Investigation on the Biosorption Potential of Alkali Pretreated Aspergillus sp. Biomass for the Removal of Copper

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Abstract: Pollution due to heavy metal becoming a major environmental threat being faced by the world today and also becomes a key concern in many developing as well as developed countries of the world. Large amount of heavy metals such as, nickel, Copper and zinc released by number of electroplating and metalworking industries. These metal pollutants are discharged directly or indirectly into waterbodies. Industrial waste wasters contain large amount of metals ions and treatment of wastewater is needed in order to avoid pollution of waterbodies before disposal. One of the important metal is copper, is beneficial to human being but in higher concentration it is highly toxic like other heavy metal. Conventional methods for the detoxification or removal of heavy metals are time consuming and highly expensive. Microorganisms like algae, actinomycetes, bacteria, fungi and yeasts have the capacity to tolerate and to accumulate heavy metal ions from the polluted environment, bioremediation by fungi is widely explored and reduction of metal ions by bioadsorption technique gained a lot of attention during the past decade. Biosorption technology mainly because of its low cost and high metal binding efficiency, offers promising alternative method for the removal of metal ions. Therefore the present investigation is based on the bioadsorption capacity of NaOH pretreated Aspergillus species namely A. flavus, A. fumigatus, A. niger and A. terreus for the removal of metal ions. Fungal growth was tested by using 3 different concentrations of $CuSO_4$ (2mM, 4mM, and 8mM) amended in PDA medium. Fungal growth was observed only at 2mM and 4mM concentrations but no fungal growth was observed at 8mM. The biosorption capacity of both living species and NaOH pretreated species of Aspergillus was compared. In the present investigation, NaOH pretreated species of Aspergillus showed highest biosorption potential in comparison to live biomass of Aspergillus. In case of live biomass, A. niger showed maximum biosorption potential in comparison to other fungal species.
Keywords: Heavy metals, environmental pollution, alkali pretreated biomass, Aspergillus species, bioadsorption, PDA medium, $CuSO_4$

I. INTRODUCTION

Presence of heavy metals and trace elements in the environment due to urbanization, industrialization and intense petrochemical works led to enormous increase in the pollution. Heavy metal pollution of the environment becomes a global concern due to their accumulation and persistence. The presence of these metal ions in air, water and soil become a growing threat to the entire biotic component. Among heavy metals copper, is one of the typical metal ion that causes substantial threat for human health even at the low concentration via the food chains. It is released and accumulates into the environment through different ways, including phosphate fertilizer, photographic materials, petrochemicals, pulp and paper, refineries, manufacturing of explosives, electronic goods and wood production and from the combustion of fossil fuel, forest fires, sewage wastewater and automobile emissions (Conrad and Hansen, 2007). Heavy metals generated in electroplating process are arsenic, cadmium, chromium, copper, mercury, nickel, silver and zinc (Germain and Patterson, 1974; Palmer and Wittbrodt, 1991). Industrial effluent contains higher concentration of heavy metals and before their disposal into the waterbodies treatment is needed for the avoidance of water pollution. The effluent removal of heavy metals from waste waters and industrial wastes still remains a major topic of present research (Veglio and Beolchini, 1997).

Long-term health risks for biotic components of ecosystems created due to the presence of higher concentration of copper in the environment. Copper, is essential to human life and health, but like all heavy metals, it is potentially toxic, especially at high concentrations (Tapiero et al., 2003). Copper finds its way to the water stream from industries like electroplating, mining, electrical/electronics, steel production, also in the non-ferrous metal industry, the printing & photographic industries, metal working and finishing processes (Birlik et al., 2006).Copper may cause serious health problems, in particular possible liver damage with prolonged exposure. Copper in water sources can also damage a variety of fish and invertebrates. Large acute doses can produce harmful, even fatal effects. These reasons suggest that, copper must be removed to very low levels from wastewater (Turkmen et al., 2009).

