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ORIGINAL ARTICLE

Evaluation of Isolated Fungal Strain from e-waste Recycling Facility for Effective Sorption of Toxic Heavy Metal Pb (II) Ions and Fungal Protein Molecular Characterization- a Mycoremediation Approach

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ABSTRACT
Electronic waste (e-waste) contamination is the serious problem since it contains toxic heavy metals. This study evaluated the efficiency of fungal strain Aspergillus fumigatus to remove Pb (II) ions, one of the toxic heavy metal present in e-waste. The parameters such as metal tolerant assay (500ppm), pH (4.0), temperature (30°C) and kinetics studies were optimized for the heavy metal removal. The maximum adsorption of 85.41% was observed during the batch sorption experiment. The fungal protein was isolated from the metal trained and untrained A. fumigatus to examine the protein variation among them.
KEYWORDS*: electronic waste; lead; biosorption; fungi; Aspergillus fumigatus*

INTRODUCTION

Electronic waste or e-waste is one of the fastest growing solid waste streams around the world growing at rate of 3-5% per annum approximately three times faster than other individual waste streams in the solid waste sector. The electronic waste disposal and recycling in developing countries is causing an increasing concern due to its effects on the environment and associated human health risks. E-waste contains more than 1000 different substances out of elements such as lead, mercury, cadmium, hexavalent chromium and brominated flame retardants are of major threat to human health and the environment. Some of the electronic wastes are recycled and the rest is dumped/ disposed into a landfill such as agriculture field and lake [1] which leads to the soil and water pollution. The presence of heavy metal contamination in aqueous streams, arising from the discharge of untreated metal containing effluent into water bodies, is one of the most important environmental issues [2, 3]. The presence of Pb (II) in drinking water is known to cause various types of serious health problems. Lead is toxic to multiple organ systems, and effects may range from enzyme inhibition and anaemia to disorders of the nervous, immune and reproductive systems, impaired kidney and cardiovascular functions, and even death. 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. Fungal sorption performs well in comparison to sorption on commercial ion-exchange resins, activated carbon, and metal oxides. Hence in this study we evaluated the fungal strain isolated from soil for its lead sorption capacity.

MATERIALS AND METHODS

Collection of soil sample

Soil samples were collected from e-parisara, Bengaluru, Karnataka, India and stored at -20°C.

Screening of the sample for lead tolerant fungi

Lead tolerant fungal strain was isolated from the soil samples using fungal medium (Potato Dextrose Broth). Potato

dextrose agar plates were prepared using 250g of potato boiled in 100 ml distilled water for 30 min and the filtrate is mixed with 2g of dextrose and 1.5 g of agar was added with this mixture in case of plates. To isolate metal resistant fungal strain the medium was amended lead ions 100mg L^{-1}. Serial dilution technique was performed to decrease the microbial load in the sample and standard spread plate method was performed. The plates were than incubated at room temperature (30-35°C) for 48 h. After 48 h incubation larger identical colonies from each plate were isolated. These isolates were characterized and further employed for heavy metal removal and tolerance studies. Morphological, physiological, and biochemical a characteristic of the isolated fungal species is given in table 1.

Heavy metal assay and biomass quantification

Lead (II) concentration was determined by PAR (2, 4-pyridile azo resorcinol) method using UV- Vis spectrophotometer (Shimadzu, Japan) at 520 nm. The linear regression of the standard graph for the estimation of Pb (II) is 0.99.

Optimization of pH for metal removal

The fungal isolate was inoculated into a series of 250 conical flasks containing 100mg L^{-1}.The pH was varied from 2 to 10 (2, 4, 6, 8 and 10). The pH of the medium was adjusted using dilute HCL or NaOH. For each pH point the percentage of lead removal was calculated using the following formula,

$$\frac{\text{(Initial lead concentration-current lead concentration)X100}}{\text{Initial lead concentration}}$$

Optimization of temperature for metal removal

The fungal isolate was inoculated into a series of 250 conical flasks containing 100mg L^{-1}.The flasks were incubated at temperatures varied from 25 to 45 (25, 30, 35, 40 and 45°C).

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

The fungal isolate was inoculated into a 250 conical flask containing 100mg L^{-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 for every two hours interval until heavy metal removal attains a saturation level.

Heavy metal tolerance assay

To elucidate the tolerance of the isolate to lead (II) ions, optimal culture conditions were used with varying initial lead concentrations. To a freshly prepared growth medium, lead was amended as Pb (II) using Lead nitrate. After 24 h incubation, the biomass was measured. The extent 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.

