

## Studies on Biosorption of Different Metals by Isolates of *Aspergillus* Species

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**Abstract:** Bioremediation of heavy metal pollution remains a major challenge in environmental biotechnology. Industrial effluents loaded with heavy metals are a cause of hazard to humans and other forms of life. In this present study the *Aspergillus* species, *Aspergillus fumigatus* (MTCC Acc. No 1399) and *Aspergillus tubingensis* (MTCC Acc. No 1398) were tested for metal sorption capacity. Maximum biosorption was observed with mercuric chloride [HgCl<sub>2</sub>] by *A.fumigatus* (85.34%) and Cobalt chloride [CoCl<sub>2</sub>] (73.05%) by *A.tubingensis*. The FTIR analysis indicated the broad and strong bands at 3500 to 3000cm<sup>-1</sup> which can be attributed to overlapping of -OH and -NH stretching. The band at 2900 attributed the C-H, at 1620 to 1590cm<sup>-1</sup> due to the C=O, and amide groups, at 1400 cm<sup>-1</sup> N-H bending in the amine groups, at 1025 cm<sup>-1</sup> to CO stretching of alcohols and carboxylic acids. Thus the *A.fumigatus* and *A.tubingensis* biomass contain hydroxyl, carboxyl and amine groups on their surface.

**Key words:** *Aspergillus fumigatus*, *Aspergillus tubingensis*, Biosorption, Fourier transform infrared spectra (FTIR).

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### I. Introduction

Mining and metallurgical waste are the most considerable sources of environmental pollution by heavy metals. Due to the health hazard caused by heavy metals, development of effective and economic removal technologies is necessary. In the case of removal of toxic metals from wastewaters, biosorption has been involved. Conventional metal removal methods are uneconomical and ineffective, therefore the rise of biological treatment methods are emerged [1]. Biosorption is a low cost and effective biological method which involves the use of dead microorganisms to detoxify and control metal contaminants in the environment [2, 3]. Bacteria, fungi, yeast and algae can remove heavy metals from aqueous solutions in substantial quantities [4,5,6]. The uptake of heavy metals by biomass can take place by an active mode (dependent on the metabolic activity) known as bioaccumulation or by a passive mode (sorption and/or complexation) termed as biosorption. Shumate and Strandberg defined biosorption as “a non-directed physico-chemical interaction that may occur between metal/radio nuclide species and the cellular compounds of biological species” [7]. Fungi and yeasts accumulate micronutrients, such as Cu, Zn and Mn, and non-nutrient metals, like U, Ni, Cd, Sn, Hg, in amounts higher than the nutritional requirement [5]. The potential of fungal biomass as adsorbent for the removal of heavy metals and radio nuclides from polluted waters was recognized [8, 9]. Metal biosorption by non-living biomass is a metabolism independent process. It is not ruled by physiological restriction.

The metal uptake by fungal biomass takes place by two basic processes. The first is by living organisms, where the metal uptake is dependent on the metabolic activity. The second process involves metal uptake by dead and living cells as a result of the chemical functional groups of the cell and, in particular, the cell wall. It should be noted that the metal uptake by the second process may also be involved during the metabolism-dependent metal uptake of growing cells [5]. The cell wall of the fungi is the first to come into contact with metal ions in solution, where the metals can be deposited on the surface or within the cell wall structure before interacting with the cytoplasmic material or the other cellular parts. In extreme cases, for the living cells, intracellular uptake may take place due to the increased permeability as a result of cell-wall rupture and subsequent exposure of the metal-binding sites [10]. The metal uptake by the cell wall has been broadly based on two mechanisms: uptake directed by functional groups like phosphate, carboxyl, amine and phosphate diester species of these compounds. The second uptake mechanism results from physicochemical inorganic interactions directed by adsorption phenomena. The removal mechanisms for radio nuclides result from the combination of the above two processes, while for other heavy metals, the first process seems to play an important role. Biosorption of heavy metals (lead, chromium, cadmium, nickel, copper and zinc) using isolates of filamentous fungus, *Aspergillus fumigatus* was reported [11].

