Trichoderma* spp.
8.	Vatva-GIDC	Circular	Green	Spherical	*Penicillium* spp.
9.	Rotten fruit	Circular	White	Globose	*A. candidus*

Total nine fungal isolates were obtained from various sources (Table 1). *Aspergillus ochraceus*, *Aspergillus flavus*, and *Aspergillus niger* were isolated from Nadiad, GIDC. *Aspergillus tamarii, Rhizopus* spp., *Trichoderma* spp. and *Penicillium* spp. were isolated from Vatva-GIDC, Ahmedabad, however *Aspergillus candidus* was isolated from rotten fruit. Several *Aspergillus* species have been used for heavy metal ion adsorption such as *A. niger*, *A. fumigatus*, *A. flavus*, *A. terreus* and *A. cristatus* etc. (Sugasini et al., 2014; Shakya et al., 2013) also reported that *Aspergillus* isolates were the most resistant to all the metals tested, namely chromium, copper, and nickel. Morales and Cristiani (2008) reported that *Trichoderma inhamatum* has capability to convert Cr (VI) to Cr (III) so it can be used in bioremediation of Cr (VI) contaminated water.

Prolong microbial exposure to metal contaminated environment may develop adaptation towards toxic concentration of heavy metals and become metal resistance. Combination of physical and chemical pretreatment modify the cell surface which is essential for biosorption by exposing more metal binding sites (Sugasini et al., 2014). Screening of isolated fungal species for chromium sorption studies was done based on tolerance of Cr and bead formation capabilities. Chromium tolerance of the fungal species was assessed using 50 ppm, 100ppm and 150ppm of potassium dichromate solution where chromium exists as Cr (VI) oxidation state.

Table 2 showing that most of the isolated fungal strains could grow at high concentration of hexavalent chromium. *A. candidus* showed no growth in 100ppm and no growth was observed in *Penicillium* spp. at all different concentration of chromium during first 24 hours of incubation. After 48 hour of incubation the growth was observed in *A. candidus* and *Penicillium* spp.

Table 2: Chromium tolerance studies of isolated fungal strains

Fungal Stains	24 hours				48 hours		
	Control	50ppm	100ppm	150ppm	50ppm	100ppm	150ppm
A. ochraceus	+	+	+	+	+	+	+
A. tamarii	+	+	+	+	+	+	+
Rhizopus spp.	+	+	+	+	+	+	+
Trichoderma spp.	+	+	+	+	+	+	+
A. candidus	+	+	+	-	+	+	+
A. flavus	+	+	+	+	+	+	+
Penicillium spp.	+	-	-	-	+	+	+

Note: (+) presence of growth, (-) absence of growth and Control: GYE without chromium

The observed variation in degree of tolerance could be the possible variation in the mechanism of tolerance. In the agar dilution method *Penicillium chrysogenum* and *Aspergillus niger* showed resistant towards hexavalent chromium level of 800ppm meanwhile in broth dilution method the resistance level of hexavalent chromium was at 512ppm (Abubacker et. al., 2013). *A. tamarii* and *Penicillium* spp. were not able to form beads, so these species were not used for hydrated biosorption kinetic studies.

Biosorption kinetic analysis presents significant information on the rate of metal uptake and hydrodynamic parameter which helps for successful adsorption of chromium metal by the fungi. Kinetic analysis offers here significant equilibrium information necessary for designing a successful adsorption method. Initial pH, absorbent dosage, and initial concentration of

Cr(VI) are important parameters to check adsorption capability of Cr(VI) absorption (Zhang et al., 2010).

