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Biosorption of heavy metals by immobilized and dead fungal cells: A comparative assessment

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Heavy metal resistant fungi were isolated from an electroplating industrial effluent samples that uses copper, cadmium and lead for plating. These isolates were tested to evaluate their applicability for heavy metal removal from industrial wastewaters. Initially the physico-chemical parameters of the samples were analyzed. The optimum conditions of pH, biomass concentration and heavy metal concentration were determined for the microbial growth on biosorbents and correlated with heavy metal removal. The observed conditions were applied for the biosorption process in immobilized and dead fungal cells. The biosorption of immobilized cells of Aspergillus sp. was 60.94% of Cu, Penicillium sp. was 97.21% of Cd and Cephalosporium sp. was 73.27% of Pb; whereas the dead cells of Aspergillus sp. was 46.91% of Cu, Penicillium sp. was 95.27% of Cd and Cephalosporium sp. was 70.67% of Pb. Experimental results reveal that all the immobilized isolates have potential application for the removal of Cu, Cd and Pb from industrial wastewater than the dead fungal cells.

Key words: Heavy metal, fungal sorption, optimization, dead fungal cells, immobilization

INTRODUCTION

Developmental progress in an industry is a major criterion of any country. Industrial creations have emerged as one of the world’s most dynamic and economic sectors, offering vast opportunities for cultural, social and economic development today, India is one of the top ten industrialized countries of the world (Guillén, 2003). Regional industrial production is outstripping global growth from one side and several highly polluting industries are also growing more rapidly in another side. Because of the rapid growth in urbanization and industrialization, the impact of foreign direct investment (FDI) due to environmental terms has largely been negative. The current pattern of industrial activity alters the natural flow of materials and introduces novel toxic chemicals into the environment (Faisal and Hasnain, 2004). Heavy metal release to the environment has been increasing continuously because of industrial activities and technological development and poses a significant threat to the environment, public and soil health. Contamination of agricultural soil with heavy metals is a major problem on industrial and defense related sites all over the world (Parameswari et al., 2010). Heavy metals include cadmium, lead, chromium, copper and nickel, which contaminate the soils, ground water, sediments and surface waters are extremely toxic to biological and ecological systems. The heavy metals are released due to the discharge of effluent into the environment by a large number of processes such as electroplating, leather tanning, wood preservation, pulp processing, steel manufacturing, etc. The concentration levels of these heavy metals vary widely in the environment. Heavy metals have a critical concern to human health and environmental issues due to their high occurrence as a contaminant, present in soluble form that are extremely toxic to biological systems, and the classification of

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several heavy metals as carcinogenic and mutagenic (Alloway, 1995; Diels et al., 2002). Moreover, the metals cannot be degraded to harmless products and hence persist in the environment indefinitely. As a result, several methods have been devised for the treatment and removal of heavy metals in contaminated sites. Conventional physico-chemical methods such as electrochemical treatment, ion exchange, precipitation, reverse osmosis, evaporation and sorption (Kadirvelu et al., 2002) for heavy metal removal are being economically expensive and have disadvantages like incomplete metal removal, higher reagent, energy requirements and generation of toxic sludge. Biological approach has the great potential that contributes for the achievement of this goal and is economical. Microbial populations in metal polluted environments adapt to toxic concentrations of heavy metals and become metal resistant (Prasenjit and Sumathi, 2005). The response of microorganisms towards toxic heavy metals is of importance in view of the interest in the reclamation of polluted sites (Shankar et al., 2007). Microorganisms uptake metal either actively (bioaccumulation) and/or passively (biosorption) (Hussein et al., 2003). Feasibility studies for large-scale applications demonstrated that, biosorptive processes are more economical than the bioaccumulative processes, because living systems (active uptake) often require the addition of nutrients and hence increase biological oxygen demand or chemical oxygen demand in the effluent (Hussein et al., 2003). Biosorption is proven quite effective for the removal of metal ions from contaminated solution in a low cost and environment friendly manner (Volesky, 1990). Fungi are known to tolerate and detoxify metals by several mechanisms including valence transformation, extra and intracellular precipitation and active uptake (Gadd, 1993). 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 (Magyarosy et al., 2002). Considering the above mechanisms of metal resistance in fungi, it is expected that screening of metal tolerant fungi may provide strains with improved metal accumulation. It is generally assumed that microorganisms concentrate accumulated metals in the cell surface. Such phenomenon results from complexation and/or ion-exchange reactions between metal ions and the charged chemical constituents of cell walls (Gupta et al., 2000). This was thoroughly studied for inactivated *Rhizopus arrhizus* during uranium biosorption (Tsezos and Volesky, 1982), *Aspergillus niger* AB10 during cadmium and *R. arrhizus* M1 during lead biosorption indicated that the cell surface functional groups of the fungus might act as ligands for metal sequestration resulting in removal of the metals from the aqueous culture media (Pal et al., 2010). Temperature, pH, biomass, heavy metal concentrations are the factors that affect the biosorption process. Particularly, pH (Gourdon et al., 1990), biomass concentration (Gong et al., 2005) and heavy metal concentration (Kiran et al., 2005) on biosorption experiments were investigated by optimization process. In this present study, the ability of isolated native microbial strains towards biosorption of Cu, Cd and Pb using immobilized and dead fungal cells were evaluated and compared. Effect of temperature, pH, biomass and tolerance to the heavy metals by the fungal isolates were carried out.