Biosorption is one of the significant properties of both living and dead microorganisms (and their components) relevant for treatment of pollutants [12, 13, 14]. Fungal biomass received attention as biosorbent

materials for metal-contaminated aqueous solutions, because of the ease with which they are grown and the availability of fungal biomass as an industrial waste product [15] e.g. *Aspergillus niger* (citric acid production) and *Aspergillus fumigatus* (phytase, pectinase and cellulase enzyme production).

## II. Materials And Methods

### 1.1 Microorganism

The fungal species *Aspergillus fumigatus* (MTCC Acc. No. 1399) and *Aspergillus tubingensis* (MTCC Acc. No 1398) were isolated and characterized [16, 17]. These cultures were maintained on PDA agar slants at 4<sup>0</sup>C and sub cultured periodically at every 15days.

### 1.2 Preparation of biosorbent

Sabarouds dextrose broth (SDB) was prepared by adding 15.0g of glucose into two 1 liter conical flasks, made up to 500ml and the flasks were autoclaved. The isolates 15F, 20E were inoculated and incubated at 35<sup>0</sup>C for 4days in orbital shaker. The biomass was separated from the broth and washed with 0.1M sodium-acetate buffer (pH 5) [18]. The biomass was dried in an oven at 60<sup>0</sup>C until constant weight obtained. Heat treatment improves the metal binding sites of dried biomass [19].

### 1.3 Metal sorption studies

In order to study the biosorption capacity of the isolates (15F, 20E) dried biomass was treated with the different metal (100mg/L) solutions - CoCl<sub>2</sub>, MgCl<sub>2</sub>, BaCl<sub>2</sub> and HgCl<sub>2</sub> (25ml/0.2g of dried biomass). The flasks were incubated for 24hrs at 35<sup>0</sup>C in orbital shaker. Control flasks were also incubated without the fungal dry biomass and only fungal dry biomass. After completion of incubation period the samples were filtered through whatman paper.

### 1.4 Analysis of adsorbed metal through Atomic adsorption spectrophotometer

The biosorbent was washed with distilled water thoroughly. The filtrate was digested with HNO<sub>3</sub> and metal analysis was carried out using Atomic adsorption spectrophotometry (AAS) [Model AA 6200 Shimadzu]. Standard metal (100mg/L) solutions were also analyzed with AAS.

Biosorption capacity was measured by employing the following formula.

$$Q = \frac{V(C_i - C_f)}{M}$$

Whereas Q=mg of metal ions uptake per gram biomass (mg/g); C<sub>i</sub>=initial concentration of the metallic ions (mg/L); C<sub>f</sub>= final concentration of metallic ions (mg/L); M=dried biomass in the reaction mixture (g); V = volume of the sample (L).

### 1.5 FTIR analysis

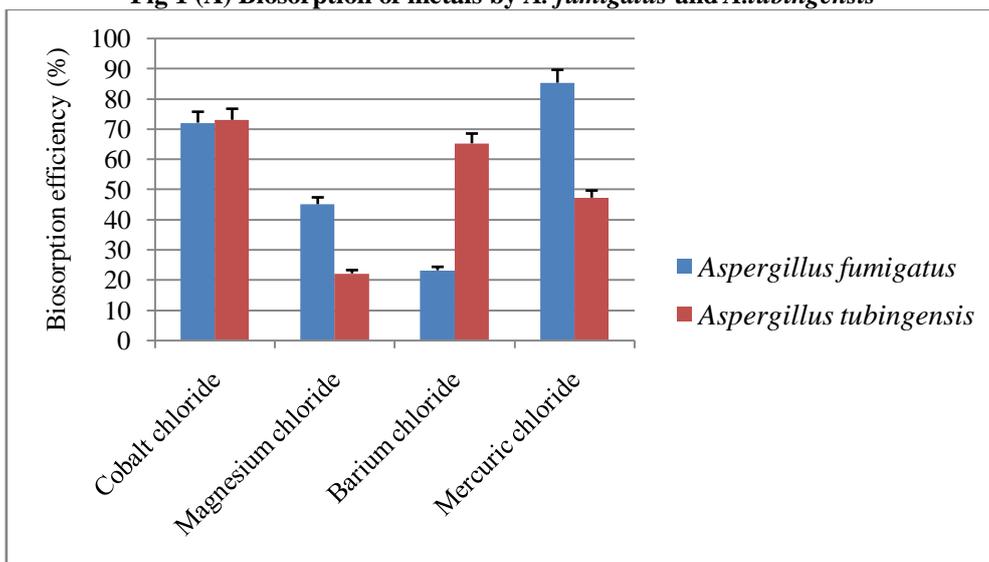
Fourier transform infrared spectra (FTIR) were used to analyze the functional groups on the surface of the dried metal adsorbed on fungal biomass (15F, 20E). The dried biomass of fungi was treated with KBr pellets, spectra were recorded with a spectrometer over the range 4,000–400 cm<sup>-1</sup> using a KBr window. In order to find out the differences between the metals treated with biomass, direct metal without biomass and only biomass without metal were also analyzed with FTIR.

