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Fungal Biosorption of Heavy Metals from Industrial Waste

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Abstract

Heavy metal contamination in ground water resources and in air is a major problem today which requires appropriate treatment before discharge into environment. Extensive use of Heavy metals in various industrial applications has cause sustainable environmental contamination. Some of these metals are useful when it is in low concentration but in nature mostly it may exceed the recommended level. Biosorption is proposed as an ideal method of removing heavy metals before discharge into the environment. Microorganisms play a significant role in biosorption of heavy metal from contaminated soil and water ecosystem. In the present study heavy metal tolerant filamentous fungi was isolated from automobile industry waste disposal area. Among the fungi isolated from industry soil which was identified as *Aspergillus* species, showed tolerance to chromium, nickel, ferrous, zinc, and lead. This selection is based in the fact that these metals are discharged in many of the industries electroplating, detergents, oil refining and others. The dominant heavy metal resistant fungi were isolated by the serial dilution and spread plate method in potato dextrose agar. The identified organism was culture at sabouraud dextrose agar and the heavy metal tolerance concentration assay was determined from the isolated organism. Hence the isolated organism of *Aspergillus* speciesis capable of bioaccumulating Chromium, Nickel, Ferric, Zinc, and lead metals. The above strains may be exploited for bioremediation strategy, of cleanup of contaminated sites.

Keywords: Heavy metals, Aspergillus, biosorption, fungi, microorganisms.

Contents

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1. Introduction

Environmental pollution is a constant threat faced by humanity. Industrial effluents entering into the surface water are one of the most important sources of toxic contamination in the environment (1). Often raw sewage is used either directly to irrigate agricultural land as a supplement of essential plant nutrients, or disposed into fresh water streams, which again can be used for agricultural purposes (2). The use of microbial biomass of fungi, bacteria and algae for the removal of toxic pollutants and heavy metals from aqueous solutions is gaining increasing attention (3).

Heavy metals mentioned in the field of bio sorption are usually classified as the following three categories: toxic metals (such as Hg, Cr, Pb, Zn, Cu, Ni, Cd, As, Co, Sn, etc.), precious metals (such as Pd, Pt, Ag, Au, etc.) and radionuclides (such as U, Th, Ra, Am, etc.), whose specific weight is usually more than 5.0 g/cm^3 (4). Fungi are known to tolerate and detoxify metals by several mechanisms including valence transformation, extra and intracellular precipitation and active uptake (5). The high surface to volume ratio of microorganisms and their ability to detoxify metals are among the reasons that they are considered as potential alternative to synthetic resins for remediation of dilute solutions of metals and solid wastes (6).

Bioremediation is the use of natural biota and their processes for pollution reduction. It is a cost-effective process and the end products are non-hazardous. In recent years, biosorption process has been studied extensively using biomass as a biosorbent for heavy metals removal. Biosorption is the binding and concentration of heavy metals from aqueous solution (even very dilute ones) by certain type of inactive, dead, microbial biomass. Pioneering research on biosorption of heavy metals has led to the identification of a number of microbial biomass types that are extremely effective in concentrating metals. Biosorption is a passive metabolism independent physio-chemical interaction between heavy metal ions and microbial surface. Microbial communities are of primary importance in bioremediation of metal contaminated soil and water because microbes alter metal chemistry and mobility through reduction, accumulation, mobilization and immobilization. There is current interest in the use of microorganisms for the removal of nitrogen, phosphorus, and metals from commercial and municipal waste (7). Several species of microorganisms are capable of accumulating metal ions up to concentrations several orders of magnitude higher than the background concentration (8). Among microorganisms, filamentous fungi are well recognized for their superior capacities to produce a wide variety of extracellular enzymes, organic acids and other metabolites, and for their capabilities to adapt to severe environmental constraints (3). For example, members of the *Deuteromycetes* such as *Aspergillus, Penicillium and Trichoderma* species are known to produce numerous extracellular enzymes, which are put to good use in biotechnology (9). Similarly, the *Basidiomycetes* white rot fungi such as *Phanerochaetechrysosporium* are noteworthy for their abilities to produce nonspecific ligninases and peroxidases which can be used to degrade pollutants both in liquid effluents and in soils (10).

The toxic characteristics of heavy meals are displayed as follows: the toxicity can last for a long time in nature; some heavy metals even could be transformed from relevant low toxic species into more toxic forms in a certain environment, mercury is such a case; the bioaccumulation and bioaugmentation of heavy metal by food chain could damage normal physiological activity and endanger human life finally; metals can only be transformed and changed in valence and species, but cannot be degraded by any methods including biotreatment; the toxicity of heavy metals occurs even in low concentration of about 1.0–10 mg/L. Some strong toxic metal ions, such as Hg and Cd, are very toxic even in lower concentration of 0.001–0.1 mg/L (11). The uptake of heavy metals by fungi is of industrial relevance for the removal of metals from waste waters for environmental protection and / or subsequent recovery of the metals. Fungi are well suited for this purpose since they often exhibit marked tolerance towards metals and other adverse conditions e.g., low pH, also they have high capacities of metals binding to cell walls and may exhibit high values of intracellular accumulation (12). The objective of this work was to isolate fungi from the contaminated soil and evaluate them for use in biosorption of heavymetals. To study the fungal biosorption of heavy metals like chromium, zinc, nickel, lead, ferrous from the industrial waste as well as their tolerance level.

