Removal of Heavy Metals in Liquid Media through Fungi Isolated from Waste Water

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Abstract: ***Wastewater mainly from paint, leather, metal and tanning industries contain huge amount of heavy metals. Microorganisms including fungi have been reported to remove heavy metals from wastewater through bioaccumulation and biosorption at low cost and in eco-friendly way. An attempt was, therefore, made to isolate fungi from sites contaminated with heavy metals for higher tolerance and removal of heavy metals from wastewater. Many fungal isolates tolerant to heavy metals like Pb, Cd, Cr and Ni were isolated from sewage, sludge and industrial effluents containing heavy metals. There are various fungi (Aspergillus foetidus,Phanerochaete chrysosporium, Aspegillus awamori, Rhizopus sp., Aspergillus flavus, Trichoderma viride) which are used for removal of different metal. The majority of the fungal isolates were able to tolerate up to 400 ppm concentration of Co,Pb, Cd, Cr,Cu and Ni. The tolerant fungi were studied for removal of heavy metals from liquid media at 50 ppm concentration. In this paper we disscuse only two fungi mainly Aspergillus flavus and Aspergillus niger and results indicated removal of varying amount of heavy metals with respect to Pb, Ni and Cr which gaves the maximum uptake of 12.44, 0.53, 0.05, and 0.55 mg/g was observed by fungi (Aspergillus flavus), Aspergillus niger, Cr10 (A. niger). This indicated the potential of these fungi as biosorbent for removal of heavy metals from wastewater and industrial effluents containing higher concentration of heavy metals.***

Keywords: Wastewater, Fungi, Bioaccumulation, Heavy metals

1. Introduction

Heavy metal pollution of water is one of the great consequences of industrialization in the sectors of mining, petroleum refining, automobiles, paints etc. Fungi are recognized for their superior ability to produce a large variety of extra cellular proteins, organic acids, enzymes and their waste biomass may be used as effective biosorbent for removal, reduction and detoxification of industrial effluents ingredients Use of wastewater in agriculture has increased in recent years due to inherent treatment capacity of soil and high contents of major and micronutrients in it. However, wastewater, particularly from industries contains high concentration of heavy metals which enter into human beings and animals through food chain. Therefore, it is desirable to remove these heavy metals from wastewater through low cost technology z methods such as reverse osmosis, solvent extraction, lime coagulation ion exchange and chemical precipitation [1, 2]. for removal of heavy metals from wastewater are very expensive and these do not remove heavy metals from wastewater up to desired limits. Recently, micro-organism has been reported as biological adsorbents to remove heavy metals from wastewater at low cost and in eco-friendly way [3–6]. The bio-sorption of heavy metals using various live and heat treated fungi has been studied. In addition, living biomass may subject to toxic effect of heavy metals at elevated concentration. To overcome the disadvantages; non-viable or dead biomass is preferred [30] .These studies showed that the bio-sorption capacity of the heat treated cells might be greater, equivalent or less than that of their living counterparts [7–10]. Heavy metal resistant micro-organism present in heavy metal contaminated source. Therefore, there is required to isolate and screen heavy metal tolerant fungi from heavy metals contaminated source. The present study attempts to isolate and screen heavy metal tolerant fungi and find out their efficiency to remove heavy metals from liquid media under laboratory conditions.

2. Materials and Methods

Sample collection

Wastewater samples were from sewage, sludge and industrial effluents were collected in sterilized containers from sewage treatment plants at Noida and industry at NCR. These samples were collected and kept in refrigerator at 4°C for further processing. Isolation of Fungi from samples of sewage, sludge and industrial