## III. Results And Discussion

### 1.6 Analysis of adsorbed metal through Atomic adsorption spectrophotometer

The fungal species *A. fumigatus* and *A.tubingensis* showed the biosorption efficiency. Maximum biosorption (85.34%) efficiency of the *A.fumigatus* was observed with mercuric chloride [HgCl<sub>2</sub>], followed by (72.1%) with cobalt chloride [CoCl<sub>2</sub>], (45.08%) with magnesium chloride [MgCl<sub>2</sub>] and minimum (23.15%) with barium chloride [BaCl<sub>2</sub>]. Maximum biosorption (73.05%) efficiency of the *Aspergillus tubingensis* with cobalt chloride [CoCl<sub>2</sub>] followed by (65.24%) with barium chloride [BaCl<sub>2</sub>], (47.25%) with mercuric chloride [HgCl<sub>2</sub>] and minimum (22.13%) efficiency with magnesium chloride [MgCl<sub>2</sub>] was observed. The results were depicted in figure 1(A). [10, 20, 21, 22, 11] reported the biosorption capacity of *Aspergillus fumigatus* against metals viz. lead (Pd), chromium (Cr), cadmium (Cd), nickel (Ni), copper (Cu) and zinc (Zn) at constant pH 5 and temperature 30°C. The highest biosorption value (76.07) exhibited by *A. fumigatus* isolate K3 against Pb, followed by Cu (69.6) and Cr (40.0). Santhi and Guru [23] reported the maximum (75.36%) percentage of biosorption of hexavalent chromium by *A.niger*. Once the adsorbent capability gets exhausted, the uptake rate is controlled by the transportation of biosorbent from exterior site to interior [24].