Therefore, the removal of Copper metal ions from drinking water, wastewater water used for agricultural purpose has gained much attention in recent years. As the result, the developments of reliable and economically acceptable treatment methods for the removal of Cu from aqueous system are particularly important. Technologies used for treatment of heavy metals are chemical precipitation (Sampaio et al., 2009), ion exchange (Alyuz, 2009), reverse osmosis (Cadotte et al., 1981), solvent extraction, membrane separation, electrolysis (Bessbousse et al., 2008), photochemical reactions (Fox and Dulay, 1993), and magnetic separation (Karapinar, 2003). However, these technologies are not economical and favorable because some technologies produces large amount of toxic compounds to remove heavy metals from industrial wastewaters (Ahalya et al., 2005).

iological treatment methods uses either living or dead microorganisms or plants, provide unique abilities to concentrate and reduce the concentration of heavy metals ions to environmental acceptable limits in economically and environmentally friendly manner (Volesky, 2001).Biosorption using biological material has gained appreciable attention for the detoxification of metal ions from aqueous system as they have various advantages such as low cost, minimization of toxic components, biosorbent regeneration and recovery of metals etc.(Ahalya et al., 2003). Fungi are having the characteristics for the detoxification of heavy metals by various mechanisms and are considered as efficient adsorbent for the removal of metal ions (Magyarosy et al., 2002).

Both living (bioaccumulation) and non-living (biosorption) biomass of microorganisms can be used as potential scavenger of heavy metals. Non-living biomass of fungi not affected by adverse conditions and has been used in various studies and can resolve highly toxic environmental conditions (Chu and Hasim, 2004). Fungal cell wall possess many metal binding sites with carboxyl, carbonyl, hydroxyl and methoxyl groups that efficiently binds with the metal ions(Gadd,2009).Several modifying agents have been extensively used for the modification of microbial cell wall to the enhancement of biosorption potential of heavy metal ions by microbial biomass. The treatment varies with the type of microbial biomass and the heavy metal ion to be adsorbed (Gupta et al., 2000).Physical methods for pretreatment are boiling, heating, autoclaving and freeze drying and the chemical treatment methods include with acids, detergents, alkalis and organic or inorganic chemicals or a combination of both physical and chemical treatment methods (Bai and Abraham, 2002; Awofolu et al.,2006; Loukidou et al.,2003). Microbial cell surfaces modified by these types of pretreatment methods which is essential for biosorption either by exposing more metal ion binding sites or by removal of groups(Gupta et al., 2000).Binding of metal ions with microbial biomass increased by alkaline treatment. Cell walls of microbes ruptured on treatment with alkali such as sodium hydroxide, potassium hydroxide, alkaline detergents or other alkaline reagents and due to these functional groups are exposed for binding with metal ions (Yan and Viraraghavan, 2000).

The objective of present study was to investigate the copper tolerance of 4 species of Aspergillus and to study the effect of alkali (NaOH) pretreatment of Aspergillus spp. on the biosorption of copper metal ion.

II. MATERIALS AND METHODS

A. Sterilization of glass ware

The sterilization of glass wares such as Petri dishes, flasks, and test tubes washed with detergent was then autoclaved at 121^0C 15psi for 20 min.

B. Isolation and Identification of Fungi

Approximately, 1kg of soils was collected from peri-urban areas of Kurukshetra, Haryana which were irrigated with untreated wastewater agriculture fields. Soil samples were then taken into fresh sterile polythene bags. The species of *Aspergillus* were isolated from soil by serial dilution methods. Potato Dextrose Agar medium was used for culturing of fungi. fungi were identified using lactophenol and cotton blue (Barnett and Hunter, 1999).

C. Screening of Aspergillus species for Copper tolerance:

The copper tolerance of A. flavus, A. fumigatus, A. niger and A. terreus was evaluated at various concentrations(2mM, 4mM and 8 mM) of copper sulphate supplemented in PDA medium and was poured in the petriplates. 6mm disc of each fungus was inoculated and incubated at 27˚C for 7 days. The culture in PDA without copper sulphate served as control. The reduction in the rate of radial growth was used as an index for metal (copper) tolerance.