effluents by serial dilution method using potato dextrose agar (Hi-Media, Mumbai, India) containing 25 ppm of Pb, Ni, and Cr individually. The 1000 ppm stock solutions of Pb, Ni and Cr were made in double distilled water using Pb(NO3)2, NiCl2_6H2O, and K2Cr2O7. The stock solution of heavy metals was sterilized through filters and after that added to sterilized potato dextrose agar (PDA) medium and makes the concentration at 25 ppm. Dilution of each sample and added in sterilized Petri plates in duplicate manner. Twenty milliliter of prepared PDA medium containing 25 ppm of one of these heavy metals was poured in these sterilized Petri plates and incubated at 28°C for 2 days. The colonies of predominant genera of fungi were identified, collected and then purified by pour plate method. The organic matter decomposing fungi namely Aspergillus niger was procured from Laboratory for this study. Another fungus namely Aspergillus flavus isolated at Yamuna River was also included in this study.

3. Identification of Fungal Isolates for Tolerance to Heavy Metals

Heavy metal tolerant (25 ppm) fungal isolates were further screened by fine methodology for tolerance to Pb, Ni, Cr and Cd at 50, 100 and 400 ppm of heavy metals individually on PDA. Various fungal isolates were streaked on PDA medium containing 50, 100 and 400 ppm of each heavy metal separately. Streaking of fungal isolates on normal PDA medium served as control (normal growth) for comparison of various growths of fungal isolates on PDA medium and observes at different concentration of heavy metals. Observations on growth of fungal isolate were made after 72 h of incubation. The growth of fungal isolates was recorded as normal growth or absent growth in comparison to control respectively.

4. Removal of Heavy Metals by Fungal Isolates from Liquid Media

The micro-organism continued to grow up to the 3rd day when the cell mass production reached a plateau. After that the cell mass quantity decreases slowly. The most tolerant fungal strain isolates at different heavy metals were evaluated for uptake of heavy metals in potato dextrose broth medium containing 50 ppm concentration of different heavy metals Pb, Ni, Cr and individually in duplicate. The Potato dextrose broth containing 50 ppm of one of that prepared heavy metals was dispensed in 100 ml lots to 250 ml conical flasks and sterilized at standard condition (15 lbs/psi) for 15 min. These flasks were inoculated with 1 ml of freshly spore suspension (104–105 spores/ml) of each fungal isolate and kept on shaker at 150 rpm at 27±2°C for 4 days. Control flasks having only PD broth of 50 ppm concentration of different heavy metals served as control. Fungal growth was harvested after 4 days through filtration using Whatman filter paper. The harvested fungal mass was washed with double distilled water 2–3 times and dried in hot air oven at 70±5°C for 1 day. The dried fungal biomass was weighed and heavy metal concentration in it was measured by digestion with nitric acid and perchloric acid (3:1 ratio). The digested fungal biomass was filtered through Whatman filter paper and made the volume of filtrate to 50 ml in volumetric flask. The heavy metals concentration in filtrate was estimated [11] by Spectrophotometer. Each experiment was conducted in duplicate and data were analyzed statistically. Tolerance and uptake of different heavy metals by fungi in PD broth containing 12.5 ppm each of Pb, Cr and Ni was studied by same method.

The uptake of heavy metal by fungal biomass was calculated using the following equation:

$$qe\ (mg/g) = C * V * 1000/W$$

qe concentration of heavy metal uptake by fungal biomass, (mg/g); C concentration of heavy metal (ppm); V (ml) the volume of the medium and W (g) is the dry weight of the fungal biomass.

5. Result and Discussion

The hyphae growth of *A. flavus and A. niger* exposed to heavy metals were mapped using the tolerance index [31]. Isolation of Heavy Metal Tolerant Fungi were isolated from samples of sewage, sludge and industrial effluent contaminated with heavy metals such as Pb, Cr and Ni using standard methods [12]. This included many strain tolerant to Pb, some tolerant to Cr and some tolerant to Ni at 25 ppm. There are many heavy metal tolerant isolates were identified as Aspergillus niger (Pb2, Cr10, Ni19, Ni27, Ni33), Aspergillus flavus (Pb7, Pb8, Ni35, Ni36) respectively by the laboratory culture.

