

Adsorption of Iron and Synthesis of Iron Nanoparticles by *Aspergillus* Species Kvp 12

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Abstract Biosorption technology has gained tremendous importance in bioremediation and microbes could become the cheapest tool in detoxification of effluent streams. *Aspergillus* sp. isolated from the soil sample collected from the area near Hyderabad Metal Plating Industry, I.D.A, Balanagar, Hyderabad, India have been investigated in this study. The growth kinetics of *Aspergillus* sp. was studied by growing the fungi at different concentration of iron ranging between 0.2mM – 12 mM (Ferrous sulphate). The culture showed considerable inhibition of growth with iron when compared to the metal free controls. The maximum amount of iron was observed in the medium containing 3.0 mM concentration and further increase in the metal concentration was found to increase metal adsorption. Transmission Electron Microscopy analysis revealed the adsorption of iron nanoparticles on the cellwall.

Keywords: *Aspergillus* species, iron adsorption, iron nanoparticles, transmission electron microscopy

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1. Introduction

Heavy metals are on the forefront of academic and regulatory concern, since millions of gallon of water containing toxic heavy metals are generated annually from several metal processing industries and discharged into the environment. Metals thus discharged into water bodies are not readily biodegraded but thus undergoes chemical or microbial transformations, creating large impact on the environment and public health [1].

Several conventional wastewater technologies have been developed and are successfully in use at a large scale, in order to reduce hazardous concentration of compounds in wastewater to lower and safe levels [2]. The need for effective and economical methods for removing heavy metals from waste water has resulted in the search for material that may be useful in reducing the levels of accumulation of heavy metals in the environment [3]. Microbial populations in metal polluted environments include microorganisms which have adapted to toxic concentrations of heavy metals and become metal resistant [4]. Biomaterials like algae, fungi, bacteria and activated sludge have been tested as biosorbents for heavy metal removal [5,6,7]. Iron a common contaminant enters the water bodies through effluents of industries such as electroplating, mining, steel processing etc. The presence of Fe (II) in water results in undesirable color, odour and taste which makes water unfit for industry and domestic consumption. The aim of the present work was to study the removal of iron from solutions and synthesis of iron nanoparticles using a strain of *Aspergillus* species isolated from soil samples collected from metal plating industry.

2. Materials and Methods

Aspergillus species isolated from the soil samples collected from Hyderabad metal plating industry, I.D.A, Balanagar, Hyderabad, A.P. were preserved at 4°C in a refrigerator.

2.1. Growth Kinetics

The isolated pure culture of *Aspergillus* species was grown in fungal media containing KH_2PO_4 –7.0 g/L, KH_2PO_4 –2.0 g/L, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ – 0.1 g/L, $(\text{NH}_4)_2\text{SO}_4$ – 1.0g/L, yeast extract–6.0 g/L, glucose–10 g/L with different concentrations of $\text{FeSO}_4 \cdot 5\text{H}_2\text{O}$ (2, 4, 6,8,10 and 12mM) and a set of broth culture devoid of iron sulphate served as a control. Growth curves were obtained using dry mass weight of fungal growth in different concentrations against time variation.

2.2. Estimation of Iron by Atomic Absorption Spectrophotometry

Aspergillus species grown in different concentration of metal were harvested during early stationary phase. To the 50ml filtrate obtained after filtration add 2ml nitric acid. Amount of iron present in the filtrate was analyzed by Atomic Absorption Spectroscopy (GBC Scientific Equipments Ltd).

2.3. TEM Analysis of *Aspergillus* Species

Aspergillus sp. was grown in 1mM Ferrous sulphate and after 48hrs. The medium was centrifuged and fungal

pellet was collected. The fungal pellet was used for TEM studies to identify the localisation of metal and to know the *Aspergillus* sp. Capacity to synthesize iron nanoparticles.

3. Results and Discussion

The organism isolated from the soil sample was identified by amplication of the 18s rRNA. The isolated *Aspergillus* sp. from the soil sample collected from industrial area is an indication that the microorganisms are capable of growing in the presence of high concentrations of heavy metals like iron.

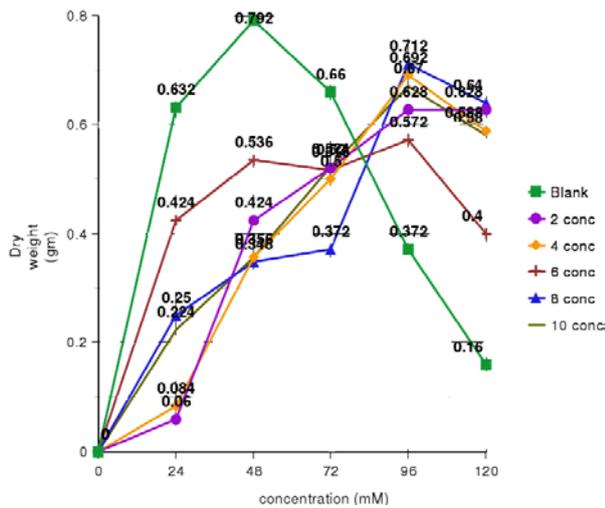


Figure 1. Growth kinetics of *Aspergillus* species in absence and presence of various concentrations of iron sulphate