Fig 1 (A) Biosorption of metals by *A. fumigatus* and *A.tubingensis*

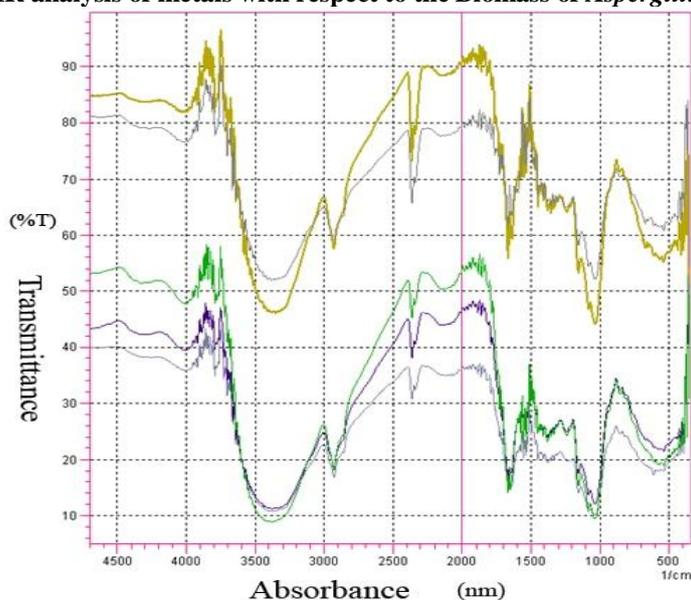


### 1.7 FTIR analysis of Metals by fungal species

In order to determine the characteristic functional groups responsible for biosorption of metal ions, FTIR spectroscopy was applied. The bonding mechanism between metal and the fungal biomass can be determined by interpreting the infrared absorption spectrum. The results of the spectra and their comparisons were depicted in the figures 1 (B), (C) and (D).

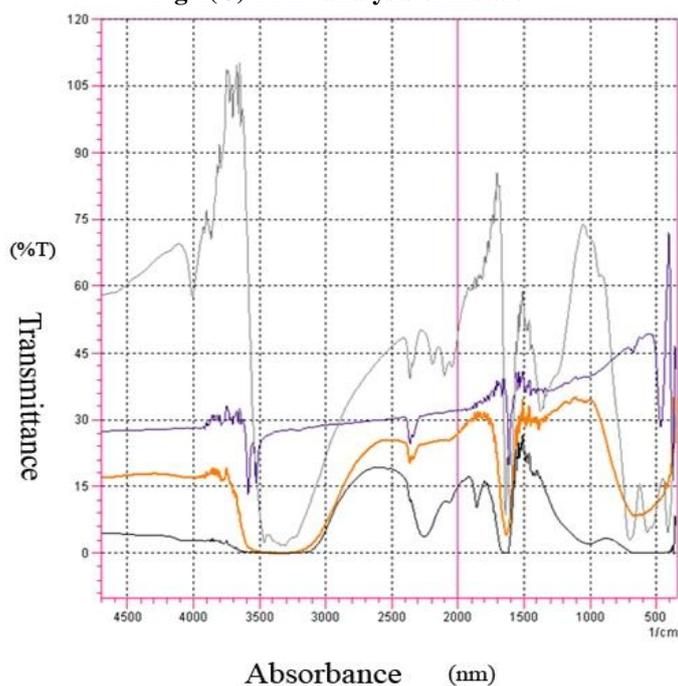
FTIR spectrum presents distinct peaks at 4500 to 500  $\text{cm}^{-1}$ . The broad and strong bands at 3500 to 3000  $\text{cm}^{-1}$  can be attributed to overlapping of  $-\text{OH}$  and  $-\text{NH}$  stretching. The band at 2900 attributed the C-H, at 1620 to 1590  $\text{cm}^{-1}$  due to the C=O, and amide groups, at 1400  $\text{cm}^{-1}$  N-H bending in the amine groups, at 1025  $\text{cm}^{-1}$  to CO stretching of alcohols and carboxylic acids. Thus the *A.fumigatus* and *A.tubingensis* biomass contain hydroxyl, carboxyl and amine groups on their surface. The stretching vibrations from the above values revealed the chemical interactions between metals and hydroxyl groups of biomass. The stretching vibrations were observed at 500 which indicates the Co-O stretching models, these similar results were reported with different metals like lead nitrate, nickel chloride, mercuric chloride [25]

Fig. 1(B) FTIR analysis of metals with respect to the Biomass of *Aspergillus fumigatus*