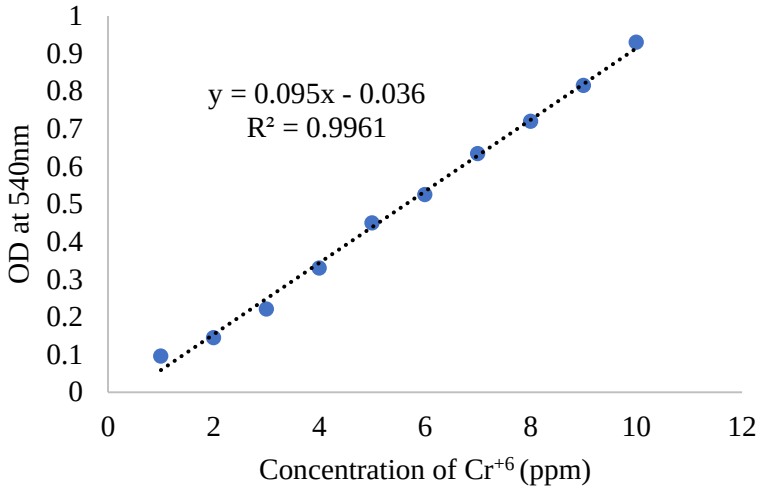

Fig 1. Standard curve for Chromium

Biosorption kinetic analysis presents significant information on the rate of metal uptake and hydrodynamic parameter which helps for successful adsorption of chromium metal by the fungi. Mechanism of biosorption depends on the physical and chemical characteristics of the adsorbent, and on the mass transfer process (Subbaiah & Yun, 2013). The linear regression of the standard chromium graph (Fig. 1) was used to determine initial and final concentration of chromium present in the aqueous solution. To compare adsorption capacity of Cr (VI) on hydrated biomass of isolated fungal strains Langmuir and Freundlich adsorption models were used. Kinetic analysis offers here significant equilibrium information necessary for designing a successful adsorption method. Efficient fungal isolates were selected for biosorption kinetic studies.

Table 3: Adsorption Kinetics of chromium at 50 ppm concentration

Fungal Species	RE* (%)	Langmuir Isotherm				Freundlich Isotherm		
		K_L	q_m	R_L	R^2	K_f	n	R^2
A. ochraceus	85.10	0.42	2.98	0.05	0.99	2.17	1.31	0.98
Rhizopus spp.	88.15	0.09	0.11	0.18	0.98	2.01	1.28	0.97
Trichoderma spp.	86.50	0.17	1.66	0.10	0.99	2.40	1.66	0.97
A. candidus	93.05	0.37	3.22	0.05	0.98	1.40	1.25	0.97
A. flavus	83.39	0.35	3.37	0.05	0.99	2.28	1.50	0.98

*RE: Removal Efficiency

Table 3 describes adsorption kinetics of chromium by Langmuir and Freundlich isotherm and removal efficiency by various fungal biosorbent. Maximum Cr removal efficiency (93.05%) was observed in *A. candidus* as compared to other fungal species. *Aspergillus flavus* showed higher adsorption capacity, 3.37mg/g with 83.39% of removal efficiency for Cr showed good potential for the removing chromium. *Rhizopus* spp. showed 88.15% Cr removal efficiency with minimum Cr adsorption capacity (0.11mg/g h^{-1}) at 50ppm Cr concentration. Metal uptake is based on physical adsorption, ion exchange and chemical sorption and is independent of the cells' metabolism (Vankar & Bajpai, 2008).

Table 4 describes adsorption kinetics of chromium by Langmuir and Freundlich isotherm and removal efficiency by various fungal biosorbents at 100ppm. The Cr removal efficiency by an isolated fungi species is in the order of *A. candidus* > *Trichoderma* spp. > *A. ochraceus* > *Rhizopus* spp. > *A. flavus*. *A. candidus* showed higher adsorption capacity, 3.56mg/g with 93.47% of removal efficiency of Cr. The Freundlich constant n value observed in the range of 1.55 to 2.19 (table 4) indicates higher intensity of adsorption. Chromium adsorption capacity value 3.56 mg/g at 100ppm for *A. candidus* was greater than *Trichoderma* spp. (2.93mg/g) and *A. flavus* (2.63 mg/g). The capturing of metal by the cell are cell surface binding at various sites is directly dependent on the complexity of microorganism's structure, extracellular precipitation, intracellular gathering, oxidation and reduction, and methylation/demethylation (Sen & Dastidar, 2010).