2. Materials and Method

Isolation of fungi

Serial dilution: A small measured volume or weight of sample was mixed with a large volume of sterile water called dilution blank. Dilutions were made in multiplies of 10. A single dilution was calculated as follows:

Dilution = Volume of the sample/ Total volume × Dilution factor

The dilution blanks were labeled as 10^{-1} to 10^{-10} and it was filled with 9 ml of sterile distilled water or saline water. Initial dilution prepared by adding 1 ml of sample into dilution blank. Then the contents were mixed thoroughly, from these diluents 1 ml of sample was transferred to the 10^{-2} dilution. The same procedure was repeated upto 10^{-10}dilution (13).

Identification of fungal species

The fungal species was confirmed by the Lacto phenol cotton (LCB) blue staining. The smear was prepared from the given sample and heat fixed. The smear was flooded with lacto phenol cotton blue for 3-4 minutes. It was washed with tap water for 3 minutes to remove excess stain. Then the smear was air dried and then observed under microscope. The fungal species were identified and characterize based on their morphological characters and microscopic analysis by using taxonomic guides and standard procedures (14, 15).

Growth rate

Growth rate (kd) was determined with the following equation: kd= D/T, where D is the experimentally determined average diameter of the fungal colony in mm exclusive of the diameter of the inoculum (8 mm) and T is the time period.

Cultivation of fungi in submerged culture

For the growth of fungi in suspension culture, the modified Vogel's mineral salts medium was used (16).Varying concentrations of sterile potassium dichromate were added directly to the cultivation media. Spores of the fungi were harvested from 7 days old culture slants by washing with 0.2% Tween-80 and inoculated into a sterile flask (autoclaved at 121˚C for 15 mins) containing 100ml fresh medium. Fungal growth was evaluated in media with dichromate concentrations of 5 – 25 mg/L.The fungi were grown in batch reactors using Erlenmeyer flasks on a shaker (200-300 rpm) at pH 5.0 and temperature of 30° C for 4-7 days.

Measurement of specific growth rate

Culture turbidity was used to determine the specific growth rates (μ) of fungal cultures (17). Erlenmeyer flasks containing 100 ml of the modified Vogel's mineral salt medium and Cr (VI) at concentrations of 5 – 25 mg/l were inoculated with 2 ml of exponential – phase culture grown in identical medium (initial culture absorbance of 0.1 – 0.4 nm). The fungal cultures were then incubated at 300C on a rotary shaker at 200 rpm and changes in turbidity were determined at 24 hours interval for five days. Culture turbidity was measured spectrophotometrically by taking the absorbance of the growing cultures at 560 nm. The specific growth rate, μ (hr-1) was determined by dividing the change in turbidity by the time interval within which growth was assessed.

Heavy Metal Tolerance Assay:

To explore the tolerance of the isolates to the heavy metals optimal culture conditions were used with varying initial heavy metal concentrations. To each freshly prepared growth medium, Chromium, nickel, zinc, ferric and lead was amended using potassium dichromate, nickel sulphate,zinc sulphate, ferric phosphate, and lead acetate respectively where the concentrations ranging from 5-25 mg/ 1000ml.

Screening of metal tolerance

The fungal isolates were evaluated for their metal tolerance and growh efficiency in the presence of varying concentrations of heavy metals that included chromium as potassium dichromate, nickel as nickel sulphate, zinc as zinc sulphate, ferric as ferric phosphate in the sabourad dextrose agar.

Estimation of potassium dichromate

A burette was washed with water and rinsed with sodium thiosulphate solution and it was filled with sodium thiosulphate solution upto zero mark. Exactly 20 ml of copper sulphate solution was pipette out into a conical flask. To this ammonium hydroxide solution was added drop by drop till a faint blue precipitate was obtained. Then acetic acid was added till the precipitate was just redissolved to get a clear blue solution. Then 10 ml of 10 % KI solution was titrated against sodium thiosulphate till the appearance of straw yellow color. Then 2 ml of starch indicator was added. The solution turns blue color. The titration was continued till the end point. The end point was just disappearance of blue color and same procedure was repeated for concordant values.

Estimation of ferrous ion

The burette was washed with water and rinsed with potassium dichromate solution and it was filled with potassium dichromate solution upto zero mark. Exactly 20 ml of ferrous ammonium sulphate solution was pipette out into a conical flask. 5ml of phosphoric acid and 3drops of diphenylamine indicator was added. This solution was titrated against potassium dichromate. The titration was continued till the end point i.e.appearance of pale permanent violet blue color. The end point was just appearance of pale permanent violet- blue and same procedure was repeated for concordant values.

