BIOSORPTION OF HEAVY METAL CHROMIUM BY LIVE AND DEAD FUNGIISOLATED FROM LEATHER TANNERY

Haseena M, Raja A, Sangavai C, Roja B, Kiruba Ranjani A,

Dhanalakshmi Srinivasan College of Arts & Science for Women (Autonomous),

Perambalur, Tamil Nadu, India.

ABSTRACT:

Hexavalent chromium Cr (VI) is one of the most common environmental metal containment released primarily from industries such as leather tanning, metal plating, alloying, wood preservation and electroplating. Chromium (VI) is known to be highly toxic, mutagenic & carcinogenic to living organisms including mammals. Biosorption is potentially cost effective way to remove toxic heavy metals from industrial waste waters. The present study was planned to evaluate the heavy metals uptake potential of newly isolated white rot fungi from metals contaminated leather tannery sites. Two white rot fungi viz., *Aspergillus niger,Rhizopus stolinifer* were selected for biosorption of Cr(VI) study. Chromium resistance was tested at different concentration.. Biosorption efficiency was estimated by live and dead fungal mass. Dead immobilized white rot fungi had the effect of biosorbents dose on metal biosorption. The cost effective potential biosorbent can be developed by these two fungal species for removing chromium from industrial effluents as well.

Key words: Biosorption, Chromium (VI), *Aspergillus niger,Rhizopus stolinifer*, Diphenyl carbazide

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INTRODUCTION

Heavy metal contamination has gotten one of the major environmental issues that pose serious health hazard. Tannery industries uses chromium compounds in the tanning process and produces spent chromium laden wastes in to the nearby places (sharma and Malavika,2014).

Hexavalent chromium Cr(VI) is one of the most common environmental metal contaminant released primarily from industries such as leather tanning, metal plating and alloying, wood

preservation, electroplating, paint and pigment manufacturing, textile and fertilizer industries.

Biosorption is the process of cation binding from industrial waste water by dead , living microbial biomass, stands for potentially cost- effective method of removing toxic heavy metals from industrial waste waters. Inactive microbial biomass frequently possess a higher relationship for metal ions compared with living biomass probably due to the absence of competitive protons synthesized during metabolism.(Sağ, 2001).

Fungal cell walls and their constituent have a significant function in biosorption and also take up Heavy metal particulates and mixture (Yan and Viraraghavan, 2000).

Dead fungal cells sequester metals through chemical functional groups of the material respect the cell and especially the cell wall which constitutes a huge level of the cellular dry weight. Fungal cell surfaces can be considered as a mosaic of various functional groups where arrangement edifices with metals can be form. Among these group are carboxyle (- COOH), amide (- NH_2), thiol (- SH), phosphate (PO4 3-), and hydroxide (- OH)(Volesky, 1990).

The present study was planned to evaluate the heavy metals uptake potential of newly isolated white rot fungi from metals contaminated leather tannery sites. The assessment of the chromium metal-binding capacity of new biosorbents has been discussed. Consequently, there is need to confine and screen heavy metal lenient fungi fromtannery effluent.

MATERIALS AND METHOD

Collection of soil sample

Effluent collected from leather tannery in a sterile container near Kottapatu, Tiruchirappalli, Tamil Nadu, India and stored in refrigerator at 4°C for future processing.

Isolation of fungi

One ml of the effluent sample was taken in a 250 ml conical flask containing 100ml of sterile distilled water and the diluted upto $10^{-5.}$. One ml of 10^{-3} dilutions was plated in Petri dishes containing potato dextrose agar medium. The pH of the medium was adjusted to 5.6 and streptomycin sulphate (100 mg/L-2) was added to the media to prevent the bacterial growth. The plates were incubated at 25±2º C for five days. The fungi were identified by

morphological observations (Emmanuel etal., 2014).

Identification of fungi

The fungi on PDA plates were identified based on characteristic features including colony morphology and reproductive structural characteristics like sporangiospore position, columella and spore shape (Nagamani et al., 2006) followed by lactophenol cotton blue staining technique.

Heavy metal tolerance assay

To determine the heavy metal tolerance of the isolates, potato dextrose agar medium was prepared with different concentrations of potassium dichromate, ranging from 100 to 1000 ppm. The fungal isolates were spot inoculated into each freshly prepared chromium plates and incubated for 24 h. After incubation, the extent of chromium was monitored *via*. the mycelial growth of the fungal isolates. The metal tolerance index (*Ti*) of the fungal isolates was determined using the formula:

$$Ti = Dt/Du \times 100$$

where, Dt is the radial extension of treated colony in cm and Du is the radial extension of untreated colony in cm.

