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Biosorption of lead using pretreated cells of *Aspergillus* species

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Abstract

Microbial bioremediation is an emerging technology for environmental cleanup. Application of living biomass for metal binding depends on nutrient type and concentration, environmental conditions and cell age. In addition, living biomass may be subject to toxic effect of heavy metals at elevated concentrations. To overcome the disadvantages; non-viable or dead biomass is preferred. To test these hypothesis three fungal strains were isolated from effluent of chemical and pharmaceutical industry using SDA agar. Identification of the above isolates was carried out and was identified to be predominant strains of *Aspergillus* i.e. (*Aspergillus niger and Aspergillus flavus*). Further preliminary test was performed to check the tolerance of the fungi to different metal salts of lead, copper, chromium, zinc, nickel, cadmium using 1mM concentration. All three fungal species showed tolerance to metal salts like lead nitrate, zinc sulphate and cupric sulphate above 20 mM. Furthermore minimum inhibitory concentration was determined against the two above species for the three heavy metals. Pretreatment of live cells of *Aspergillus* strain was carried out .This dried biomass was then used for optimization of various parameters like concentration of metals, biomass concentration, pH, temperature of incubation and contact time. The filtrate was then analyzed after proper digestion and dilution by Atomic Absorption Spectrophotometer. The availability of variety of biomass and their metal binding potential makes it economical and sustainable option for developing effluent treatment process for removal and recovery of heavy metals.

Keywords*: Biosorption, Lead, Aspergillus flavus, atomic absorption spectrophotometer*

Introduction

In developing countries like India, wastewater treatment is of utmost importance. The degree of treatment may range from a main process for seriously polluted industrial waste to a polishing process for removing the trace concentrations which remain after the main treatment. In this light, biological materials have emerged as an ecofriendly and economic option.For a long time, peat has occupied the place of prominence among biosorbents, but since it is not available everywhere, microbial biomass is the other option. Treatment of effluents with heavy metals following biotechnological approaches is simple, comparatively inexpensive and environment-friendly. Biosorption can be defined as the selective sequestering of metal soluble species that result in the immobilization of the metals by microbial cells. Metal sequestering by different parts of the cell can occur via various processes: complexation, chelation, coordination, ion exchange, precipitation, reduction. It is a process with some unique characteristics and can effectively sequester dissolved metals from very dilute complex solutions with high efficiency. This makes biosorption an ideal candidate for the treatment of high volume low concentration complex waste-waters.Fungal cell walls are typically composed of the polysaccharides chitin and cellulose, and the cell walls of algae and plants are composed mainly of the polysaccharide cellulose. These biopolymers, constituents of the cell wall and the other parts of the cell possess functional groups that have a significant potential for metal binding. Furthermore, intracellular biopolymers such as proteins and DNA may also contribute to metal immobilization. In many cases, extra cellular polymeric substances such as exo- polysaccharides (EPS) that are closely related to the cell membrane can also participate in metal immobilization.The appropriate selection of metals for biosorption studies is dependent on the angle of interest and the impact of different metals, on the basis of which they would be divided into four major categories: toxic heavy metals, strategic metals, precious metals and radio nuclides. In terms of

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environmental threats, it is mainly toxic heavy metals and radio nuclides that are of interest for removal from the environment and/or from point source effluent discharges.(Sanyal et al ., 2005).
Lead(Pb), a heavy metal produced as a byproduct of fossil fuel combustion in its organic compound form, as well as a variety of industries and at solid waste dump sites , has a debilitating effect on the human body. Lead even in low doses, may cause development disorders in fetuses, infants and the young as well as brain damage, behavior changes/abrupt mood swings with violent tendencies , juvenile delinquency, irritation of the respiratory tract, intoxication of the central nervous system, and gastrointestinal complications. In some cases elevated Pb levels in the blood and seminal fluids has been linked to unexplained male infertility. (Tamer Akar *et al*. 2006).The current study is aimed to carry out biosorption of lead using pretreated cells of *Aspergillus* species.

