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Biosorption Potential of Alkali Pretreated Fungal Biomass for the Removal and Detoxification of Lead Metal Ions

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Nowadays biosorption technology is primarily used as a potent tool for solving the environmental pollution, as compared to conventional methods because of its low cost, and environmental safety. It is not associated with secondary pollutions during its operation. The present study is based on checking the capacity of live and pretreated biomass of *Aspergillus* species for the biosorption of lead metal ions. Among the five species tested, the best results were obtained for *A. niger*. While the minimum and maximum removals of lead metal ion by live *A. niger* biomass were 3.84 and 16.42 mg/g at 2 mM and 9 mM concentration respectively, it was increased to 31.25 and 48.44 mg/g respectively at same base concentration for pretreated biomass. Overall, it was observed that pretreated alkali biomass of test fungal species is a potent biosorbent for the metal ions.

Keywords: *Aspergillus* sp., Biosorbent, Live biomass, Pretreated biomass

Introduction

Environmental pollution is the pollution of air, soil, and water. One such example of environmental pollution is the presence of a higher level of heavy metal ions.[1,2] Metals that have a high atomic number, atomic weight and relatively high density ranging from 3.7–7 g/cm^3 are termed as heavy metals, and these metal ions are the major pollutants of the aquatic system and soils.[3] Arsenic, cobalt, cadmium, chromium, iron, lead, manganese, mercury, nickel, and zinc are the common toxic metal ions that are directly related to the health of living components of the environment[4] and created a poisonous effect on the living being.[5] Heavy metals are not biodegraded, remains in the environment due to their chemical persistence and hence, continuously biomagnified into the food chains.[6] Industrial wastes, domestic wastewater and stormwater runoff from urban areas, the metal content of agricultural runoff and fossil fuel combustion, chemical processing and extensive use of fertilizers are the primary artificial source of these heavy metal pollutions.[7] Therefore, the present study was undertaken to determine the biosorption potential of alkali pretreated fungal biomass for the removal and detoxification of lead metal ions.

Materials and Methods

Isolation and identification of fungi

For the separation, soil samples (1 kg) were collected into sterilized polythene bags from the agricultural fields irrigated with untreated wastewater and were taken into the laboratory for experimentation. The Serial dilution plate method[8] was used to isolate the fungi from soil samples. Soil sample weighing 20 g was placed in 200 ml sterilized distilled water for 15–20 minutes to get a stock solution of 1:10 dilution. 10 ml from this solution then transferred into a conical flask containing 90 ml of sterilized distilled water to get a suspension of 1:100 dilutions. This procedure was repeated to prepare serial dilution of 1: 1000 and 1: 10,000. The suspension of 1:10 dilution was discarded. Aliquots measuring 1 ml from the suspension of each of the remaining three dilutions, i.e., 1:100, 1:1000, 1:10000 were transferred to each of a set of three Petri dishes containing 20 ml sterilized and cooled PDA medium.

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Four concentrations (3 mM, 5 mM, 7 mM, and 9 mM) of lead nitrate supplemented in the PDA medium was poured in sterilized Petri dishes. After that mycelial disc of 6 mm diameter of each of the four *Aspergillus* sp. were centrally inoculated and then incubated at room temperature for six-seven days.

Estimation of the lead tolerance potential

For the estimation of the lead tolerance potential, medium without lead nitrate served as control. The decrease in radial growth was used as a parameter to examine metal tolerance Also, TI (tolerance index) was calculated to compare the metal tolerance capacity of each species of test fungi as the ratio of rate of radial growth in the metal treatment to the rate of radial growth in the control.

Biosorbent preparation and experiment

Six mm mycelial disc of each fungal sp. was transferred to the medium. Live biomass (50 g) was treated with NaOH (0.5 N) for 30 minutes and then washed with sterilized distilled water. Alkali pretreated biomass was then dried in a hot air oven and then powdered by mortar and pestle. Live and alkali pretreated biomass of each of the four-test fungal spp. was injected at the rate of 0.1g per 100 ml solution containing 3 mM, 5 mM, 7 mM, and 9 mM of lead nitrate. After centrifugation (at 10,000 rpm for about 15–20 minutes) of the metal solution, the content of the supernatant was examined by the AAS (atomic absorption spectrophotometer).