To compare the heavy metal tolerance of each species, a parallel Index of Tolerance (T.I) was calculated as a percentage value from the ratio:

$$T.I. = \frac{\text{Radial growth rate in metal treatment}}{\text{Radial growth rate in control}}$$

The isolates exhibiting better growth after incubation were considered as copper tolerant (Tahir,2012). The tolerance studies were conducted in duplicates and the mean values were used to compare the tolerance *of Aspergillus* spp.

D. Preparation of biosorbent

1) *Preparation of live biomass:* 6 mm disc of each fungus culture was transferred to PDA broth and incubated at 27^0C for 7 days.
2) *Alkali (NaOH) pretreated biomass:*50g of live harvested biomass was treated with 0.5 N NaOH for half an hour then washed with distilled water. The pretreated biomass was then autoclaved and was then dried at 60^0C in hot air oven and then powdered using mortar and pestle (Ahmad *et al*., 2005).

E. Biosorption Experiment:

0.1 gm of live and NaOH pretreated A. flavus, A. fumigatus, A. niger and A. terreus was inoculated into 100 ml solution of metal containing 2mM, 4mM and 8 mM of $CuSO_4$. The flasks were then kept on rotary shaker 125 rpm for 18-20 hr at 30˚C. Then the solution was centrifuged at 10,000 rpm for 15 min. The content of the supernatant was analysed after proper digestion and dilution by atomic absorption spectrophotometer. Metal solution without biomass served as control.

Biosorption capacity, i.e. amount of metal ions (mg) biosorbed per g of biomass was calculated with the help of following equation:

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

Where,
Q = mg of metal ion bioadsorbed per gm of biomass,
Ci = initial metal ion concentration mg/l,
Cf = final metal ion concentration mg/l,
m = mass of biomass in the reaction mixture gram,
V = volume of the reaction mixture (1) (Ahmad, 2005)

III. RESULTS AND DISCUSSION

In the present study, 15 fungi were isolated from soil samples(table 1).This include 8 species of Aspergillus,2 species of Penicillium,Trichoderma viride, Graphium sp., Fusarium oxysporum, Cladosporium cladosporioides and C. sphaerospermum. Shazia et al. isolated 19 fungi from metal contaminated soils, also reported that species of Aspergillus (12 out of 19) were frequently encountered in comparison to other fungi. Ahmad et al.(2005) and Zafar et al.(2007) also reported that Aspergillus species were the most commonly occurring fungi in the heavy metal contaminated soils in their studies.

Several species of Aspergillus have been used for the Biosorption of heavy metals such as A. cristatus(Hassan and Kassas,2012), A. flavus (Akar and Tunali,2006), A. fumigatus (Al-Garni,2009), A. niger (Srivastava and Thakur,2006), A. niveus (Karaca,2006), A. terreus (Sun et al.,2010) and A. versicolor (Cabuk et al.,2005) etc. Sen and Dastidar (2007) studied the biosorption of hexavalent chromium ion by Aspergillus species isolated from industrial wastewater. Shazia et al.,(2013) also observed in their study that Aspergillus isolates were the most resistant to cadmium, and nickel.

Hence, in the present work, four Aspergillus species viz., A. flavus, A. fumigatus, A. niger and A. terreus were selected under study.

In metal polluted environment microbes become metal resistant as they adapt the poisonous level of heavy metals.Therefore in the present work, copper tolerance of the Aspergillus species was checked using 2mM, 4mM and 8mM concentration of copper sulphate. The fungal growth was observed in 2mM and 4mM but at high conc. i.e. 8mM there was no fungal growth. Inhibition of fungal growth could have resulted due to copper toxicity at higher concentration.