6. Analysis of Fungal Isolates for Tolerance to Heavy Metals

Many fungal strain isolates and some of these fungi (Aspergillus niger, Aspergillus flavus,) tolerant to Pb at 25, 50, 100 and 400 ppm of Pb. Observation shows decrease in number of isolates tolerant to Pb at higher concentration of Pb. Out of various fungal isolates tolerant to Pb at 25 ppm, only some isolated strain could tolerate to Pb at 400 ppm. Similar observation was scene in their tolerance to Cr and Ni. This observation indicates that inhibition of some of the fungal isolates at higher concentration of heavy metals due to the various biological factors. And this toxic effect of higher concentration of heavy metals on growth of fungi have been reported [13, 14].

7. Growth and Uptake of Pb by Fungal strain

The maximum dry wt (0.46 g) was observed by Aspergillus niger in PD broth containing 50 ppm of Pb. The minimum dry wt (0.22 g) was observed by fungus Aspergillus flavus. The dry weight of A. niger was statistically significant in comparison to all other fungi (Table 1). The maximum uptake (17.35 mg/ g) of Pb was observed in Aspergillus flavus. Minimum uptake Pb (7.76 mg/g) found in A. niger (Table 1). Wherever there was less growth, there was maximum uptake of Pb and vice versa. The highest uptake of Pb (17.35 mg/g) by A. flavus indicated that having more binding sites on cell wall of this fungus and its potential as biosorbent to remove Pb from industrial wastewater containing higher concentration of Pb. Similar results with respect to differential Pb uptake by different fungi were reported by earlier workers [15–20].

Table 1: Pb uptake by different fungi from liquid medium at 50 ppm lead

Fungi	Dry wt (g)	uptake (mg/g)
Pb2 (Aspergillus niger)	0.46	7.76
Pb7 (A. flavus)	0.21	12.44
Pb8 (A. flavus)	0.22	17.35
CD at 5%	0.03	2.66

8. Growth and Uptake of Cr by Fungal strain

The maximum dry wt (0.36 g) was observed in A. niger. The minimum dry wt (0.32 g) was found in Cr10 (A. niger) which was statistically significant in comparison to other one. The maximum uptake (0.05 mg/g) and minimum uptake of Cr (0.04 mg/g) was observed in (Table 2) Cr10 (A. niger) and Aspergillus niger in PD broth containing 50 ppm of Cr respectively. The highest uptake of Cr (0.05 mg/g) by Cr10 (A. niger) indicated its efficiency to remove Cr from aqueous solution containing higher concentration of Cr. These results with respect to uptake of Cr by fungi are in agreement with those reported earlier worker [25–28].

Table 2: Cr uptake by different fungi from liquid medium at 50 ppm Cromium

Fungi	**Dry wt (g)**	**Uptake (mg/g)**
Cr10 (A. niger)	0.32	0.05
Aspergillus niger	0.36	0.04
CD at 5%	0.03	0.02

9. Growth and Uptake of Ni by Fungal strain

The maximum dry wt (1.62 g) was observed in (Table 3) fungal isolate Ni36 (A. flavus) in PD broth containing 50 ppm of Ni. The minimum dry wt (1.01 g) was found in Ni35 (Aspergillus flavus). The dry weight of Ni33 (A. niger) and Ni36 (A. flavus) was statistically significant in comparison to all other fungi. The maximum uptake (0.53 mg/g) and minimum uptake of Ni (0.32) from PD broth containing 50 ppm of Ni was observed by isolates Ni27 (A. niger) and Ni36 (A. flavus) respectively. The uptake of Ni by A. niger was most significant in comparison to all other tolerant fungi. Similar results with respect to uptake of Ni by fungi have been reported earlier worker [7, 29].