Table 4: Adsorption Kinetics of chromium at 100 ppm concentration

Fungal Species	RE* (%)	Langmuir Isotherm				Freundlich Isotherm		
		K_L	q_m	R_L	R^2	K_f	n	R^2
A. ochraceus	91.11	0.13	1.92	0.07	0.97	1.57	1.67	0.97
Rhizopus spp.	87.03	0.05	1.17	0.17	0.99	1.87	2.19	0.98
Trichoderma spp.	93.33	0.11	2.93	0.08	0.97	1.40	1.80	0.96
A. candidus	93.47	0.15	3.56	0.06	0.98	1.44	1.73	0.96
A. flavus	84.69	0.28	2.63	0.03	0.98	2.43	1.55	0.96

*RE: Removal Efficiency

Table 5 shows higher value of correlation coefficient (R^2 > 0.96-0.99) which indicates data are best fitted in Freundlich & Langmuir isotherms. At 150ppm Cr concentration, removal efficiency and adsorption capacity were observed in the order of *A. candidus* > *A. ochraceus* > *A. flavus* > *Rhizopus* spp. > *Trichoderma* spp. *A. candidus* showed higher adsorption capacity, 5.49 mg/g with 98.75% of removal efficiency. The adsorption capacity of hexavalent chromium is higher than the biosorbent used by other researchers i.e., 2 mg/g for dry biomass of *Aspergillus foetidus* (Prasenjit & Sumathi, 2005) and 3.63mg/g for *A. niger* at 60 mg/lf; 2.43 mg/g for *A. sydoni* (Kumar et al., 2008). One of the reasons to obtain lower q_e is growing hydrated fungal cells used for Cr removal which is having greater mass compared to dry fungal biomass.

Table 5: Adsorption Kinetics of chromium at 150 ppm concentration

Fungal Species	RE* (%)	Langmuir Isotherm				Freundlich Isotherm		
		K_L	q_m	R_L	R^2	Kf	n	R^2
A. ochraceus	98.27	0.65	2.70	0.01	0.99	1.19	2.03	0.98
Rhizopus spp.	72.00	0.0	0.76	1	0.99	4.72	3.22	0.98
Trichoderma spp.	53.19	0.01	0.12	0.4	0.98	1.25	3.90	0.99
A. candidus	98.75	0.12	5.49	0.05	0.98	1.07	1.91	0.96
A. flavus	79.66	0.04	1.08	0.14	0.99	2.28	2.32	0.99

*RE: Removal Efficiency

Most of the chromium removal reported studies have been carried out using either using

dry biomass or resting cells of various fungi and due to chromium toxicity very limited information is available on the use of growing cells. Biotechnological exploitation of biosorption technology for removal of heavy metals depends on the efficiency of the renewal of bio sorbent following metal desorption. Therefore, non-destructive recovery by mild and cheap desorbing agents is desirable for regeneration of biomass for use in multiple cycles.

CONCLUSION

Total nine fungal isolates were obtained from various sources and were used to check chromium tolerance and chromium removal efficiency. *Aspergillus ochraceus, Aspergillus flavus,* and *Aspergillus niger* were isolated from Nadiad, GIDC. *Aspergillus tamarii, Rhizopus* spp., *Trichoderma* spp. and *Penicillium* spp. were isolated from Vatva-GIDC, Ahmedabad, however *Aspergillus candidus* was isolated from rotten fruit. At 150ppm Cr concentration, removal efficiency and adsorption capacity were observed in the order of *A. candidus* > *A. ochraceus* > *A. flavus* > *Rhizopus* spp. > *Trichoderma* spp. Growing hydrated cells of *A. candidus* showed maximum removal efficiency and adsorption capacity of Cr at 150 mg/l. The R_L value for Langmuir isotherm for all the three concentration was less than 1, so this depicts favorable sorption and for Freundlich isotherm, the value of 1/n exceeds more than 1, so all the processes show co-operative or similar type of adsorption.

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The present research did not receive any financial support.

CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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