Protein expression assays in heavy metal trained and untrained fungi

Proteomes from the fungal isolate was extracted and purified using microbial lysis method. Cell samples was taken from mid log phase of cellular growth (at optical density of 03 0.4) under the conditions of the experiments to measure the kinetics of the cellular growth and heavy metal removal. The isolated protein was quantifies by Bradford method and the pattern of proteomic expression was analyzed by 10% SDS- PAGE using Laemmli's method [4]

RESULTS AND DISCUSSION

Isolation of lead tolerant fungi

The number of fungal colonies decreased in the plates with increase in lead concentration. This shows the toxic nature of lead and also the tolerant nature of fungi. The isolated fungal culture was tolerant to a lead concentration of 100 ppm. It was further characterized and used in Pb removal batch mode studies.

Identification of lead tolerant fungal isolates

Identification of the isolated fungal species 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 species Table 1.

Optimal pH for heavy metal removal by isolated fungi

Adsorption of lead increased along with the increase of pH of the adsorbate solution. Maximum adsorption (85.41%) occurred at pH 4, which is shown in Fig 1. From the same figure, it was found that the optimum pH for fungal growth was also pH 4.

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 the ionization state of the functional groups. Fungal surfaces have a negative charge in the pH range of 2-6 [5]. 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 [6]. 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 8.0, precipitations of the ions as hydroxides occur [7]. Similar results have been reported for the removal of lead using *Penicillium digitatum* and *Rhizopus nigricans* [8].

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

Morphological, physiological and biochemical characteristics	Isolated fungal species
Colony diameter	25 µm
Conidial color	Blue-green
Hyphae	Septate and hyaline
Conidial shape	Globose
Conidial heads	Columnar
Conidiophores	Flask shaped
Conidiophores color	Green
Vesicle	Flask shaped, 20 µm in diameter, Green
Sterigmata	Green, Conidia in chains
No. of sterigmata	Present in single series

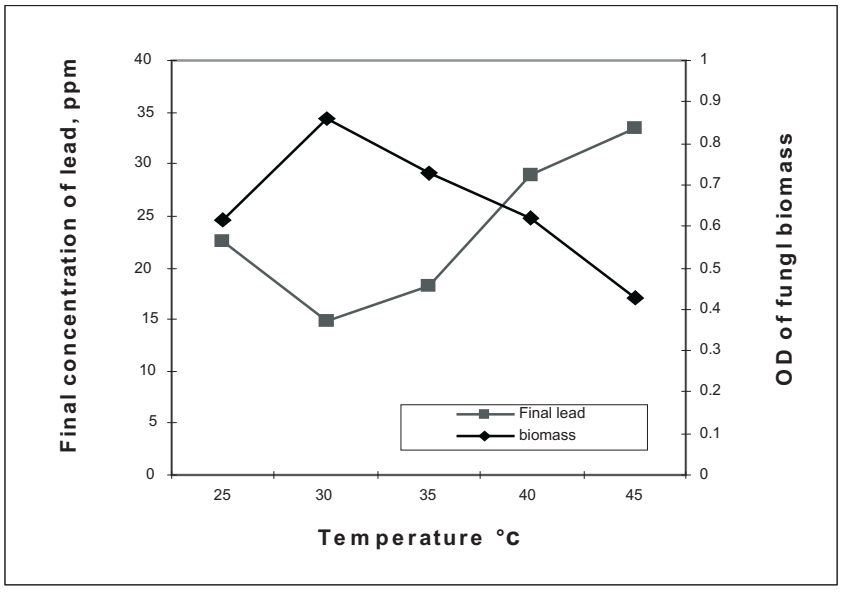

Figure 2 Cellular growth and lead removal by *Aspergillus* sp. in response to various temperatures. Concentration of Pb (II):100 mgL^{-1}, pH: 4, incubation time: 24h

Optimal pH for heavy metal removal by isolated fungi

Bioaccumulation of Pb (II) by the isolated fungal species, *Aspergillus fumigatus* appears to be temperature dependent. Maximum removal of lead (85.25%) was observed at 30°C for *A.fumigatus* (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 [9], whereas, some examples of endothermic adsorption have also been reported [10]. In case of exothermic biosorption processes, an increase in temperature has been found to reduce the biosorption capacity of the biomass [11, 12]. This is attributed to the increase in temperature may increase metal desorption

tendency from the interface to the solution [13]. At that temperature, 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 [14]. However, physical damage to the biosorbent can be expected at higher temperature. Sometimes the adsorption may be temperature independent. The adsorption of Hg (II), Cd (II) and Pb (II) by the calcium alginate and immobilized algal preparations appears to be temperature independent over the temperature range tested (5-40^{o}C) [15].