The growth kinetics (Figure 1) of *Aspergillus* species grown in iron sulphate showed the fungi growing in lower concentration of iron showed increased log phase as compared to high concentration of iron. Lag phase was not observed when the fungi was grown in different concentrations of iron. The presence of iron was found to decrease the growth of *Aspergillus* sp as compared to the growth in the media without iron. As the external iron concentration is increased the growth of the organism is not affected. This may be due to decreased the bioavailability of the toxic metals/metalloids through extracellular complexation, precipitation, and binding to cell wall constituents [8].

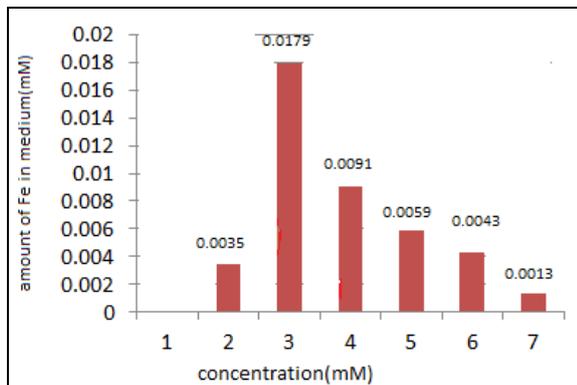


Figure 2. The amount of iron present in the medium

As shown in (Figure 2) Atomic Absorption Spectrophotometer analysis shows that the amount of metal in the medium is more at 3.0 mM range and afterwards it is decreased. As the iron concentration in the broth was increased the sequestration of iron on to the cell wall increased. Iron is an essential metal and requires a proper uptake, intracellular transport and storage to avoid the deleterious consequences of the appearance of free iron in either the cytosol or the cell organelles. In filamentous fungi, intracellular siderophores like ferricrocin and hydroxyferricrocin have been reported to keep excess iron in a thermodynamically inert state [9,10,11].

But TEM observations of *Aspergillus* sp. cells exposed to iron solution showed electron dense metal nanoparticles are adsorbed on the cell walls. (Figure 3). The size of the nanoparticles are in range of 50-200nm. As reported [8] ferrous ions may bound to the cellwall to regulate metal stress. Guerinot stated that microbes have developed various strategies for acquiring iron and at the same time protecting themselves from potential toxic effects of iron [12]. Beveridge and Murray [13] related the bacterial metal interactions to the anionic character of specific functional groups situated on membrane components (e.g. teichoic acids, peptidoglycan, phospholipids). *A. Ferroxidans* growing in iron rich environment accumulated uranium on the cell wall [14]. Brown precipitates of Fe(III) adhering to the hyphae surface in cultures of *A. alternata* (SA), *C. cladosporoides* (TO), and *Ph. Glomerata* (SA) are reported [15].

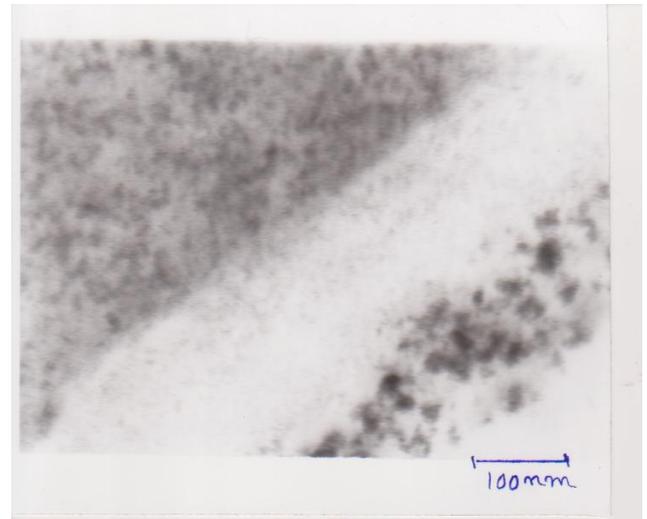


Figure 3. Transmission electron micrograph of *Aspergillus* sp. grown in iron sulphate

4. Conclusion

This study has shown that the iron can be removed from waste water using *Aspergillus* sp. Hence *Aspergillus* species can be considered for use in Biological filter systems for removal of iron present in industrial effluents and Waste water. Considering the wide array of molecular genetic tools available in genetic engineering modified, *Aspergillus* strains be created for use in future in the versatile metal removal technologies and *Aspergillus* based nanoparticle producing technologies is foreseeable.

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