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Original article

Toxic cadmium ions removal by isolated fungal strain from e-waste recycling facility

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Abstract:

The use of biomaterials for removing heavy metals from contaminated wastewater has emerged as a potential alternative method to conventional techniques. Fungi have been investigated as a biosorbent because of its capability to sequester metal ions from aqueous solutions. In this study, the cadmium tolerant fungus, *Aspergillus* sp was isolated from sample of an e-waste recycling facility. The optimal parameters for removal of Cd such as metal concentration, pH, temperature and time were studied. In the controlled conditions it was demonstrated that the maximum of 88% cadmium (100 mgL^{-1}) was removed from aqueous solution by *Aspergillus* sp. at an optimum pH 4 and temperature 30°C. The observations reveal that the biomass of isolated *Aspergillus* sp. has the potential to be used as biosorbent for heavy metal cadmium removal from contaminated wastewater.

1. INTRODUCTION

The presence of heavy metal contamination in water streams arising from the discharge of untreated metal containing effluents from industries into water bodies is one of the most important environmental issues (Dursan, 2008; Ghaedi, 2006). Heavy metals such as Cu, Zn, Ag, Cd, In, Sn, Sb, Hg, Pb, and Bi was detected in higher concentration in e-waste recycling facility soil samples (Ha et al. 2009). Due to presence of heavy metals in soil, microbes have evolved mechanisms to tolerate the presence of heavy metals by efflux, complexation, or reduction of metal ions (Gadd, 1990). Conventional techniques commonly applied to recover heavy metal from wastewaters have several disadvantages whereas biosorption has good metal removal performance from large volume of effluents. Among the biomaterials, fungi have been reported as an efficient economic source for removal of toxic heavy metals from aqueous solutions because fungal cell wall has different functional groups which are involved in metal binding and fungal biomass is easily available which can be isolated from environment for metal sorption purposes (Ramasamy et al. 2011). Hence, in this study we aim to isolate cadmium tolerant fungi from e-waste recycling facility heavy metal contaminated soil and also to evaluate its capability for removal of cadmium from aqueous solution.

2. MATERIALS AND METHODS

2.1 Collection of sample

Soil sample was collected from e-waste recycling facility, Bengaluru, Karnataka, India and stored at -20°C.

2.2 Screening of cadmium tolerant fungal strain

Cadmium tolerant fungal strain was isolated from the sample using fungal medium (Potato Dextrose Broth). The medium was amended with different concentrations of cadmium (10-100 mgL^{-1}). Serial dilution was carried out to decrease the microbial load in the sample and standard spread plate method was performed. The plates were then incubated at room temperature (30-35°C) for 48 h. After 48 h incubation fungi from each plate were isolated.

2.3 Heavy metal assay and biomass quantification

Cadmium concentration was determined by Pyronine G method with cadmium chloride as standard at 470 nm

spectrophotometrically (APHA, 1992). The fungal biomass was quantified at 405 nm (Ramasamy et al. 2011).

2.4 Removal isotherms

To evaluate the metal removal capacity of the biosorbent "***n***" used as removal rate (%). Tests were conducted with initial heavy metal levels of 100 mgL^{-1}.

$$n = [(Ci - Ce) / Ci] \times 100\ \%,$$

where '**Ci**' is initial heavy metal level in solution (mgL^{-1}), '**Ce**' heavy metal level remaining in solutions after removal (mgL^{-1}) .

2.5 Optimization of pH for cadmium removal

The fungal isolate was inoculated into a series of 250 mL conical flasks containing cadmium chloride (100 mgL^{-1}). The pH was varied (2, 4, 6, 8 and 10) and kept in orbital shaker (120rpm) for 24 h incubation. The cadmium removal and fungal biomass was measured spectrophotometrically.

2.6 Optimization of temperature for cadmium removal

The fungal isolate was inoculated into a series of 250 mL conical flasks containing cadmium chloride (100 mgL^{-1}). The flasks were incubated at different temperatures (25, 30, 35, 40 and 45°C) and kept in orbital shaker (120rpm) for 24 h incubation. The cadmium removal and fungal biomass was measured spectrophotometrically.

2.7 Kinetics of broth cellular growth and cadmium removal

The fungal isolate was inoculated into a 250 mL conical flask containing cadmium chloride (100 mgL^{-1}). The flask was kept in orbital shaker (120 rpm) at optimum pH and temperature for 26 h. During the incubation period, heavy metal concentration and biomass were monitored at a regular interval of two hours until heavy metal removal attains a saturation level.

