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Biosorption of cadmium and nickel by pretreated *Aspergillus* spp. biomass

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Industrial effluents contaminated with the heavy metals pose threat to the environment and its habitants. Biosorption is an effective and eco-friendly method for sequestration of heavy metals from such effluents. Fungi, with their remarkable metabolism-independent metal uptake systems, are efficient natural biosorbents of heavy metals. Therefore, we explored fungal biomass (*Aspergillus* spp.) pretreated with formaldehyde (solvent) and sodium hydroxide (alkali) for sequestration of metals cadmium (Cd) and nickel (Ni) from the aqueous solutions contaminated with heavy metals. The results have shown significant increase in the sequestration of Cd and Ni by the *Aspergillus* spp. biomass pretreated with formaldehyde and sodium hydroxide and thereby demonstrated its potential in cleaning the environment polluted with heavy metals.

Keywords: Bioremediation, Compost, Degradation, Heavy metals, Lignocellulose, Sequestration

Increased industrialization and human activities have contaminated the environment with heavy metals through waste disposal. Mine drainages, metal industries, refining, electroplating, dye and leather industries, domestic effluents, landfill leachate, agricultural runoff, and acid rain contribute to such contamination[1]. Heavy metals such as copper and cadmium are reported to induce production of reactive oxygen species, and negatively affect the population growth of ciliates[2]. Cadmium intake is reported to cause oxidative stress and have adverse effect on neural tissues[2,3]. On the other hand, in nature, microorganisms *viz*. algae, bacteria, fungi and yeast are capable of accumulating the heavy metals and thereby make the environment relatively clean and better[4-6]. They act as efficient biosorbents.

Biosorption, non-directed physicochemical interaction that occurs between metals and microbial cells, is reportedly a better ecofriendly alternative over the conventional physical and chemical methods[6,7]. It is reported to be influenced by various factors and processes, such as temperature, pH, initial concentration of the metal ions, biosorbent dose, and speed of agitation[7]. The cadmium uptake capacity of green algae biomass from the aqueous solutions has been shown to be affected by pH, temperature, biomass dosage and initial metal concentration[8]. Further, the biosorption capacity of biomass can be modified by physical and chemical pretreatment[7]. Potential of filamentous fungi in bioremediation of heavy metals from the industrial effluents and wastewaters has been reported from different parts of the world[9]. Bioaccumulation of heavy metals from aqueous solution using *Aspergillus flavus* and *Rhizomucor pusillus* has also been reported[10]. Cai *et al*.[11] have demonstrated removal of heavy metals from the aqueous solutions using immobilized biomass of *Penicillium janthinillum*. Works on bioaccumulation of cadmium by green algae[8] and *Aspergillus flavus*[12]; and Cd, lead and nickel accumulation by *Spirulina maxima*[13] are also available.

The specific mechanisms of uptake differs with the species; the origin of the biomass and its processing[14]. The hyphal wall is the primary site of metal ion accumulation. This accumulation is attributed to various chemical groups (the acetamido group of chitin, amino and phosphate groups in nucleic acids, amino, amido, sulfhydryl and carboxyl groups in proteins, and hydroxyls in polysaccharides) that sequester the metal ions[15]. Biomass of fungi, *viz*. *Absidia*, *Cunninhamella*, *Mucor*, *Penicillium chrysogenum*[16], *Streptomyces pimprina* and *Rhizopus* exhibit excellent metal ion uptake due to the high chitin and chitosan cell wall content[17]. Fungi have been proven more efficient and economical for sequestration of toxic metals because of their filamentous morphology and cell wall composition[18].

Fermentation industries all over the world generate huge amounts of waste biomass which are used in animal feed or organic manure if not incinerated. The food and beverage industries, chemical industries (e.g., citric acid), enzymes industries that produce array of enzymes and pharmaceutical industries involved in steroid transformation, generate large

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amount of microbial biomass. In a day, antibiotics fermentation industries generate fungal biomass of about 5000 tons[16,19]. The lysine fermentation industries also generate large amount of biomass of *Corynebacterium glutamicum*[20].