Following equation was used for the calculation of biosorption efficiency and amount of metal ion adsorbed by biosorbent:

$$Q = (Ci - Cf)V/M$$

Where,

Q is the uptake capacity of metal ion (mg/g),

Ci is the initial concentration of metal (mg/g),

Cf is the final concentration of metal in solution (mg/g),

M is the dry wt. of the biomass (biosorbent) (g), and

V is the volume of the solution (lt)

Results and Discussion

Total of sixteen fungal species were isolated from the metal contaminated soil samples by dilution plate method (Table 1). Out of the sixteen, one species of *Cladosporium cladosporioides, Dactylium dendroides, Melanospora lagenaria, Penicillium*, two species of *Fusarium, Trichoderma*, and seven species of *Aspergillius* were reported. From this study it was found that *Aspergillus* sp. were present in large number in the contaminated soils, similar to results of other workers.[9–11] Solat *et al.*[12] reported that NaOH pretreated biomass of *Aspergillus niger* was quite useful material for the biosorption of uranium. Javid *et al.*[13] found that the pretreated biomass of *Aspergillus niger* effectively adsorbed copper and nickel from aqueous solution. Several species of *Aspergillus* have biosorption potential for the removal and detoxification of metal ions.[14–16]

Present work was focused on the biosorption capability of five species of *Aspergillus* for the sorption of lead metal ions. In this study, metal tolerance of *Aspergillus* species was investigated at 3 mM, 5 mM, 7 mM, and 9 mM concentration of lead nitrate solution. At the 3 mM, 5 mM and 7 mM concentration of the metal solution, fungal growth was observed, but no fungal growth was noticed at the 9 mM concentration. It was found that fungal colonies could not tolerate and survive at higher concentration of the metal solution because of the increasing toxicity of lead nitrate solution. The tolerant indices of test fungal species are shown in Table 2. The metal tolerant index of *Aspergillus flavus* and *A. sulphureus* was found to be similar, i.e., 0.6 at the 3 mM concentration of metallic solution. The lead tolerant index was 0.7, 0.5 and 0.9 for *A. japonicus, A. nidulans and A. niger*, respectively. The tolerance index for *A. flavus* and *A. sulphureus* decreases, but the similarity was observed, i.e., 0.5 and 0.5 at 5 mM concentration. Tolerance index of 0.6, 0.4, and 0.7 was observed respectively for *A. japonicas, A. nidulans and A. niger* at 5 mM concentration of

Table 1 — List of the fungal species isolated from the soil samples by dilution plate method.

Sr. no.	Fungal species
1.	*Aspergillus flavus*
2.	*Aspergillus fumigatus*
3.	*Aspergillus japonicus*
4.	*Aspergillus nidulans*
5.	*Aspergillus niger*
6.	*Aspergillus sulphureus*
7.	*Aspergillus terreus*
8.	*Cladosporium cladosporoides*
9.	*Dactyliumdendroides*
10.	Fusarium *nivale*
11.	*Fusarium* sp.
12.	*Melanosporalageneria*
13.	*Penicillium* sp.
14.	*Trichodermaviride*
15.	*Trichoderma lignorum*
16.	Dark brown mycelium

Table 2 — Lead tolerance index of test fungal species

Fungal species	Lead tolerance index (T.I)			
	3 mM	5mM	7mM	9 mM
Aspergillus flavus	0.6	0.5	0.3	NG*
Aspergillus japonicus	0.7	0.6	0.4	NG
Aspergillus nidulans	0.5	0.4	0.4	NG
Aspergillus niger	0.9	0.7	0.5	NG
Aspergillus sulphureus	0.6	0.5	0.3	NG

*NG indicates no growth (At 9 mM concentration of lead nitrate)

lead nitrate. Also, the tolerance index for *A. niger* was 0.5 at 7 mm concentration while for *A. flavus and A. sulphureus* it was a lower value of 0.3. Similar tolerance index (0.4) was noticed for *A. japonicus and A. nidulans*.

Following the tolerance study, the organisms were tested for their biosorption potential. Live and alkali pretreated biomass of the five *Aspergillus* species were used as biosorbent for the adsorption of lead metal ions. Concentration of lead nitrate solution tested was 3 mM, 5 mM, 7 mM and 9 mM. The results are given in Table 3 which showed that with the increasing concentration of the lead metal solution, the biosorption of lead metal ion increased. In comparison to the live biomass of all five test fungal species, pretreated biomass found to perform better.