Metal tolerance index was calculated for all the 4 Aspergillus sp. (Table 2).For 2 mM $CuSO_4$ concentration, the copper tolerance index of A. flavus and A. niger was same, i.e., 0.6, whereas it was 0.3 and 0.4 for A. fumigatus and A. terreus respectively. For 4mM concentration of $CuSO_{4,}$ the tolerance index for A. flavus and A. niger was decreased to 0.4, but the tolerance index for A. terreus remained same. A decrease in tolerance index was noticed for A. fumigatus, i.e., it was observed to be 0.02.This variation in degree of tolerance was most probably due to the potential variation in the mechanism of tolerance.

In the present work two types of biosorbent (live biomass and NaOH pretreated biomass) of the Aspergillus species were used for the removal $CuSO_4$.Biosorption potential of both live biomass and NaOH pretreated biomass of A. flavus, A. fumigatus, A. niger and A. terreus was analyzed using metal solution containing $CuSO_4$ at various concentrations of 2, 4 and 8 mM at pH 7 as suggested by Ahmad et al.

Table 1. List of fungi isolated from metal contaminated soil samples

Sr. No.	Name of fungi
1	Aspergillus flavus
2	Aspergillus flaviceps
3	Aspergillus fumigatus
4	Aspergillus japonicus
5	Aspergillus nidulans
6	Aspergillus niger
7	Aspergillus terreus
8	Aspergillus versicolor
9	Cladosporium cladosporoides
10	Cladosporium sphaerospermum
11	Fusarium oxysporum
12	Graphium sp.
13	Penicillium frequentans
14	Penicillium oxalicum
15	Trichoderma viride

Table 2. Metal tolerance index of *Aspergillus* species

Name of the fungi	Metal tolerance index		
	2mM	4mM	8mM
Aspergillus flavus	0.6	0.4	no growth
Aspergillus fumigatus	0.3	0.02	no growth
Aspergillus niger	0.6	0.4	no growth
Aspergillus terreus	0.4	0.4	no growth

The amount of copper removed by live biomass and NaOH pretreated, A. niger, A. fumigatus, A. flavus and A.terreus was given in figure 1-4 respectively. The results showed that biosorption of copper was increased with increase in the concentration of metal ion.

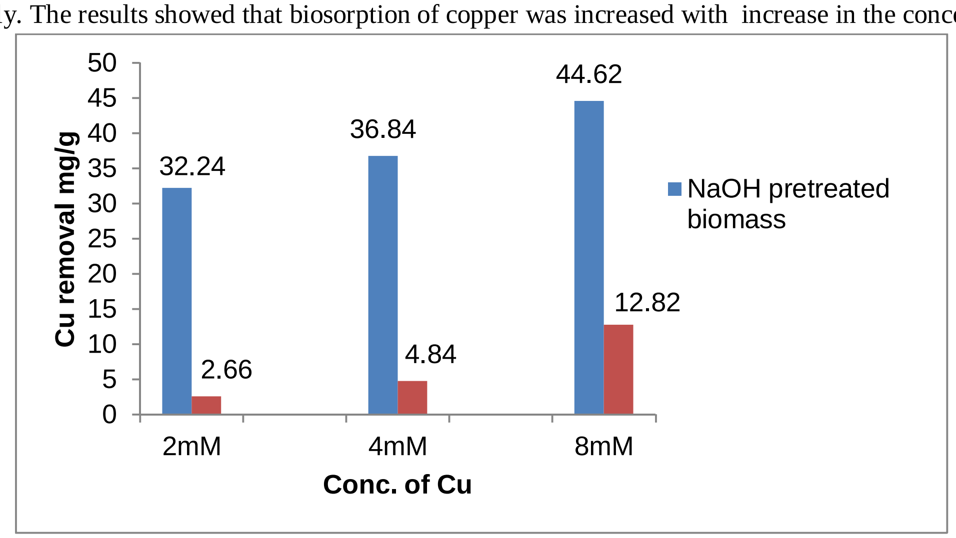

Figure 1. Comparison of copper biosorption potential of NaOH pretreated biomass and live biomass of A. niger