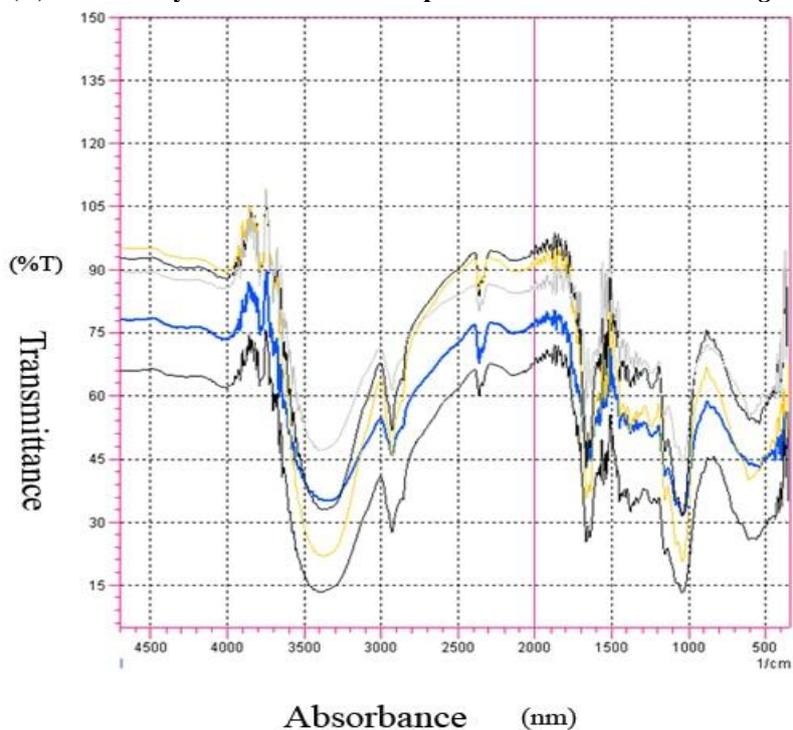
1.  $\text{HgCl}_2$ - *Aspergillus fumigatus* (Lemon yellow color)
2.  $\text{CoCl}_2$ - *Aspergillus fumigatus* (Ash color)
3. *Aspergillus fumigatus* (Green color) without metal
4.  $\text{MgCl}_2$ - *Aspergillus fumigatus* (violet color)
5.  $\text{BaCl}_2$ - *Aspergillus fumigatus* (Grey color)

Fig.1(C) FTIR analysis of metals



1. BaCl<sub>2</sub> (Grey color)
2. HgCl<sub>2</sub> (Violet color)
3. CoCl<sub>2</sub> (Orange color)
4. MgCl<sub>2</sub> (Black color)

Fig.1(D) FTIR analysis of metals with respect to the biomass of *A.tubingensis*



1. CoCl<sub>2</sub>-*Aspergillus tubingensis* (Yellow color)
2. BaCl<sub>2</sub>-*Aspergillus tubingensis* (Grey color)
3. *Aspergillus tubingensis* (Ash color) without metal
4. HgCl<sub>2</sub>-*Aspergillus tubingensis* (Blue color)
5. MgCl<sub>2</sub>-*Aspergillus tubingensis* (Black color)

#### IV. Conclusion

Biosorption of metals by both the species was performed on different metals- mercuric chloride [HgCl<sub>2</sub>], Cobalt chloride [CoCl<sub>2</sub>], Barium chloride [BaCl<sub>2</sub>] and magnesium chloride [MgCl<sub>2</sub>]. Maximum biosorption was observed with mercuric chloride [HgCl<sub>2</sub>] by *A.fumigatus* (85.34%) and Cobalt chloride [CoCl<sub>2</sub>](73.05%) by *A.tubingensis* and least sorption was observed with Barium chloride [BaCl<sub>2</sub>] (23.15%) by *A.fumigatus* and with magnesium chloride [MgCl<sub>2</sub>] (22.13%) by *A.tubingensis*. FTIR spectrum presents distinct peaks at 4500 to 500 cm<sup>-1</sup>. The broad and strong bands at 3500 to 3000cm<sup>-1</sup> can be attributed to overlapping of –OH and –NH stretching. The band at 2900 attributed the C-H, at 1620 to 1590cm<sup>-1</sup> due to the C=O, and amide groups, at 1400 cm<sup>-1</sup> N-H bending in the amine groups, at 1025 cm<sup>-1</sup> to CO stretching of alcohols and carboxylic acids. Thus the *A.fumigatus* and *A.tubingensis* biomass contain hydroxyl, carboxyl and amine groups on their surface. The stretching vibrations from the above values revealed the chemical interactions between metals and hydroxyl groups of biomass. The stretching vibrations were observed at 500 which indicates the Co-O stretching models.

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