2.8 Cadmium tolerance assay

To elucidate the tolerance of the isolate towards cadmium, optimal culture conditions were used with varying initial cadmium concentrations. To a freshly prepared growth medium, cadmium was amended (10-100 mgL^{-1}). After 24 h incubation, the biomass was measured. The extent of tolerance was compared and the normalized biomass was calculated, i.e., biomass at each heavy metal concentration per biomass using a control. All the experiments were carried out in triplicates.

3. RESULTS AND DISCUSSION

3.1 Screening of cadmium tolerant fungal strain

The number of fungal colonies decreased in the plates with increase in cadmium concentration. This shows the toxic nature and tolerant nature of fungi. The isolated fungal strain showed maximum cadmium tolerance up to the concentration of 100 mgL^{-1}.

3.2 Identification of cadmium tolerant fungal strain

Identification of the isolated fungal strain was done by observing the colony morphology on PDA plates and by performing Lacto phenol Cotton Blue Mounting with the pure culture of the isolated strain (Table 1).

Morphological, physiological and biochemical characteristics	**Isolated fungal strain**
Conidial color	Orange
Hyphae	Septate and hyaline
Conidia	1.7- 2.3
Conidial shape	Globose
Conidial heads	Columnar and biseriate
Conidiophores	Smooth walled and hyaline
Vesicle	Sub-spherical, biseriate
Sterigmata	Green, Conidia in chains
Type of strain	***Aspergillus***

Table 1. Morphological, physiological and biochemical characteristics of the isolated fungal strain

3.3 Optimal pH for heavy metal removal by isolated fungal strain

Removal of cadmium increased (83%) with increased in pH up to 4 and further the cadmium removal decreased (Fig. 1) at alkaline conditions. Most of the microbial surfaces are negatively charged due to the ionization of the functional groups, thereby contributing to metal binding. The pH of the biosorption medium affects the solubility of the metal ions and ionization state of functional groups. Fungal surfaces have a negative charge in the pH range of 2-6 (Shankar et al. 2006). The proton concentration is high at lower pH (<2) and heavy metal biosorption decreases due to the positive charge density on metal binding sites, i.e. hydrogen ions compete effectively with metal ions in binding to the sites. The negative charge density on the cell surface increases with increasing pH due to deprotonation of the metal binding sites. The metal ions then compete more effectively for available binding sites, which increase biosorption (Kapoor et al. 1999). Decrease in biosorption at higher pH (>6) is due to the formation of soluble hydroxylated complexes of the metal ions and their competition with hydroxyl ions for active sites. Beyond pH 8, precipitations of the ions as hydroxides occur (Martins et al. 2006).

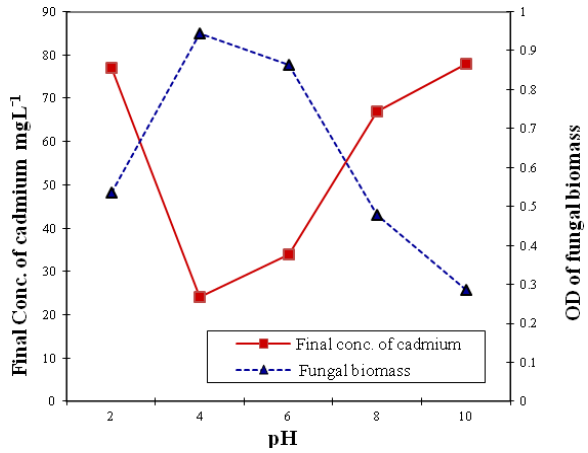

Figure 1. Cellular growth and cadmium removal by *Aspergillus* sp. in response to various pH. Temperature: 35°C, incubation time: 24h, concentration of cadmium:100 mgL^{-1}

3.4 Optimal temperature for heavy metal removal by isolated fungal strain

Removal of cadmium by the isolated *Aspergillus* sp. appears to be temperature dependent. Maximum removal of cadmium (83%) was observed at 30°C (Fig 2). The temperature of the adsorption medium could be important for energy dependent mechanisms in metal adsorption by microbial cells. Mostly adsorption is an exothermic process (Martins et al. 2006), whereas, some examples of endothermic adsorption have also been reported (Davis et al. 2003). In case of exothermic biosorption processes, an increase in temperature has been found to reduce the biosorption capacity of the biomass (Mamrei et al. 1999; Suhasini et al. 1999). This is attributed to the increase in temperature may increase metal desorption tendency from the interface to the solution (Sari et al. 2007). At that temperature 30°C, the fungal growth was also higher. During endothermic biosorption processes, the extent of adsorption processes increases with increasing temperature. This effect may be due to the fact that at higher temperatures, an increase in active sites occurs due to bond rupture (Hawari and Mulligan, 2006). However, physical damage to the biosorbent can be expected at higher temperature.