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

Lead adsorption by *Aspergillus fumigatus* increased upto 18 hours of incubation. After that, adsorption reached equilibrium. The maximum adsorption capacity of *Aspergillus fumigatus* was found to be 85.65 % (Fig 3). In case of lead biosorption by *Pleurotus platypus*, the removal efficiency reached equilibrium at 120 min and that by *Agaricus bisporus* and *Calocybe indica* reached equilibrium at 240 and 180 min respectively [7].

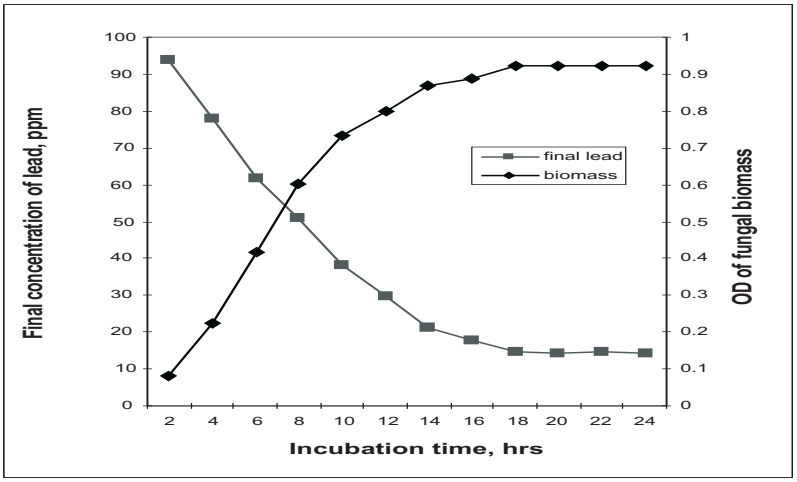

Figure 3 Kinetics of cellular growth and lead removal by *Aspergillus* sp. Concentration of Pb (II):100 mgL^{-1}, pH: 4, temperature: 35°C

Heavy metal tolerance assay

The tolerance of *Aspergillus fumigatus* to lead was studied for the lead concentrations ranging from 100-500 ppm and the extent of tolerance was compared. When the concentration of lead increased in the media, the absorbance of the fungal culture decreased. It implies that the increase in lead concentration results in the decrease in fungal growth. Maximum growth was obtained in the media containing 100 ppm lead (Fig 4).

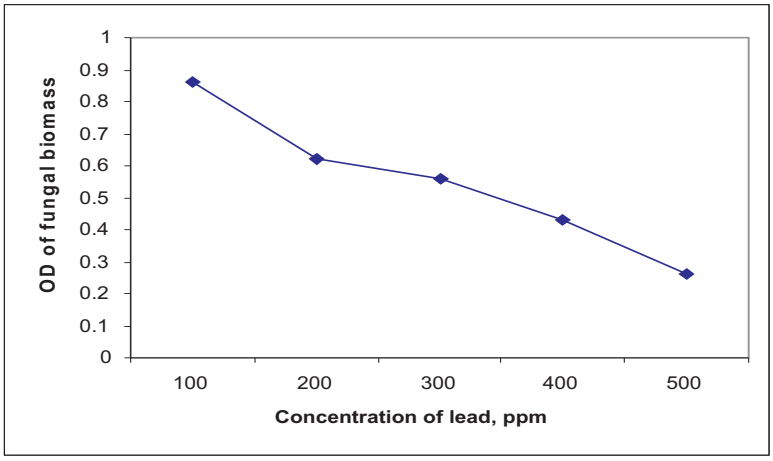

Figure 4 Normalized biomass measure at 24h incubation time in response to varying initial concentrations of Pb(II) concentration range: 100-500 mgL^{-1}, temperature: 35°C

Protein expression assays in heavy metal trained and untrained fungi

The isolated protein was run in 13% SDS PAGE. The cell samples were taken from the mid-log growth phase of the kinetics experiments, that is, the time required for cells to adapt heavy metal toxicity [5]. When compared with metal untrained, the *A. fumigatus* expressed considerable amount of polypeptide (protein) on 66kDa regardless the test heavy metal (Fig 5). This indicates the speculation that 66kDa protein may involve in response to heavy metal and probably pervasively exists in heavy metal resistant fungi. This was probably attributed to a higher degree of functional diversity fungi [5].