2. Material and Methods

2.1 Isolation of Fungal strains

The composite soil samples (10gm) and the industrial effluent samples (10ml) from pharmaceutical and chemical industry each were suspended in 100 ml of sterilized Normal saline solution (NSS). Subsequently 1 ml of this suspension was serially diluted to 10^6 with NSS. Different dilutions (0.1 ml) was spread on Sabouraud's dextrose agar(SDA) plates containing 100 µg of broad spectrum antibiotic (chloramphenicol) to inhibit bacterial growth. The inoculated plates were incubated at 29^{o}C for 72 hrs and fungal colonies were isolated. Fungal isolates were maintained in the laboratory by sub-culturing and refrigerating at 4^{o}C.

2.2 Identification of Fungal strains

Slide culture Technique: An agar block (7 x 7) mm) small enough to fit under a coverslip was cut using a sterile scalpel. The block was flipped up onto the surface of the agar plate. The four sides of the agar block were inoculated with spores or mycelial fragments of the fungus to be grown. A flamed coverslip was placed centrally upon the agar block. The plates were incubated at 26^{o}C until growth and sporulation occurred.

Lacto phenol Cotton blue mount: The cover slip was removed from the agar block. A drop of 95% alcohol was applied as a wetting agent. A flamed coverslip was gently placed onto a drop of Lacto phenol cotton blue on a clean glass slide and observed under high power objective.

2.3 Screening and Selection of Heavy metal resistant fungi

Purified isolates were screened on the basis of their tolerance to Cr^{6+}, Pb^{2+}, Zn^{2+}, Cd^{2+} and Cu^{2+}. Metal salts used were potassium dichromate, lead nitrate, zinc sulphate, cupric sulphate, and cadmium sulphate.A disk of mycelium was inoculated aseptically on SDA plates supplemented individually with 1mM of heavy metal. The inoculated plates were incubated at 29^{o}C for 7 days.

2.4 Determination of Minimum Inhibitory Concentration

Resistance of the selected isolates to Pb^{2+}, $Zn^{2+}$and Cu^{2+} was determined by the dilution plate method. Metal ions were added separately to SDA plates at concentrations of 1mM to 25mM.The plates were inoculated with 8mm agar plugs from young fungal colonies pre-grown on SDA. Three replicates of each concentration and controls were used. Inoculated plates were incubated at 29^{o}C for at least 5 days. Minimum inhibitory concentration was observed as the lowest conc. of metal that inhibits visible growth of the isolate.

2.5 Biomass Harvestation and Pretreatment of Live Biomass

Spores of 6-7 day old culture grown in Sabourauds agar plate was inoculated in SAB broth. The culture on incubation under shaker conditions formed spherical pellets. After 3-4 days the harvested broth was washed with deionised water. Live harvested mycelial biomass was treated with 0.5 N NaOH for 30 mins.It was followed by washing with adequate amount of distilled water until the pH reached to neutral range (pH 6.8-7.0).It was then autoclaved at 15 lb/inch^2 for 20 min. The pretreated biomass was dried at 60^{o}C for 24 hr in hot air oven and converted into powder form by grinding in mortar or pestle.

2.5 Optimization of Various Parameters for Biosorption

0.02 grams of powdered biomass was inoculated into 20 ml metal solution containing (2, 5, 10 15 mM) lead nitrate in deionised water. The flasks were kept on rotary shaker for 24 hrs at 30°C. Solution along with dead biomass was centrifuged. Content of the supernatant was analyzed after proper digestion and dilution by Atomic Absorption Spectrophotometer. The experiments were repeated by using various biomass concentrations. (0.02, 0.05 & 0.1gms) and contact time. (24, 48 & 72hrs