Biosorption studies

The Cr stock mixture was embattled by dissolving 1000mg of salt in 1 L of deionized water. From stock, Cr particle mixture was ready in 2 sets at the concentration of 50mg/L and therefore the pH was adjusted to seven. About 0.5 g of the live and autoclaved flora biomass wereadded individually to the 100mL ofchromium ion mixture and therefore the reaction solutions were incubated in shaker for 60min. when incubation, the Cr concentration within the reaction solution was calculated (John etal., 2017).

Estimation of chromium

About five millilitre of the on top of reaction mixture was centrifuged at 4000 rpm for fifteen min and also the supernatant was used for the estimation of Cr. To one millilitre of the supernatant, 9 mL of 0.2 M sulfuric acid and 0.2 mL of 0.25% diphenyl carbazide in acetone were poured, and also the absorbance of the pink color developed was read at 540 nm using Dis.H_2O as blank. The linear regression of the standard graph was used for the estimation of Cr identifiedt within the mixture. The Cr removal proportion was calculated using the subsequent formula:

$$E = (Ci - Cf / Ci) \times 100$$

Where, E = Percentage removal of heavy metal; Ci = initial metal ion concentration, mg/L; Cf = final metal ion concentration, mg/L

RESULTS AND DISCUSSION

The main purpose of this work was to evaluate the Chromium(VI) removal by two different fungi *A.niger and R.stolinifer* fungi that are present in tannery effluent(plate 1 a). The colony forming unit was 3 X 10^3 and the isolates were produces black and white cotton colonies (plate 1b). Based on spore morphology given in table 1 the isolates were found to be *A.niger* and *Rhizopus stolinifer*. In soil, they frequently are the most prevalent culturable fungi.

The isolate *A.niger* exhibited MIC for Cr(VI) as 100 ppm and *R.stolinifer* showed tolerance upto 1000ppm (plate 2 and 3).The results indicated that some native fungi have a marked adaptation to heavy metals underneath constant metal stress for an extended time, and therefore the heavy metals were even used as micronutrients by these growth stimulated fungi. Similar to our findings, *Aspergillus niger, Aspergillus lentulus, Penicillium* sp., and *Fusarium solan i*solated from contaminated sites have been reported to tolerate 1000 ppm Cr(VI)(Sen et al., 2012).

The adsorption phenomenon was called biosorption as fungal species were used for the adsorption. The fungus can have remarkable ability to remove high concentrations of Chromium(VI) when grown in plates containing glucose as the sole carbon and energy source, under aerobic conditions. In this respect the remediation of chromium containing water has been carried out by two different kind of fungal sp., i.e., *A.niger sp.* and *R.stolinifer* . The purpose of selecting two different kinds of fungi is ability to adopt high concentration of chromium.(Padma etal., 2015).

The biosorption capacity was also calculated in the live and dead form at 100 ppm level (table 2). The treatment of biosorption with*R.stolinifer*in shake flask experiment resulted in the reduction of 83 % and 45% by dead fungi mat. Another experiment for chromium biosorption wit *A.niger* was found to be moderate at dead and less significant at live status (Plate 4&5).

The biosroption efficiency was 25 and 45. The effect of biosorbents dose on metal biosorption by dead immobilized white rot fungi was studied at 1000 ppm (Fig. 1). Increase in biomass 0.1, 0.2, 0.5,1 g dose in a fixed volume of solution (100 mL) at constant pH (5), initial metal concentration (100 mg/L)

and temperature (30 ºC) reduced the metal sorption capacity of biomass. This decrease in uptake capacity of biosorbents can be attributed to poor utilization of biomass/ lower efficiency at higher biomass concentrations.

At higher biomass dosage agglomeration of biomass cells occurs which reduce inter-cellular distance significantly. There is a need of optimal inter-cellular distance to ensure optimal electrostatic interaction between fungal cells. Optimal internuclear distance is a significant factor in metal biosorption studies. Similar type of results are reported by Saifuddin and Raziah (2007)

Table 1:Frequency of isolates from tannery effluent

Number of colonies	Colony morphology	Genus
3 X 10^3	Black spore	*Aspergillus niger*
	Cottony white	*Rhizopus stolinifer*

Table:2. Chromium biosorption by fungal isolate

Fungi		pH	OD Value	Percentage of Biosorption
LIVE	Control	2.85	0.24	_
	Black	2.81	0.18	25%
	White	2.85	0.04	83.3%
DEAD	Black	2.85	0.04	83.3%
	White	2.70	0.13	45.83%