Table 3: Ni uptake by different fungi from liquid medium at 50 ppm Nickel

Fungi	**Dry wt (g)**	**Uptake (mg/g)**
Ni19(A. niger)	1.06	0.40
Ni27(A. niger)	1.05	0.53
Ni33(A. niger)	1.36	0.40
Ni35(A. flavus)	1.01	0.47
Ni36(A. flavus)	1.62	0.32
CD at 5%	0.03	0.02

10. Growth and Uptake of Multiple Metals by Fungi

The maximum growth in the presence of different metals was observed by Aspergillus niger. However, there was significant higher uptake of Pb (0.70 mg/g), Cr (0.66 mg/g) by Aspergillus flavus and Ni (0.63 mg/g) by fungal isolate Pb8 (A. flavus) (Table 4). The dry weight of A. niger was most significant in comparison to all other fungi. The uptake of Pb by A. niger, Cr by A. flavus and Ni by Pb8 (A. flavus) was statistically significant in comparison to all other fungi. Fungi Pb8 (A. flavus) was tolerant to all the four heavy metals and biosorbed substantial amount of the heavy metals from PD broth containing 125 ppm each of three heavy metals (Pb, Cr, Ni). There was lower uptake of all the three heavy metals (Pb, Cr, Ni) by these fungi in combination in comparison to individual heavy metal uptake. This was mainly due to competition of heavy metals for same and limited adsorption sites on fungal cell wall of these fungi. These result indicated the potential of these fungi to remove heavy metals from liquids media and industrial wastewater containing higher concentration of heavy metals. Similar observations regarding reduction of uptake of heavy metals in comparison to individual heavy metal have been reported earlier worker [7, 9]. The above data

(Tables 2, 3 & 4) of dry weight of fungi and uptake of heavy metal by fungi indicated that where there is maximum dry wt of fungus, there is minimum uptake of heavy metal and vice versa. The higher uptake of heavy metal was due to less weight of fungi at a particular concentration of that heavy metal. Similar results have been reported by earlier workers [7, 29].

11. Conclusion

The ability of A. niger A. flavus biomass to bind and remove heavy metals, i.e. Ni2þ, Pb2þ, Cr2þ from real wastewater was investigated. Various fungal isolated from sewage, sludge, and industrial effluents and in which some identified fungi were screened for their tolerance to four heavy metals (Pb, Cr and Ni) in PDA medium containing heavy metal from 25 to 400 ppm. There was decrease in number of fungi for their tolerance to heavy metal with increase in concentration of heavy metal from 25 to 400 ppm. Majority of the fungal isolates were able to tolerate heavy metals up to 400 ppm. The most heavy metal tolerant (400 ppm) fungi were further screened for removal of heavy metals from PD broth containing 50 ppm of individual heavy metal. Data revealed that some of the fungi removed substantial amount of heavy metals (17.35 mg/ g) of Pb in Aspergillus flavus and maximum uptake (0.53 mg/g) of Ni by Ni27(A. niger) was observed. This indicated the potential biosorption capacity of these fungi to remove heavy metals from wastewater.

Table 4: Tolerance and uptake of heavy metals by fungi from medium having 125 ppm of Cd, Pb, Cr and Ni

Fungi	Dry wt (g)	Pb uptake (mg/g)	Cr uptake (mg/g)	Ni uptake (mg/g)
Pb2 (Aspergillus niger)	0.51	0.46	0.74	0.64
Ni35 (A. flavus)	0.4	0.70	0.44	0.43
Aspergillus flavus	0.39	0.51	0.66	0.23
Pb7 (A. flavus)	0.42	0.64	0.56	0.58
Pb8 (A. flavus)	0.41	0.56	0.54	0.63
Ni27 (A. niger)	0.45	0.67	0.49	0.33
Ni30 (A. niger)	0.48	0.61	0.49	0.31
Ni33 (A. niger)	0.48	0.58	0.62	0.45
Ni36 (A. flavus)	0.34	0.43	0.56	0.39
CD at 5%	0.04	0.03	0.03	0.03

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