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Bioremediation of toxic heavy metal cadmium by using metal tolerant fungi

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ABSTRACT

Heavy metals also occur naturally, but rarely at toxic level. Excess heavy metal accumulation in soil is toxic to human and other animals. Exposure to heavy metals are normally chronic (exposure over a longer period of time), due to food chain transfer. Acute (immediate) poisoning from heavy metal is rare through ingestion or dermal contact, but is possible. Preventing heavy metal pollution is critical because cleaning contaminated soil is extremely expensive and difficult. Application of industrial waste or sludge must abide by the regulatory limits set by the U.S. Environment Protective Agency (EPA).

KEYWORDS

Metal-Cadmium;
Bioremediation;
Biosorption;
Fungus.

INTRODUCTION

In recent years, heavy metal pollution has become one of the most serious problems. Presence of heavy metals even in traces is toxic and determined both flora and fauna. With the rapid development of many industries (mining, surface finishing, energy and fuel producing, fertilizer, pesticide, metallurgy, iron and steel electroplating, electrolysis, electro osmosis, leather, synthetic compounds, waste nuclear liquids, photography, electric appliance manufacturing, metal surface treating) and aerospace and atomic energy installation has cause a pollution. Metallurgical wastewaters are considered to be a major source of he metal contamination. Waste containing metals are directly or indirectly being discharged into the environment causing serious environmental pollution and even threatening human life. Several methods are being used for the removal of heavy metal ions from aqueous solution waste (chemical precipitation, ion exchange, electro chemical treatment, membrane technologies, adsorption on activated carbon, bioremediation).

Excess heavy metal accumulation in soil is toxic to human and other animals. Exposure to heavy metals are normally chronic (exposure over a longer period of time), due to food chain transfer. Acute (immediate) poisoning from heavy metal is rare through ingestion or dermal contact, but is possible.

Chronic problem associated with long-term heavy metal exposures are:

- Cadmium-affects kidney, liver and GI tract
- Arsenic-skin poisoning, affects kidneys and central nervous system
- Cadmium is non – essential, toxic and non-benefi-

cial to plants and animals, heavy metal that is a common contamination in soil.

The major sources of cadmium are industrial wastes. It was found in batteries, welding, electroplating, pesticide, fertilizer, CdNi batteries, nuclear fission plant, cigarette smoke (1cigarette contain 1-2 microorgans of cadmium). Cadmium in soil is known to originate from geogenic (natural) and anthropogenic (industrial) source[7].

The daily intake of cadmium in the most heavily contaminated areas amounted to 600-2000 μg/day; in other less heavily contaminated areas, daily intakes of 100-390μg/day has been found[20].

Cadmium is mainly stored in the liver and kidneys. Skeletal damage is another critical effect of chronic cadmium exposure. Excretion is slow, with a very long half-life (decades) in the human body. Cadmium concentrations in most tissues increase with age.

MATERIALS AND METHODS

Sample collection

Stored soil sample from the laboratory of Molecular Bioremediation, Department of Environmental Biotechnology, Bharathidasan University, Tiruchirappalli was used for this study. The soil samples were stored in -20°C.

Isolation and screening of cadmium resistant fungal species

To a series of sterilized 8 test-tubes with 9 ml of distilled water was taken and labeled as 10^{-1} to 10^{-8}. One gram of soil sample was dissolved in 10^{-1}dillution. One ml of 10^{-1} dilution was transferred carefully to next tube using a sterilized pipette and repeated up to 10^{-8} test tubes. Dilution 10^{-3} and 10^{-7} was chosen for isolating cadmium resistant fungi. The Petri plates were prepared with 15 ml of containing PDA with cadmium in different concentration ranging from 10-100 ppm .From the 10^{-3} and 10^{-7}dillution 0.1 ml of sample was transferred to the Petri plates and using an L-rod the sample was spread evenly in the Petri plates. The Petri plates were kept in inverted position in room temperature for 3-4 days.