3.5 Measurement of the kinetics of broth cellular growth and heavy metal removal

Cadmium removal by *Aspergillus* sp. increased upto 24 hours of incubation. After that, sorption reached equilibrium. The maximum removal capacity of cadmium in aqueous solution by *Aspergillus* sp. was found to be 88 % (Fig. 3). In case of cadmium biosorption by *Pleurotus platypus*, the removal efficiency reached equilibrium at 60 min and that by *Agaricus bisporus* and *Calocybe indica* reached equilibrium at 240 and 180 min respectively (Vimala and Das, 2009).

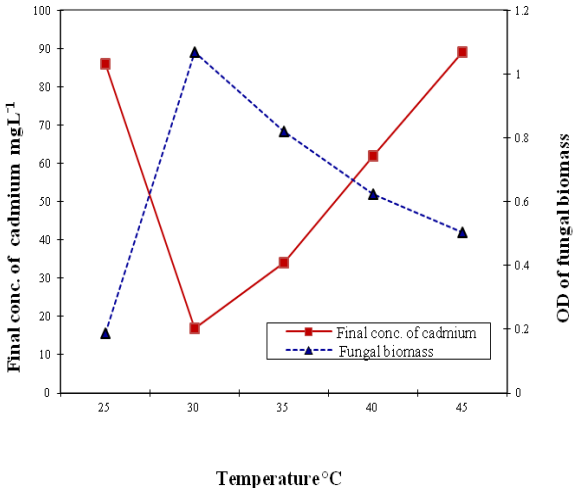

Figure 2. Cellular growth and cadmium removal by *Aspergillus* sp. in response to various temperatures. Concentration of cadmium:100 mgL^{-1}, pH: 4, incubation time: 24h

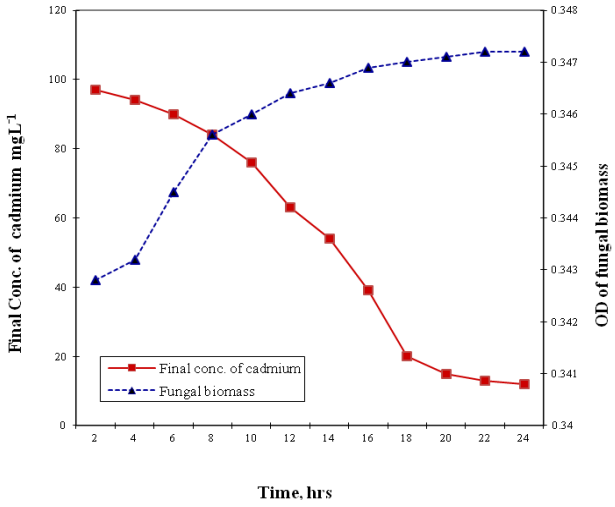

Figure 3. Kinetics of cellular growth and cadmium removal by *Aspergillus* sp. Concentration of cadmium:100 mgL^{-1}, pH: 4, temperature: 35°C

3.6 Heavy metal tolerance assay

The tolerance of isolated *Aspergillus* sp. to cadmium was studied for the cadmium concentrations ranging from 10-100 mgL^{-1} and the extent of tolerance was compared. When the concentration of cadmium increased in the media, the absorbance of the fungal culture decreased. It implies that the increase in cadmium concentration results in the decrease in fungal growth. Maximum growth was obtained in the media containing 10 mgL^{-1} cadmium (Fig. 4).

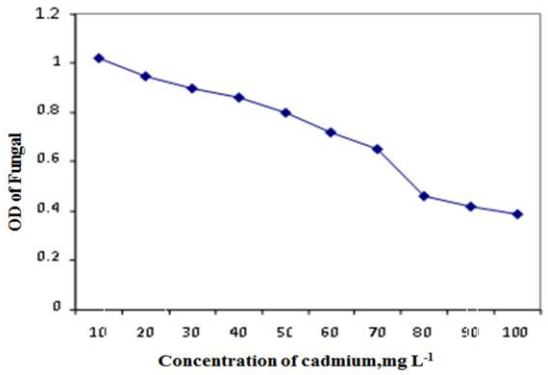

Figure 4. Normalized biomass (measured at 24h incubation time) in response to varying initial concentrations of cadmium concentration range: 10-100 mgL^{-1}, temperature: 35°C, pH: 4

4. CONCLUSION

In this study cadmium tolerant fungi was isolated from heavy metal contaminated environments, and the applicability of their heavy metal removal from a simulated environment was evaluated at a laboratory scale. The optimum conditions for both the growth and heavy metal removal were determined. The tolerance data revealed the cadmium tolerant *Aspergillus* sp. can tolerate cadmium toxicity up to 100 mgL^{-1}. The study demonstrated that the newly isolated heavy metal resistant *Aspergillus* sp. from e-waste contaminated site has potential application for the removal of cadmium from industrial waste waters.

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