The biosorption of lead was 2.46, 2.92, 5.21 and 6.62 mg/g at 2 mM, 5 mM, 7 mM and 9 mM concentration respectively by live biomass of *A. flavus*. It was observed that 15.82, 16.85, 19.25 and 20.52 mg/g lead biosorbed at 2 mM, 5 mM, 7 mM and 9 mM concentration respectively by alkali pretreated biomass of *A. flavus*. The minimum and maximum biosorption of lead was 1.46 and 3.96 mg/g at 2 mM and 9 mM concentration by live biomass of *A. japonicus*. In the case of pretreated biomass, minimum and maximum biosorption was 12.87 and 19.43 mg/g at 2 mM and 9 mM concentration. Whereas, the minimum and maximum removal of test metal ion were 2.46 and 7.65 mg/g at 2 mM and 9 mM concentration respectively by live *A. nidulans* biomass, in case of pretreated biomass, the values were 18.25 and 27.65 mg/g at 2 mM and 9 mM concentration respectively. It was observed that minimum and maximum removal of lead metal ion was 3.84 and 16.42 mg/g at 2mM and 9mM concentration respectively by live *A. niger* biomass while in case of pretreated biomass, minimum and maximum metal uptake was 31.25 and 48.44 mg/g at 2 mM and 9 mM concentration respectively. On the other hand, the minimum and maximum removal of test metal ion

Table 3 — The biosorption of lead metal ions by live, and alkali pretreated biomass of *A. flavus, A. japonicus, A. nidulans, A.niger and A. sulphureus*

	Lead ion removal (mg/g)			
	3mM	5mM	7mM	9mM
A. flavus				
NaOH pretreated fungal biomass	15.82	16.85	19.25	20.52
Live Fungal Biomass	2.46	2.92	5.21	6.62
A. japonicus				
NaOH pretreated fungal biomass	12.87	13.51	15.48	19.43
Live Fungal Biomass	1.46	2.41	3.05	3.96
A. nidulans				
NaOH pretreated fungal biomass	18.25	23.25	25.82	27.65
Live Fungal Biomass	2.46	3.46	5.52	7.65
A. niger				
NaOH pretreated fungal biomass	31.25	37.68	46.42	48.44
Live Fungal Biomass	3.84	5.67	24.86	16.42
A. sulphureus				
NaOH pretreated fungal biomass	13.25	15.82	17.84	18.85
Live Fungal Biomass	1.84	2.26	3.15	4.42

were 1.84 and 4.42 mg/g at 2 mM and 9 mM concentration respectively by live *A. sulphureus* biomass while in case of pretreated biomass, minimum and maximum metal uptake was 13.25 and 18.85 mg/g at 2 mM and 9 mM concentration respectively.

The present investigation indicated that pretreated biomass of *A. niger* showed maximum biosorption capacity and all values were higher than the live and pretreated biomass of other fungal species. Netpae[17] reported that the NaOH pretreated biomass of *Humicola* sp. have better biosorption potential in comparison to the untreated and acid-treated biomass. Javaid *et al.*[13] also observed that the pretreatment of fungal biomass increases metal removal potentiality. Awofolu *et al.*[18] reported that the modified biomass of *Aspergillus* spp. can be utilized as a potent biosorbent for the elimination of metal ions from the aquatic system. Ilhan *et al.*[19] reported that physical and chemical pretreatment extensively increased the biosorption efficiency of fungal biomass for the lead and copper biosorption. Yan and Viraraghavan[20] observed that pretreated biomass of *Mucor rouxii* efficiently adsorbed lead, cadmium, nickel and zinc metal ions.

Conclusions

Biosorption is an eco-friendly alternative for remediation of environmental health hazard. In the present study pretreated biomass of *A. niger* showed maximum biosorption capacity than the live and pretreated biomass of other fungal species. Hence,

A. niger can be a potential agent for biosoption of lead from aquatic environment as well as for treatment of wastewater. The metal uptake capacity of biological material becomes quite useful after the physical and chemical pretreatment in comparison to the non-treated biomass because the more metal-binding site is exposed after pretreatment. The pretreated biomass significantly removes the metal ion contaminants and pollutants from the aquatic environment. The processes of biosorption and bioaccumulation have been proved as economical, low cost and efficient methods and these can efficiently remove the minute concentration of heavy metals in comparison to conventional methods.