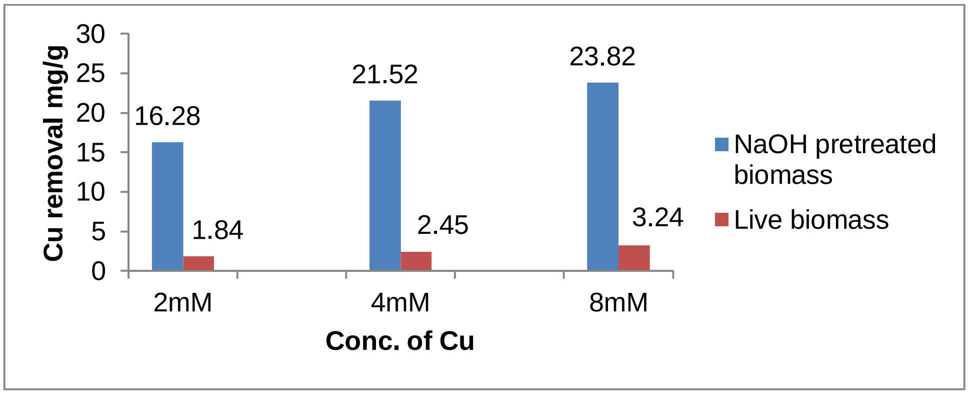

Figure 2. Comparison of copper biosorption potential of NaOH pretreated biomass and live biomass of A. fumigatus

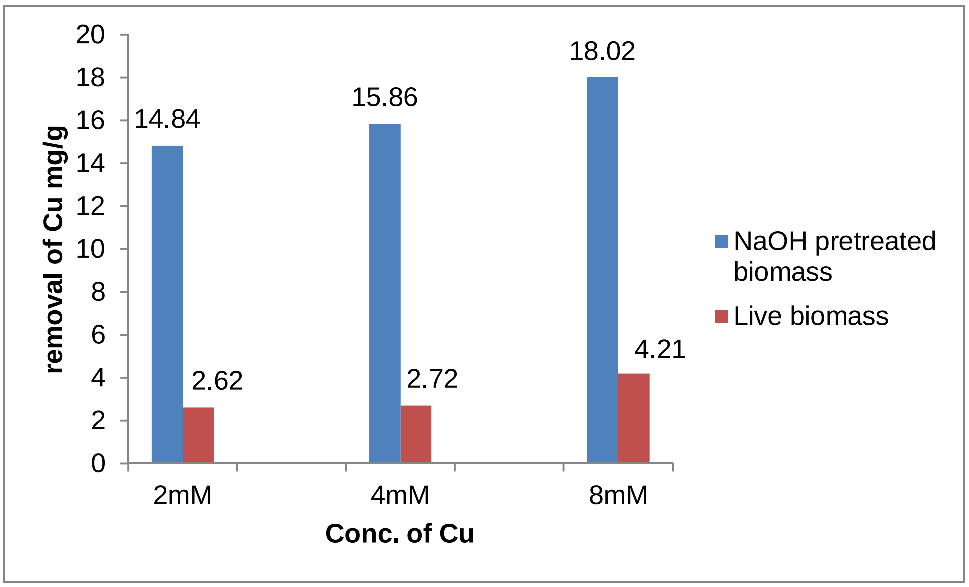

Figure 3. Comparison of copper biosorption potential of NaOH pretreated biomass and live biomass of A. flavus.