Chemical pretreatment using acids, alkali, solvents and organic chemicals has shown enhancement in metal biosorption[21]. Pretreatment of fungal biomass has been found to improve their biosorption capacity[22]. However, reports on pretreating the *Aspergillus* spp. biomass to improve biosorption capacity are limited. Hence, in this study, we explored the effect of pretreatment of *Aspergillus* biomass with solvent i.e., formaldehyde (HCHO) and alkali i.e., sodium hydroxide (NaOH) on sequestration of cadmium (Cd) and nickel (Ni) from the aqueous solution.

Materials and Methods

Fungi for sequestration of metals

The fungi were isolated by viable count method from the distillery spent wash-based composts and compost yard soil. The distillery spent wash based composts and compost yard soil were obtained from the sugar factories from Theur, Malegaon and Baramati, Pune, Maharashtra, India. The distillery spent wash based composts and compost yard soil were weighed (1 g) each, and added to 9 mL sterile distilled water. The serial dilutions were made and 0.1 mL of 10^{-4} dilution was spread plate on Potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) medium, respectively. The plates were incubated at 28°C for 48 h and the colony forming units (CFU)/g were noted[8,9].

Characterization of fungi by slide culture technique

Half strength sterile CDox agar medium [CDox (g/L): sucrose: 4.0, $NaNO_3$: 0.2, $MgSO_4.7H_2O$: 0.05 and K_2HPO_4: 0.1 g/L, pH 6.8, agar: 30.0 g, distilled water: 1000 mL] was prepared. Agar blocks (1 cm^2) were cut and placed on sterile glass slide. The fungal isolates were inoculated to the four corners of the agar block. The slides were incubated at 28°C for 3 days in the Petri plate on the glass triangle kept on the filter paper moistened with 20% glycerol. Staining was done by lactophenol cotton blue [lactophenol cotton blue (g/100 mL), phenol 100.0, cotton blue 0.25, glycerol 200 and lactic acid 100 mL] and observed under phase contrast microscope.

Colony characters on different media

The three fungal isolates were spot inoculated on the CDox and malt extract agar [malt extract (g/L): malt extract 20.0, pH 6.5, agar 30.0 and distilled water 1000 mL] plates and incubated at 28°C for 6 days and observed for colony characters.

Preparation of the fungal biomass

The spore suspension (5×10^5 spores/mL) of the Three fungi was used as inoculum for the preparation of biosorbents. The collected spores were aseptically transferred to 500 mL conical flask containing 100 mL Yeast Peptone Glucose (YPG) [YPG (g/L): yeast extract 3.0, peptone 10.0, glucose 30.0, pH 4.5 and distilled water 1000 mL] and incubated on a shaker (125 rpm) at 28°C for 3 days. After incubation, the biomass was harvested and washed with a copious amount of deionized water and then further ground using a mortar and pestle. The prepared fungal biomass was used to study the effect of pretreatment with HCHO and NaOH on the sequestration of heavy metals by the fungal biomass.

Effect of pretreatment with HCHO and NaOH on the sequestration of heavy metals by fungal biomass

For pretreatment with HCHO, the live fungal biomass (2 g) each, was treated with 100 mL 10% (v/v) HCHO solution and agitated for 3 h on a shaker at 28°C and for NaOH pretreatment, the biomass (2 g) each were boiled in 50 mL 0.5 N NaOH solution for 15 min[23]. After this, the biomass was washed thrice with distilled water and mixed with 50 mL of individual heavy metal solutions of Cd and Ni, respectively of 1000 ppm and pH 5.0 in 250 mL conical flasks. The flasks were kept at 38°C for 20 min contact time period. The biomass without any treatment served as control. After 20 min contact time, the solutions were filtered through Whatman filter paper No.1 and the filtrates were analyzed for metal sequestration using Atomic Absorption Spectrophotometer (AAS) (Varian SpectrAA, Germany).