The isolated colonies were grown in PDB and these colonies were further characterized and employed for heavy metal tolerance studies.

Isolation of pure culture

The pure culture was isolated from the Petri plate by inoculating the individual colonies into the sterile Potato Dextrose Agar plates by Dot plate technique. The plates were incubated at 37°C for 48 h.

Identification of metal tolerant fungi

The metal tolerant fungus was examined by Lacto phenol Cotton Blue method. Placed a drop of LCB on a clean glass slide over it a loop full culture was spread uniformly. A cover slip was placed over it, with out air bubble observed under microscope of 45 X resolution.

Estimation of cadmium (APHA-AWWA-WEF, 1998)

Cadmium concentration was determined by Pyronine G method (APHA- AWWA-WEF, 1998) with cadmium chloride as standard. Different aliquots of standard were taken in the concentration range of 10 to 100 mg / ml. To this 2.5ml of citrate buffer (pH 4), 2.5 ml of potassium iodide, 2.5ml of pyronine G and 1 ml of 1% gelatin was added, mixed thoroughly and allowed to stand for 20 minutes at room temperature. The absorbance was read at 405 nm in a spectrophotometer. A standard graph was plotted against the concentration of cadmium observance. From the standard graph, the concentration of sample can be calculated.

Biomass quantification

Fungal biomass was quantified by withdrawing 2.5 ml of broth culture from the fungal medium and the absorbance was measured using spectrophotometer at 405 nm. Uninoculated growth medium was used as blank[18].

Biosorption studies for cadmium removal using the isolated metal resistant fungi

(1) Optimization of ph for metal removal

The fungal isolates were incubated into a series of 50 ml conical flasks containing PDB amended with 100 mg L^{-1} of cadmium. The pH was varied from 2 to 10 (2, 4, 6, 8, 10) using dilute HCL or NaOH. The cultures were shaken (120 rpm) at 35°C for 24 hours. After 24 hours incubation, the adsorbate were separated by centrifugation was determined spectrophotometrically (APHA-AWWA-WEF, 1998). The initial final concentration of cadmium was calculated by esti-

mating the concentration of cadmium spectrophotometrically.

(2) Optimisation of temperature for metal removal

The fungal isolates were inoculated into a series of 50 ml conical flasks containing PDB amended with 100 mg L^{-1} of cadmium. The temperature was varied from 25 to 45°C (25, 30, 35, 40 and 45°C). The cadmium concentration was determined spectrophotometrically (APHA-AWWA-WEF, 1998). And the cadmium removed by isolated fungus was determined.

(3) Extraction of extra cellular protein from isolated cadmium resistant fungi

Protein from the fungi was extracted and purified using microbial lysis method. The cadmium resistant *Aspergillus terreus* was inoculated into a 50 ml Glucose-peptone broth and was incubated 24-36 h at 37°C. Then 15 ml of sample was taken from the falcon tube and it was shaked and centrifuged at 6000 rpm for 10 min. The supernatant was discarded, and then washed three times with normal saline buffer. The supernatant was discarded.

To the pellet, 200µl of 10%SDS and 50 mM Tris glycine buffer was added and mixed well. The mixed pellet was transferred to microfuge tube and sonicated at 3 bonts each of 30 seconds, then centrifuged at 5000 rpm for 10 min. The supernarant was collected; the same procedure was followed for the isolation of protein from the cadmium unexposed *Aspergillus terreus*.

RESULTS AND DISCUSSION

Soil analysis

Atomic absorption spectrophotometer (AAS) analysis of cadmium in soil revealed a high level of 0.26 ppm.

Isolation of cadmium resistant fungal species

The soil samples was collected were enumerated for fungi subjected to serial dilution and plating methods. For enumeration of cadmium resistant fungi, the dilute soil samples were inoculated in PDA plates with different concentrations of cadmium ranging from 10-100 mg/L. The colonies were grown when the concentration of metal at a concentration of 100 mg/L. From this concentration, the identical large colonies were picked and isolated for pure culture. The isolated culture was tolerant to cadmium concentration of 100 mg/ L. The isolated colonies were further characterized and used in Cd removal batch mode studies (Figure 1).