**MATERIALS AND METHODS**

**Sampling**

Effluent sample was collected from an electroplating industry at Tiruchirappalli district, Tamil Nadu, India that uses copper, cadmium and lead for plating. The collected sample was transferred to a sterile plastic container, taken immediately to the laboratory and maintained at 4°C for further studies. The characteristics of electroplating industrial effluent was carried out following the method of APHA, 1998; Saxena, 1998. Heavy metal concentrations were analyzed using 400/HGA 900/AS 800–Perkin Elmer Atomic Absorption Spectrophotometer (AAS).

**Isolation and identification of heavy metal-resistant fungi from the effluents**

Cu, Cd and Pb-resistant fungi were isolated from the effluent using Sabouraud's dextrose agar (SDA) medium and was prepared using peptone (10 g/L), dextrose (40 g/L) and agar (15 g/L). The metal resistant fungal isolates were amended in SDA medium with 100 mg/L of Cu, Cd and Pb individually and pour plate was performed. The fungal plates were incubated at 28°C for 72 h. After incubation, larger identical colonies from each plate were isolated. Based on the morphological characteristics the isolated of fungal isolates were identified by using A Manual of Soil Fungi (Gilmann, 1975).

**Determination of heavy metal-resistant fungal isolates by plate diffusion method**

Heavy metal resistant fungi were determined by plate diffusion method (Hassen et al., 1998). Heavy metal salt solutions were prepared in different concentrations as 10, 20, 40, 60, 80 and 100 mg/L. Each plate was spread with 72 h cultures of appropriate organisms. To each plate, 100 µL of appropriate metal salt solutions were added in each well of 10 mm in diameter and 4 mm in depth. Fungal plates were incubated at 28°C for 72 h. After incubation (Sub-Zero. IN-60; Serial No. 243-10-08, Lab Instrument, Chennai), the zone of inhibition was measured. A zone size less than 1 mm scored as resistance strain.

**Antibiotic resistance test**

The disc diffusion method was used to determine antifungal resistance of the isolates. Fluconazole, itraconazole, ketoconazole and nystatin were the antibiotics used (10 mcg). Zone of inhibition was measured and were classified as resistance or sensitive.
Table 1. Characteristics of electroplating industrial effluent.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.97</td>
</tr>
<tr>
<td>Electrical conductivity (mS/Cm)</td>
<td>240</td>
</tr>
<tr>
<td>Total Dissolved Solids (mg/L)</td>
<td>151.2±0.86</td>
</tr>
<tr>
<td>Organic carbon (%)</td>
<td>3.5±0.59</td>
</tr>
<tr>
<td>Organic matter (%)</td>
<td>6.13±0.74</td>
</tr>
</tbody>
</table>

**Heavy metals (mg/L)**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copper</td>
<td>2.8860±0.75</td>
</tr>
<tr>
<td>Cadmium</td>
<td>1.9820±1.40</td>
</tr>
<tr>
<td>Lead</td>
<td>1.2720±0.37</td>
</tr>
</tbody>
</table>

* Value represents mean of triplicates. ^ Values in parentheses represent standard deviation.