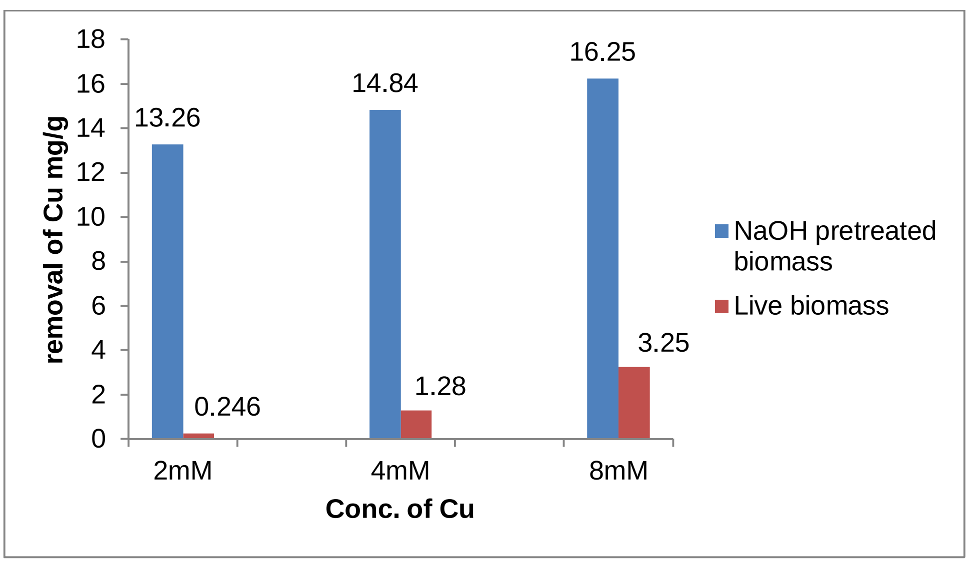

Figure 4. Comparison of copper biosorption potential of NaOH pretreated biomass and live biomass of A. terreus

Live biomass of A. niger showed maximum removal of copper i.e., 2.66 mg/g at 2mM concentration and minimum removal of Cu i.e., 0.246 mg/g exhibited by A. terreus. In case of NaOH pretreated biomass, maximum removal of Cu (32.24 mg/g) was reported by A. niger whereas A. terreus showed lowest biosorption (13.26 mg/g) at 2mM concentration. At 4mM conc. maximum biosorption (36.84 mg/g) was showed by A. niger and minimum biosorption (14.84 mg/g) was exhibited by A. terreus at the 4mM concentration of $CuSO_4$ in case of NaOH pretreated biomass. Living biomass of A. niger showed maximum removal i.e., 4.84 mg/g of Cu whereas living biomass of A. terreus showed minimum biosorption i.e., 4.84 mg/g at 4mM concentration. Maximum (44.62 mg/g) and minimum (16.25 mg/g) biosorption was exhibited by NaOH pretreated biomass of A. niger and A. terreus respectively at 8 mM concentration. In case of living biomass, Maximum (12.82 mg/g) and minimum (3.25 mg/g) biosorption was exhibited by A. niger and A. terreus respectively at 8 mM concentration.

In the present study, out of 4 Aspergillus species selected for copper biosorption, living biomass and NaOH pretreated biomass of A. niger exhibited highest biosorption efficiency at higher concentration in comparison to other fungal species.

Raja Rao and Bhargavi (2013) also studied biosorption of lead and nickel by sodium hydroxide by pretreating A. niger. The maximum removal of lead was observed around 75% - 80% at pH 6 - 7 with maximum adsorbent dose of 0.2 g/ml. Maximum biosorption of nickel was observed around 50% - 60% at pH 5 - 8.

Das et al. (2007) investigated the biosorption capacity of NaOH pretreated biomass of Pleurotus florida for the adsorption of Cd metal ions. Maximum increase on biosorption of Cd metal ions by alkali pretreated biomass was observed approximately three times in comparison with living biomass (from 3.21 to 9.76 mg/g).

In the present work, even though the metal tolerance index of A. flavus and A. niger is more or less similar, A. niger showed excellent copper biosorption capacity than A. flavus. Zafar et al. (2007) observed similar results.

IV. CONCLUSION

This investigation concludes that fungal species isolated from soil samples of agricultural fields (irrigated with untreated wastewater) have the ability to resist higher concentrations of copper. This may be due to development of fungal adaptions and fungal tolerance against heavy metal ions. Out of the selected 4 fungal species of Aspergillus, A. niger showed excellent biosorption capacity for copper. Biosorption efficiency of fungal biomass increased after the pretreatment with NaOH. Therefore this study clearly indicates that NaOH pretreated biomass of A. niger could be effectively utilized for the detoxification and removal of copper metal ions.

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