Identification of cadmium tolerant fungi isolates

The isolated colonies showed blue-green shade with velvety structure. The surface is appeared granular; large quantities of pigmented spores are produced and the white apron is seen along the edge of active growth .The camel is formed on the surface of colonies. The fungi was tolerant to a cadmium ion concentration of 100 mg/L. Microscopic view of the fungi with Lactophenol Cotton Blue staining showed that the isolated strain was *Asspergillus terreus*. They having Hyphae are septate and hyaline. Conidial heads are biseriate (containing medulla that support phialides) and columnar (conidia form in long columns from the upper portion of the vesicle in mostly globose vesicles. Conidia are small (2-2.5 µm), globose, and smooth. Globose, sessile, hyaline accessory conidia (2-6 µm) frequently produced on submerged hyphae (Figure 2).

Biosorption studies for cadmium removal using isolated metal resistant organisams

(1) Cadmium removal by isolated fungal species at different pH

Heavy metal bioremediation process involving biosorption do not require growing cells, so it is not necessary to add extra nutrients to the biosorption mixture. In the absence of nutrients, no complexing capacity is expected in the fungal environment, so the metal is completely available to interact with cells. Biosorption studies were carried out to establish experimental conditions and to select the proper fungal strain for the process design. pH was evaluated since it affects the number of cellular surface sites available to bind cations, as well as metal speciation. Cadmium adsorption increased along with the increase of pH of the absorbate solution which is shown in graph 1. The maximum removal of cadmium was around 76 % at pH 4.0 at the concentration of 100 mg/ L. pH changes during a biosorption process when it was carried out with out pH regulation. In this work, buffered solutions are used for pH control during the biosorption assay to ensure reliable results.

According to several authors, it is expected that

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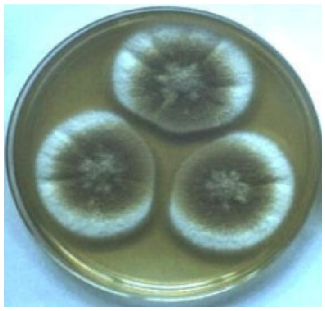


Figure 1 : Isolated fungi from heavy metal contaminated soil

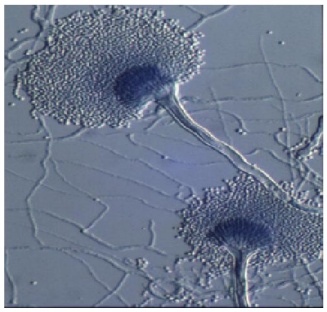
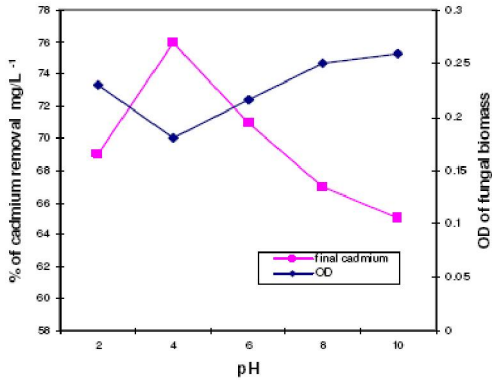
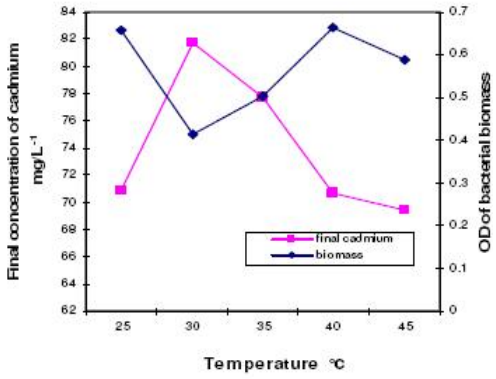