References:

1 Jaishankar M, Tseten T, Anbalagan N, Mathew B B, & Beeregowda K N, Toxicity, mechanism and health effects of some heavy metals, *Interdiscip toxicol*, **7(2)** (2014) 60–72.

2 Kampa M & Castanas E, Human health effects of air pollution, *Environ pollut*, **151(2)** (2008) 362–367.

3 Duffus J H, "Heavy metals" a meaningless term? (IUPAC Technical Report), *Pure Appl chem*, **74(5)** (2002) 793–807.

4 Nagajyoti P C, Lee K D, & Sreekanth T V M, Heavy metals, occurrence and toxicity for plants: a review, *Environ chem lett*, **8(3)** (2010) 199–216.

5 Zahir F, Rizwi S J, Haq S K, & Khan R H, Low dose mercury toxicity and human health, *Environ Toxicol and Pharmacol*, **20** (2) (2005) 351–360.

6 Wuana R A &Okieimen F E, Heavy metals in contaminated soils: a review of sources, chemistry, risks and best available strategies for remediation, *ISRN Ecol*, (2011), https://doi.org/10.5402/2011/402647.

7 Micó C, Recatalá L, Peris M, & Sánchez J, Assessing heavy metal sources in agricultural soils of an European Mediterranean area by multivariate analysis, *Chemosphere*, **65(5)** (2006) 863–872.

8 Warcup J H, On the origin of colonies of fungi developing on soil dilution plates, *Trans Br Mycol Soc*, **38(3)** (1955) 298–301.

9 Zafar S, Aqil F, & Ahmad I, Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agricultural soil, *Biores Technol*, **98(13)** (2007) 2557–2561.

10 Atlas R M, Microbial degradation of petroleum hydrocarbons: an environmental perspective, *Microbiol rev*, **45(1)** (1981) 180–209.

11 Srinivasan M, Gonzales C A, George C, Cevallos V, Mascarenhas J M, Asokan B, Wilkins J, Smolin G, Whitcher J P, Epidemiology and aetiological diagnosis of corneal ulceration in Madurai, south India, *Br J Ophthalmol*, **81(11)** (1997) 965–971.

12 Sana S, Roostaazad R, & Yaghmaei S, Biosorption of uranium (VI) from aqueous solution by pretreated *Aspergillus niger* using sodium hydroxide, *Iran J Chem Chem Eng (IJCCE)*, **34(1)** (2015) 65–74.

13 Javaid A, Bajwa R, & Javaid A, Biosorption of heavy metals using a dead macro fungus Schizophyllum commune fries: evaluation of equilibrium and kinetic models, *Pak*, **42(3)** (2010) 2105–2118.

14 Manguilimotan L C & Bitacura J G, Biosorption of cadmium by filamentous fungi isolated from coastal water and sediments, *J Toxicol*, (*https://www.hindawi.com/journals/jt/2018/7170510/*), (2018).

15 Huang C & Huang CP, Application of *Aspergillus oryze* and *Rhizopus oryzae* for Cu (II) removal, *Water Res*, **30(9)** (1996) 1985–1990.

16 Sun Y-M, Horng C-Y, Chang F-L, Cheng L-C, & Tian W-X, Biosorption of lead, mercury and cadmium ions by *Aspergillus terreus* immobilized in a natural matrix, *Polish J Microbiol*, **59(1)** (2010) 37–44.

17 Netpae T, Cd^{2+} biosorption by pretreatment biomass of highly cadmium resistant fungus *Humicola* sp., *Electron J Biol*, **11(1)** (2015) 13–16.

18 RafiuAwofolu O, Okechukwu Okonkwo J, Badenhorst J, & Jordaan E, A new approach to chemical modification protocols of *Aspergillus niger* and sorption of lead ion by fungal species, *Electron J Biotechnol*, **9(4)** (2006) 1–7.

19 Ilhan S, Cabuk A, Filik C, & Caliskan F, Effect of pretreatment on biosorption of heavy metals by fungal biomass, *Trakya Univ J Sci*, **5(1)** (2004) 11–17.

20 Yan G & Viraraghavan T, Heavy-metal removal from aqueous solution by fungus *Mucor rouxii*, *Water Res*, **37(18)** (2003) 4486–4496.