Fungal biomass immobilization

The spores were obtained by the procedure of Anderson et al. (1973). For cell immobilization, the modified method of Shide et al. (2005) was used. In brief, the cells of exponentially growing mycelia of the culture were harvested aseptically into a 1 L capacity blender, using a spatula. The harvested cells were homogenized and 17.5 ml of the cell homogenate was added to 87.5 ml of distilled water into a 250 ml conical flask and mixed thoroughly. The mixture was allowed to settle and after 10 min, exactly 3.063 g of sodium alginate was added into the supernatant (concentrated cells). The mixture was subsequently pumped through a 5 ml syringe drop wise, into a flask containing sterilized 100 ml of 0.12 M calcium chloride solution. This mixture was allowed to settle for 1 h to complete precipitation that formed spherical beads. The immobilized cells were removed and stored until use at 4°C in 5 mM CaCl₂ solution. The known quantity of immobilized fungal beads was maintained in the conical flask containing 50 ml of samples for 72 h incubation and centrifuged at 6000 rpm for 20 min. The supernatants of the samples were analyzed and the percentage of each metal was determined by atomic absorption spectrophotometer. The experiments were carried out in triplicate and the results are presented as mean values.

RESULTS AND DISCUSSION

The characteristics of electroplating industrial effluent are listed in Table 1.

Heavy metal resistance efficiency

In plate diffusion method, results of zone formation indicate the ability of the isolates as heavy metal-resistant or sensitive (Duxbury, 1981). Heavy metal-resistant isolates show no inhibition of growth for higher concentration of heavy metals, whereas heavy metal-sensitive isolates show inhibition of growth for higher concentration of heavy metals. Based on this concept, Aspergillus sp., Penicillium sp. and Cephalosporium sp. were identified (Table 2) as efficient strains that were resistant to Cu, Cd and Pb respectively (Figure 1). The identified efficient strains were selected for further studies.

In antibiotic resistance test, copper resistant Aspergillus sp. was resistant to flucanazole, itraconazole, ketoconazole and Aspergillus sp. was sensitive to nystatin. Cadmium resistant Penicillium sp. and Pb resistant Cephalosporium sp. were resistant to all three antibiotics except nystatin (Table 3). All these results showed that nystatin was sensitive to all these heavy metal resistant fungal strains. In the present study, fungal isolates were resistant to flucanazole, itraconazole and ketoconazole but sensitive to nystatin. Earlier works...
Table 2. Morphological characteristics of the isolated fungi.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Aspergillus sp.</th>
<th>Penicillum sp.</th>
<th>Cephalosporium sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conidial colour</td>
<td>Black</td>
<td>Gray green</td>
<td>Brown</td>
</tr>
<tr>
<td>Conidial shape</td>
<td>Globose</td>
<td>Elliptical</td>
<td>Round</td>
</tr>
<tr>
<td>Vesicle shape</td>
<td>Globose</td>
<td>Globose</td>
<td>Globose</td>
</tr>
<tr>
<td>Colonial reverse</td>
<td>Whitish grey</td>
<td>Yellow</td>
<td>Yellow</td>
</tr>
<tr>
<td>No. of sterigmata</td>
<td>Present in two series</td>
<td>Two alternate branches</td>
<td>Simple</td>
</tr>
</tbody>
</table>

A - *Aspergillus* sp. resistant to Cu; B - *Penicillum* sp. resistant to Cd; C - *Cephalosporium* sp. resistant to Pb

Figure 1. Fungal growth in different concentrations of heavy metal concentrations.

revealed that there is an interrelationship between the antibiotic and heavy metal resistance capacities of all the microbes (Harnett and Gyles, 1984; McEntee et al., 1986; Schwarz and Hobel, 1989; Belliveau et al., 1991). Timoney et al. (1987) reported that the metal tolerance and antibiotic resistance are often closely associated with many clinical isolates. Harnette and Gyles (1984) have reported that the resistance to both can be transferred among organisms through conjugation or transduction.