The amount of metal biosorbed per gram of the biomass was calculated as[24]:

$$Q = [(C_i - C_f) / m] \times V$$

where, Q = metal ion bioadsorbed (mg/g), C_i = initial metal ion concentration (mg/L), C_f = final metal ion concentration (mg/L), m = biomass in the reaction mixture (g) and V = volume of the reaction mixture (L)

Results and Discussion

Fungal biomass for sequestration of heavy metals

Fungal population in compost and compost yard soil

Distillery spent wash is rich in nutrients (mg/L) such as NH_3-N (636.25), P (28.36), K (6500),

Ca (920), Na (420) and metals (mg/L) like Mg (753.25), Fe (6.3), Mn (1429), Zn (1.09), Cu (0.265), Cr (0.067), Cd (0.036) and Co (0.08)[25]. The fungal population on SDA medium was more than that on the PDA medium. The fungal population in compost from distillery spent wash of Theur, Malegaon and Baramati on SDA agar medium was found to be 24.60, 6.80 and 21.00 × 10^4 CFU/g, respectively (Table 1), whereas the fungal population in compost yard soil on SDA agar medium was 3.73 × 10^4 CFU/g (Table 1).

It was also observed that the fungal population was more in the distillery spent wash based composts of Theur and Baramati (Table 1). About 15 fungi were isolated from the distillery spent wash based composts and compost yard soil. These fungi were studied for their lignocellulose degrading properties and the most potential fungi were further selected for their biosorption studies.

Characterization and identification of fungal isolates

Slide culture and staining by lactophenol cotton blue showed the fungal species isolated from the compost and compost yard soil samples were *Aspergillus clavatus*, *Aspergillus oryzae* and *Aspergillus fumigatus*[26]. *A. clavatus* conidiophores were erect, simple with clavate conidial head. The conidiophores were 125 µm tall, vesicles 20 µm and phialides 6.5 × 2.0 µm in diameter, respectively. The conidia were 2.5 µm in diameter. *A. oryzae* conidiophores were hyaline, simple, 75 µm tall, vesicles 40 µm and phialides 8.0 × 4.5 µm in diameter, respectively. The conidia were ellipsoidal measuring 4.5-8.0 µm in diameter. *A. fumigatus* conidiophores were hyaline, simple, inflated at the apex forming nodded vesicles bearing conidial heads. The conidiophores were 80 µm tall, vesicles 16 µm and phialides 4.9 × 2.5 µm in diameter, respectively. The conidia were 2.7 µm in diameter.

Table 1 — Fungal population in distillery spentwash composts and compost yard soil on different media

Medium	Fungal population (CFU/g) × 10^4			
	Distillery spentwash composts			Compost yard soil
	Theur	Malegaon	Baramati	
PDA	18.0±0.00	9.0±1.47	19.7±1.20	5.80 ± 0.90
SDA	24.6±0.00	6.8±0.80	21.0±3.00	3.73 ± 0.35
CDox	-	-	-	5.90 ± 0.00

[CFU, colony forming unit; PDA, Potato dextrose agar; SDA, Sabouraud dextrose agar. Each data point represents average of triplicate ± SD]

Colony characters on different media

On CDox solid agar media plates, *A. clavatus* and *A. fumigatus* showed dark blue-green colour colonies with diameter 3.5 and 4.0 cm, respectively, while *A. oryzae* showed olive-yellow coloured colony with diameter 5.0 cm after incubation at 28°C for 6 days. Colonies on malt extract agar media grew fast and showed similar growth characteristics.

Effect of pretreatment on sequestration of heavy metals by fungal biomass

Pretreatment of *Aspergillus* spp. biomass with HCHO increased the biosorption capacity for both Cd and Ni. In case of Cd, the biosorption capacity of *A. clavatus*, *A. oryzae* and *A. fumigatus* had increased from 67.68 to 99.72, 31.24 to 99.12 and 79.76 to 99.24%, respectively (Table 2). Whereas in case of Ni, increase for *A. clavatus*, *A. oryzae* and *A. fumigatus* was from 41.52 to 73.92, 37.48 to 70.40 and 39.36 to 70.68%, respectively (Table 2).