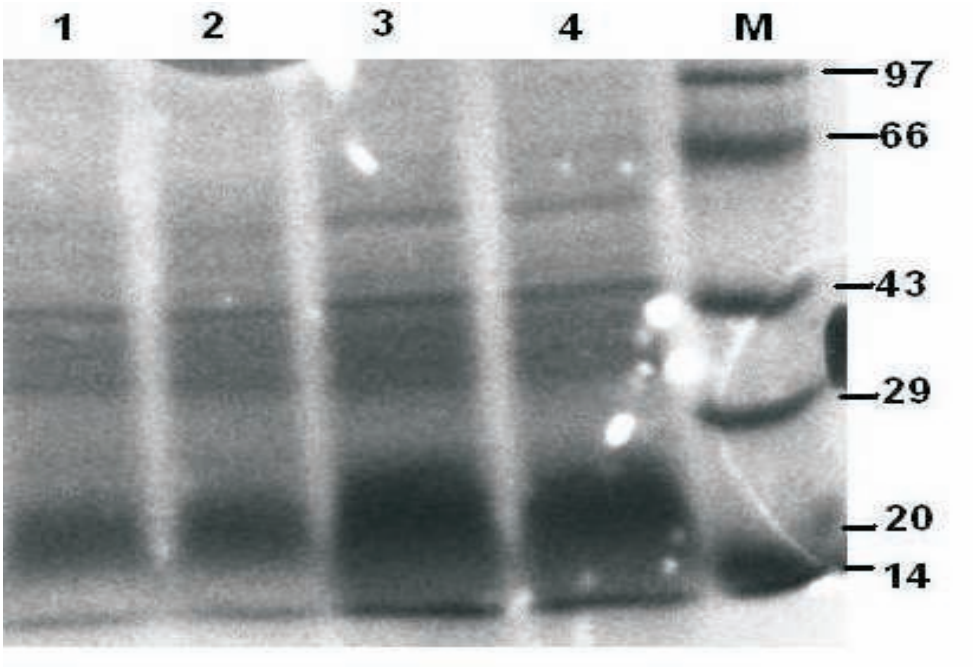

Figure 5 SDS- PAGE protein expression patterns in heavy metal trained (resistant) and untrained; Lane M Marker; Lane 1 and 2 Heavy metal untrained *Aspergillus fumigatus;* Lane 3 and 4 Heavy metal trained *Aspergillus fumigatus*

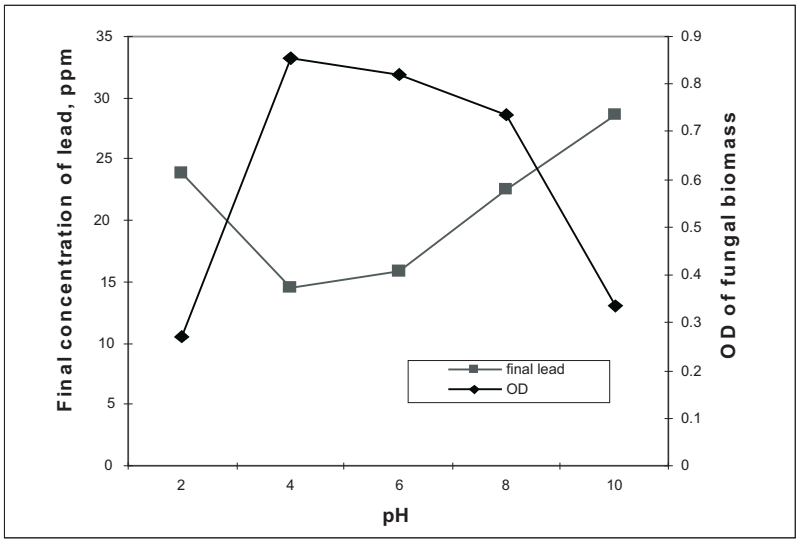

Figure 1 Cellular growth and lead removal by *Aspergillus* sp. in response to various pH. Temperature: 35°C, incubation time: 24h, concentration of Pb (II):100 mgL^{-1}

CONCLUSION

The aim of this work was to show the ability of the fungal species isolated from soil from e-waste recycling facility. The soil may contaminate with toxic heavy metals. Environmental factors, *i.e.* pH, temperature and ionic concentration showed significant effects on lead biosorption on *Aspergillus fumigatus* with maximum efficiency at pH 4.0, temperature of 30°C and the maximum tolerability of 100 ppm. *Aspergillus fumigatus* was found to be suitable biosorbent for Pb 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 lead trained fungi than the untrained. 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 fumigatus* isolated from e-waste recycling facility soil could be employed as an effective adsorbent for the removal of lead from aqueous solution.

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