Optimization for heavy metal removal

In the pH range studied (5 to 9), when 0.1 ml of fungal isolates was inoculated in the Sabouraud’s dextrose broth, the growth of *Aspergillus* sp. and *Cephalosporium*
Table 3. Anti-fungal susceptibility of heavy metal resistant fungi.

<table>
<thead>
<tr>
<th>Antibiotics</th>
<th>Disc potency (mcg)</th>
<th>Aspergillus sp.</th>
<th>Penicillium sp.</th>
<th>Cephalosporium sp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flucanazole</td>
<td>10</td>
<td>R*</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>10</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Itraconazole</td>
<td>10</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>Nystatin</td>
<td>10</td>
<td>S*</td>
<td>S</td>
<td>S</td>
</tr>
</tbody>
</table>

*R- Resistant strain, S- Sensitive strain

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Figure 2. Cellular growth of fungi in response to various pH at temperature: 28°C and incubation time: 72h.

Sp. increased at initial pH 5 and their growth decreased gradually at increased pH. Whereas the growth of Penicillium sp. increased at pH 6 and its growth decreased at increased pH. Aspergillus sp., Penicillium sp. and Cephalosporium sp. has the ability to adsorb maximum Cu, Cd and Pb at pH 5, 6 and 5 respectively (Figure 2). According to Geddie and Sutherland, (1993) the pH was reported to be the most important factor for all ions uptake, and the removal capacity was shown to increase with pH but the upper limit of working pH was limited by hydroxide precipitation. Yan and Viraraghavan (2003) describes most of the microbial surfaces are negatively charged due to the ionization of functional group thereby contributing to metal binding. Penicillium sp. showed the highest growth and adsorption of Cd at pH 6, which is similar to the result reported by Yan and Viraraghavan (2000) for the removal of Pb, using Penicillium digitatum. Yakup et al. (2004) stated that the increase in percentage removal is due to the strong relations of bioaccumulation to the number of surface negative charge, which depends on the dissociation of functional group. In Aspergillus sp. maximum growth was observed at pH (5) and gradually decreased to pH 9. Nassari et al. (2002) reported that the maximum removal of chromium by Aspergillus sp., where the maximum removal was observed at pH 5, which is suitable for the living cells of fungi and bacteria, and were able to grow significantly. With increase in pH, the removal percentage of metal was decreased. Further increased in the pH beyond five, the heavy metal removal decreased which might be due to the osmotic changes and hydrolyzing effect (Shankar et al., 2007). The variation of heavy metal adsorption at various pH is on the basis of metal chemistry in solution and the surface chemistry of the sorbent. Based on all these reviews, all fungal strains have potential application for the removal of heavy metals like Cu, Cd and Pb.

Fungal strains of Aspergillus sp., Penicillium sp. and Cephalosporium sp. showed the maximum biomass at optimized pH of 5 in the presence of Cu, Cd and Pb at the level of 2, 3 and 4% respectively (Figure 3). From this study we know that when the biomass concentration increases there will be reduction in the growth of organisms and adsorption of heavy metals. Pons and Fuste (1993) explained that the high biosorbents concentrations are known to cause cell agglomeration and consequent reduction in the inter-cellular distance. This is reported to produce a ‘screen effect’ among the dense layer of cells, leading to ‘protection’ of the binding sites from metal ions. Itah et al. (1975) reported that the metal uptake is higher when the inter-cellular distance is more (at low biosorbents concentration), as this condition
ensures optimal electrostatic interaction between cells, a significant factor of biosorption.