Similarly, pretreatment of *Aspergillus* spp. biomass with NaOH had also increased the biosorption capacity for both Cd and Ni. In case of Cd, the biosorption capacity was increased from 67.68 to 99.72, 31.24 to 99.76 and 79.76 to 99.80% for *A. clavatus*, *A. oryzae* and *A. fumigatus*, respectively (Table 2). In case of Ni, the increase was from 41.52 to 72.08, 37.48 to 55.12 and 39.36 to 65.52% for *A. clavatus*, *A. oryzae* and *A. fumigatus*, respectively (Table 2). The increase in biosorption capacity by the biomass of *Aspergillus* spp. may be attributed to the removal of surface impurities, rupture of cell membranes and exposure of available binding sites after pretreatment.

Kapoor & Viraraghavan who studied on site biosorption of Cd by *A. niger* noted that the biosorption was more efficient after HCHO

Table 2 — Effect of pretreatment of HCHO and NaOH solution on sequestration of heavy metals by fungal biomass

Fungal cultures	Sequestration of heavy metals (%)			
	Cd		Ni	
	Test	Blank	Test	Blank
A. clavatus	99.16±0.00/99.72±0.00	67.68±0.01/67.68±0.00	73.92±0.01/72.08±0.00	41.52±0.00/41.52±0.00
A. oryzae	99.12±0.00/99.76±0.01	31.24±0.00/31.24±0.00	70.40±0.00/55.12±0.01	37.48+0.00/37.48±0.01
A. fumigatus	99.24±0.01/99.80±0.01	79.76±0.01/79.76±0.01	70.68±0.01/65.52±0.01	39.36±0.00/39.36±0.01

[Each data point represents average of triplicate]

treatment[21]. Similar report has been shown in case of biosorption of Cd by *Pleurotus* florida as well[23]. Yan & Viraraghavan have also demonstrated that *Mucor rouxii* when pretreated with NaOH improved their bioadsorption capacity of Cd^{2+}, Ni^{2+} and Zn^{2+} metal ions[24]. Biosorption of Cu (II) and Ni (II) ions by pretreated biomass of *A. niger* has been reported, where pretreatment with NaOH increased its adsorption capacity as compared to the untreated biomass[27].

The NaOH treated biomass of *Penicillium digitatum* has also been reported to enhance Cd (II) bioadsorption[28] and by *Pleurotus florida*[23]. Holan & Volesky have also reported that pretreatment with alkali improved the metal biosorption capacity[15]. The enhancement of Cr biosorption capacity of dead *A. niger* fungal biomass by pretreatment and its use in a column mode[29] has also been studied. Pretreatment with NaOH has been shown to have increased the biosorption capacity of Pb(II) by *Botrytis cinerea* from 43.41 to 51.73 mg/g[30].

There is a report on the biosorption of Cd (II) by three *Candida* sp. from ripe fruit peels in the Philippines and were found to tolerate 230 mg/L of Cd (II)[31]. Cadmium removal from the aqueous solution by heated inactivated and formaldehyde treated biomass of *Aspergillus nidulans* has also been studied. The highest removal of cadmium was obtained at pH 6.0 and with biomass subjected to heat inactivation treatment[12]. The blue green algae *Spirulina maxima* demonstrated biosorption of lead (II) prominently over cadmium (II) and nickel (II)[13]. The nanocellulose fibers have also studied for biosorption of cadmium, nickel and lead ions from aqueous solution, where the nanocellulose fibers showed removal efficiency of 9.7 mg/g Cd (II), 9.42 mg/g Pb (II) and 8.55 mg/g Ni (II) ions from 25 mg/L metal solution[33].

Conclusion

The above results have shown that biomass of *Aspergillus clavatus*, *Aspergillus oryzae* and *Aspergillus fumigatus* pretreated with solvent formaldehyde (HCHO) and alkali sodium hydroxide (NaOH) improves sequestration of cadmium (Cd) and Nickel (Ni) from the aqueous solutions contaminated with these heavy metals. Therefore, it can be suggested that the biomass of *Aspergillus* spp. pretreated with HCHO and NaOH may be used for effective cleaning of aquatic environment contaminated with heavy metals Cd and Ni.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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