Estimation of Zinc ion

A burette was washed with water and rinsed with EDTA solution and it was filled with EDTA solution upto zero mark. Exactly 20 ml of calcium chloride solution was pipette out into a conical flask. 5ml of ammonia buffer and 5 drops of EBT indicator were added to it and it became the wine red in color. This solution was titrated against EDTA. The titration was continued till the end point. The end point was the change of color from wine red to blue and the same procedure was repeated for concordant values.

3. Results and Discussion

Identification of fungal isolates:

The isolated fungal species from soil was identified as *Aspergillus* species. Growth conditions for *Aspergillus* species were optimized. On the basis of the results, further studies for heavy metal ions removal were performed at the optimum pH 5.0 and 28°C.

Effect of Cr on fungal growth

The growth rates of *Aspergillus* species was slightly higher at 0.5% chromium treatment compared to the values obtained for chromium free control. As the treatment concentrations increased from 0.5 – 3%, the growth rate for

Aspergillus became significantly lower than those for the control, with no growth at 2.5%. The observed decrease in growth rates with increasing concentrations of chromium treatment indicates that chromate is inhibitory to the growth of the organisms. Chromates are known to inhibit the growth of most organisms, although resistance to the toxic metal may be developed by the selection of resistant variants to cope with such toxicities

Effect of Fe on fungal growth

The growth rates of *Aspergillus* species was slightly higher at 0.5% chromium treatment compared to the values obtained for ferric free control. As the treatment concentrations increased from 0.5 – 5%, the growth rate for *Aspergillus* became significantly lower than those for the control, with no growth at 5.0%. The observed decrease in growth rates with increasing concentrations of ferric treatment indicates that ferric is inhibitory to the growth of the organisms. Ferric are known to inhibit the growth of most organisms, although resistance to the toxic metal may be developed by the selection of resistant variants to cope with such toxicities.

Effect of Ni on fungal growth

The growth rates of *Aspergillus* species was slightly higher at 0.5% nickel treatment compared to the values obtained for nickel free control. As the treatment concentrations increased from 0.5 – 5%, the growth rate for *Aspergillus* became significantly lower than those for the control, with no growth at 2.5%. The observed decrease in growth rates with increasing concentrations of nickel treatment indicates that nickel is inhibitory to the growth of the organisms. Nickel is known to inhibit the growth of most organisms, although resistance to the toxic metal may be developed by the selection of resistant variants to cope with such toxicities.

Effect of Pb on fungal growth

The growth rates of *Aspergillus* species was slightly higher at 0.5% lead treatment compared to the values obtained for lead free control. As the treatment concentrations increased from 0.5 – 3%, the growth rate for *Aspergillus* became significantly lower than those for the control, with no growth at 2.5%. The observed decrease in growth rates with increasing concentrations of lead treatment indicates that lead is inhibitory to the growth of the organisms. Lead is known to inhibit the growth of most organisms, although resistance to the toxic metal may be developed by the selection of resistant variants to cope with such toxicities.

Effect of Zn on fungal growth

The growth rates of *Aspergillus* species was slightly higher at 0.5% zinc treatment compared to the values obtained for zinc free control. As the treatment concentrations increased from 0.5 – 3%, the growth rate for *Aspergillus* became significantly lower than those for the control, with no growth at 2.5%. The observed decrease in growth rates with increasing concentrations of zinc treatment indicates that zinc is inhibitory to the growth of the organisms. Zinc is known to inhibit the growth of most organisms, although resistance to the toxic metal may be developed by the selection of resistant variants to cope with such toxicities. The amount of different metal ions present in 3% concentration is shown in fig 1.

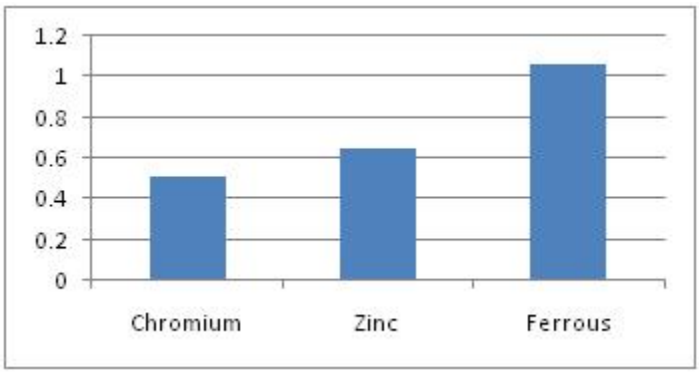

Figure 1: Amount of metal ions present in 3% concentration

From the titration the amount of potassium dichromate present in the 3% concentration was calculated as 0.51, from the titration the amount of zinc present in the 3% concentration was calculated as 0.649g, and from the titration the amount of ferrous present in the 3% concentration was calculated as 1.066g.

4. Conclusion

The present study indicated that fungal populations isolated from heavy metal-contaminated sites have the ability to resist higher concentrations of metals. The tolerance and the resistance of the isolates depended much more on the fungus tested than on the sites of its isolation. *Aspergillus* species was resistant to most of the metals tested such as chromium, nickel, ferric, zinc and lead which had the ability to remove metals form contaminated environments. *Aspergillus* species having promising biosorption capacity for the various metals.

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