The effect of heavy metal concentrations on Cu, Cd and Pb adsorption by Aspergillus sp., Penicillium sp. and Cephalosporium sp. were showed in Figure 4. The concentrations of Cu, Cd and Pb (20, 40, 60, 80 and 100 mg/L) at the appropriate levels of pH (5, 6 and 5) were studied. The adsorption of Cu metal by Aspergillus sp., Cd metal by Penicillium sp. and Pb metal by Cephalosporium sp. showed maximum growth at 80, 20 and 60 mg/L respectively, and thereafter remained stable as the concentration increased up to 100 mg/L. Similar results were reported by Gulay, (2003) in the case of Trametes versicolor for Cu, Pb and Zn removal. Salehizadeh et al. (2003) describes the Bacillus firmus as also having less ability to remove Pb, Cu and Zn. All these results clearly reveal the existence of infinite heavy metal reduction capacity possibly due to heavy metal toxicity towards the cells.

**Biosorption of heavy metal by immobilized fungal cells**

The biosorption of immobilized fungal cells like
Aspergillus sp., Penicillium sp. and Cephalosporium sp. adsorbed Cu (60.94%), Cd (97.21%) and Pb (73.27%) respectively (Figure 5). All these results describe the adsorption capacities of the immobilized fungal cells was greater than that of dead cells. Johncy Rani et al. (2010) found similar adsorption capacity of Cu, Cd and Pb in the immobilized cells of Bacillus sp., Pseudomonas sp. and Micrococcus sp. respectively. Leusch et al. (1995) explained this is because dead fungal cells consist of small particles with low density, poor mechanical strength and little rigidity. Hence, the immobilization of biomass is necessary on before subjecting to biosorption. Holan and Volesky (1994) explained immobilized cells offers many advantages including better reusability, high biomass loading and minimum clogging in continuous flow systems. Adsorption of heavy metal was also dependent on cell density in calcium alginate beads.

Kratochvil and Volesky (1998) explained the mechanisms involved in biosorption are metal-microbe interactions that should be further studied with great efforts by utilizing various techniques. Baik et al. (2002) reported that Aspergillus sp. has the ability to adsorb maximum level of Cu when treating the cell fraction with NaOH. Volesky and May-Philips (1995) explained this is due to microbial biomass consisting of poor mechanical strength and little rigidity. However, biosorbents are hard enough to withstand the application of pressures, water retention capacity, porous and transparent to metal ion sorbate species.

Conclusions

The results of this study revealed that all the immobilized fungal cells have a greater potential application for the removal of Cu, Cd and Pb from industrial wastewater than the dead fungal cells by harvesting the fungal cells at log phase to avoid lyses. Further research will be scoped to study the desorption process for the management of heavy metal laden biomass as an environmental friendly method of disposal.

Biosorption of heavy metal by dead fungal cells

Biosorption is at laboratory scale inspite of its development before few decades (Wang and Chen, 2006). In the present study, the dead fungal cells of Aspergillus sp., Penicillium sp. and Cephalosporium sp. were used as the biosorbents for the adsorption of Cu, Cd and Pb respectively. Aspergillus sp., Penicillium sp. and Cephalosporium sp. showed the maximum adsorption of 46.91% of Cu, 95.27% of Cd and 70.67% of Pb respectively (Figure 5). The adsorption capacities of these organisms were expressed as Cd>Pb>Cu. Kratochvil and Volesky (1998) explained the mechanisms involved in biosorption are metal-microbe interactions that should be further studied with great efforts by utilizing various techniques. Baik et al. (2002) reported that Aspergillus sp. has the ability to adsorb maximum level of Cu when treating the cell fraction with NaOH. Volesky and May-Philips (1995) explained this is due to microbial biomass consisting of poor mechanical strength and little rigidity. However, biosorbents are hard enough to withstand the application of pressures, water retention capacity, porous and transparent to metal ion sorbate species.

REFERENCES

Faisal M, Hasnanin S (2004). Microbial conversion of Cr (VI) into Cr (III)

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