Figure 1. Effect of inoculum on Biosorption

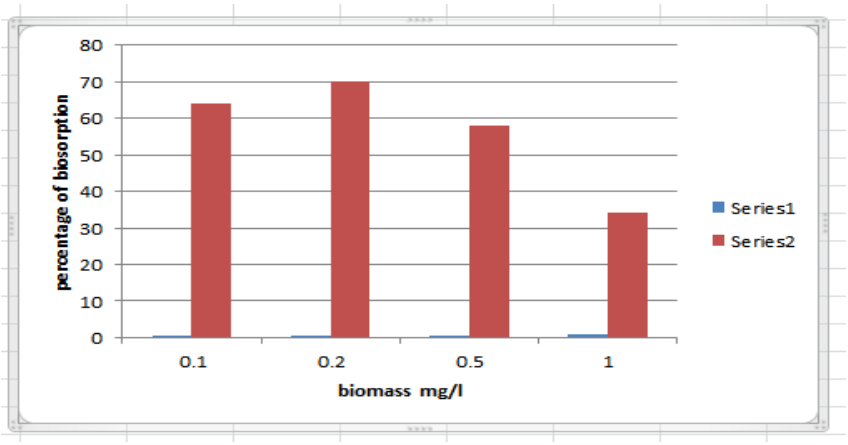
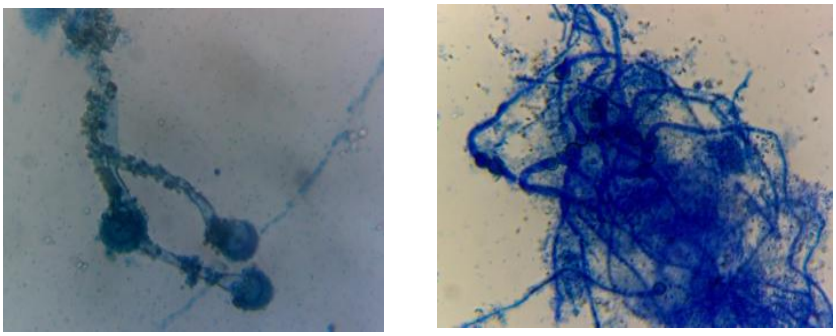


Figure 2: Isolation of fungi from Tannery effluent

a)Effluent b) Isolated fungi



c) *A.niger* d)*R.stolinifer*

Fig.3: Growth of fungi at 100 ppm Cr

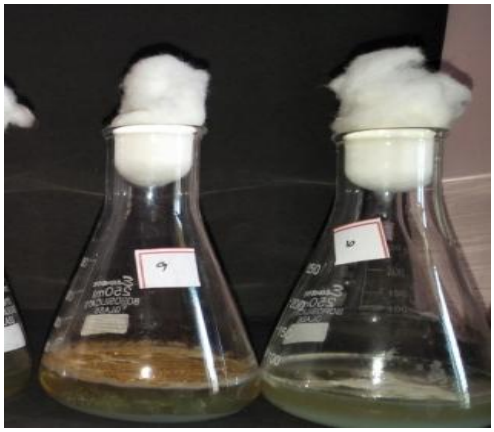


Fig.4:Growth of fungi at 1000 ppm Cr

Fig.5: Dead Fungal Mat

Fig.6:Chromium Absorption by Live and Dead Fungal mat

Fig.7::Biosorption of chromium

SUMMARY

Fungi makes important contribution in natural remediation of heavy metals and xenobiotic compound. Out of two different fungi isolated one isolates were found to be chromium resistant. Fungus belonging to genera Rhizopus sps. were highly resistant to chromium up to 1000 ppm..Thedifferent sized fungal specieswereinvestigatedtothe biosorptionof the hexavalent chromium ion (Cr (VI)) on their cell surface inan aerobiccondition(Patel Shriram, 2014).

The new biosorbents has been discussed by the assessment of the metal-binding capacity. Batch experiments were conducted with various initial concentrations of chromium ions between 100 -1000 ppm to obtain the tollerence capacity of fungal isolates. Out of two tested fungal named *A.niger and R.stolinifer* only thae later one withstands and grown at 1000 ppm.

CONCLUSION

The results obtained at biosorption of chromium at minimal broth were 83.3% reduction by *live* and 45 % reduction by dead *R.stolinifer*. The cost effective potential biosorbent can be developed by these two fungal species for removing chromium from industrial effluents as well.

Another important facet of this study is that the future use of those two fungous strains in biosorption of alternative heavy metal species typically present in numerous industrial effluents.

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