Figure 2 : Identification by Lacto phenol cotton blue stain



pH-4, Incubation time-24 h, Concentration of cadmium-100mgL^{-1}

Figure 3 : Cellular growth and cadmium removal by *Aspergillus terreus* in response to various pH



Temp-35°C, Incubation time-24 h, Conc. of cadmium-100mgL

Figure 4 : Cellular growth and cadmium removal by *Aspergillus terreus* in response to various temperatures

the adsorption of metals degreases at low ph values because the competition for binding sites between cations and the products of acid hydrolysis. Proton and oxonium ions (H_3O^+) concentrations are relatively high at low pH and compete with metals for ion exchange. The other report also confirmed that the effect of initial pH on the biosorption of cadmium (II) ions onto *P. platypus, A. bisporus* and *Calocybe indica*[19].

(2) Biosorption of Cadmium by isolated fungal species at different temperature

Maximum removal of cadmium (81%) was observed at 30ºC for the isolated *Aspergillus terreus*. The fungal biomass grown on the nutrient media have a significant influence on the temperature. The optimum growth of fungal biomass was seen at temperature 30ºC which is shown in graph 2. At temperature above 35ºC, the fungal does not attain the growth due to its insignificant environmental condition. Temperature seems to affect biosorption only to a lesser extent with in the range from 20-35°C, higher temperatures usually enhance sorption due to the increased surface activity and kinetic energy of the solute however, physical damage to the biosopbent can be expected at higher temperatures. Due to the exothermic nature of some adsorption processes, an increase in temperature has been found to reduce the biosorption capacity of the biomass). It is always desirable to conduct/ evaluate biosorption at room temperature, as this condition is easy to replicate.

(3) Biosorption of Cadmium and cellular growth of the isolated fungal species

The metal adsorption by *Aspergillus terreus* increased and become constant beyond 24 hours and 82 % cadmium removal was observed graph 3. Similar results was obtained in the biosorption of cadmium by *P. platypus,* the removal efficiency reached equilibrium at 60 min and that by *Asspergillus bisporus* and *Calocybe indica* reached equilibrium at 240 min[19].

(4) Cadmium tolerance response by isolated fungal species

The biosorption capacities of Cd (II) by *Asspergillus terreus* as shown in Grap 4. Among the different concentrations, the *Asspergillus terreus* has shown tolerance upto 100 mg/L (100 PPM). The

biosorption capacity if biomass increased first with increasing of the initial concentration of metal ions and reached a saturated value. The similar results were obtained in the case of *Funalia trogii* for Hg^{2+}, Cd^{2+} and Zn^{2+} All these data clearly reveals that the existence of a finite heavy metal reduction capacity possibly due to heavy metal toxicity towards cells.

SUMMARY AND CONCLUSION

Metal accumulation by appropriate biological substrates can counteract metal mobilization into the environment. Microorganisms provide a large contact area that can interact with metals in the surrounding environment. Biosorption has received great attention during the last years, due to the potential use of microorganisms for cleaning metal-polluted soil, water or wastewater streams. The aim of this work was to show the ability of several microorganisms, isolated from metal-polluted soils to biosor and remove toxic metals from of aqueous solutions. Environmental factors, *i.e.* pH, temperature and ionic concentration showed significant effects on cadmium biosorption on *Aspergillus terreus* with maximum efficiency at pH 4.0, temperature of 30°C and the maximum tolerability of 100 ppm. Aspergillus terreus found to be suitable biosorbent for Cd ions, especially when the metal content in the aqueous solution was in the concentration of 100 mg/l.

A significant differential expression of some polypeptides was seen in cadmium resistant fungi than the control (without cadmium). This was probably attributed due to a higher degree of functional diversity among the fungi.

The present investigation concluded that the heavy metal resistant *Aspergillus terreus* isolated from contaminated soil could be employed as an effective adsorbent for the removal of cadmium from aqueous solution.

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