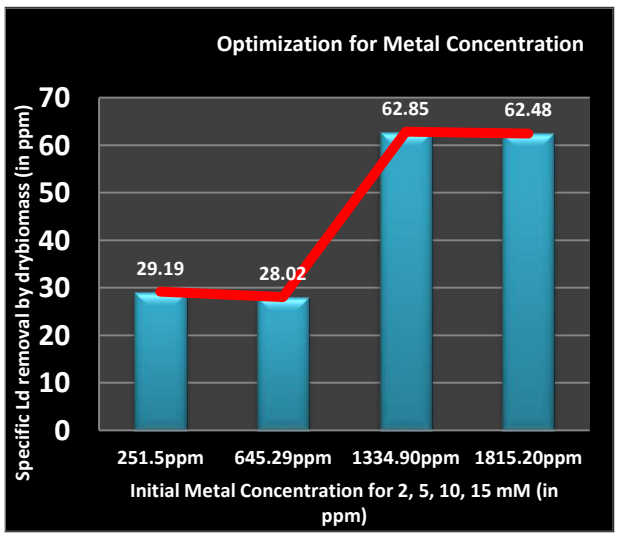
2.6 Digestion Procedure for Atomic Absorption Spectrophotometer

1ml of supernatant /centrifuged biomass was treated with concentrated HNO_3 for 30 mins. Digestion was carried out inside the fumehood. Then 60%perchloric acid was added until the volume reduced to half the total volume. Small amount of volume was then subjected to Atomic Absorption Spectrophotometer analysis.

Results and Discussion

Four fungal species were isolated from waste water effluent of chemical and pharmaceutical industry and soil. On the basis of slide culture technique, lacto phenol cotton blue mounting and colonies obtained on Sabourauds dextrose agar the isolates were identified to be *Aspergillus niger and Aspergillus flavus*. Heavy metal tolerance was showed by *Aspergillus flavus* isolated from waste water effluent of chemical industry and *Aspergillus niger* isolated from soil and waste water of chemical industry.The higher amounts of heavy metals in the soil are likely due to long-term application of the wastewater containing the heavy metals. Soil fungi able to grow in the presence of heavy metals were isolated. The fungi most frequently encountered from the soil samples are *A.niger*, *P. chrysosporium* and *T.viride*. (E. Parameswari et al.,2010).In the present study, isolation of two species of fungi was done namely three isolates of *Aspergillus niger* and one isolate of *Aspergillus flavus* from waste water industrial effluent and soil. They were found to be resistant to heavy metals like lead, zinc and copper.Amongst the microbial flora present in the effluent, fungi were selected for the present study, due to the ease they offer for removal from liquid substrates. *A. niger RH 17* and *A. niger RH 18* showed the highest tolerance, 6000 and 7000 mg/L, respectively, warranting them to be successful candidates for metal detoxification. (Rani faryal et al., 2007).Both the *Aspergillus* species were checked to determine minimum inhibitory concentration .It was found that the order of resistance of the isolates to heavy metals was Pb>Zn>Cu. *Aspergillus flavus* showed more tolerance to lead than *Aspergillus niger*, the highest tolerated concentration being 8000 mg/L which is quite comparable and promising.Assessments revealed that the fungal biomass exposed to alkaline supplements/salts exhibited significantly higher biosorption efficiency in comparison to untreated biomass. In current research work, an increase in biosorption of Pb (II) ions was noticed as a result of alkali pretreatments, particularly NaOH. Similar enhancement in metal uptake capacity of the fungal biomass regarding alkali pretreatment was recorded in comparison to live cells used for preliminary testing.The biosorption of Pb(II) and Cu(II) ions by A. flavus increased with increasing initial concentration of metal ions, becoming saturated at 200 and 150 mg/l for Pb(II) and Cu(II) ions, respectively.(Tamer Akar et al.,2005).Initial metal concentration plays an important role in determining the biosorptive capacity of the absorbent. The initial metal ion concentration used which shows maximum biosorption is at 3300 ppm i.e.10mM.

Fig 1- Optimization for metal concentration



The experiment on metal uptake reveals that the metal uptake decreases when biomass concentration rises. Therefore it is not useful to increase the biomass beyond 0.02-0.25 grams per 100 ml to sequester metal ions from 100 ppm of lead solution.

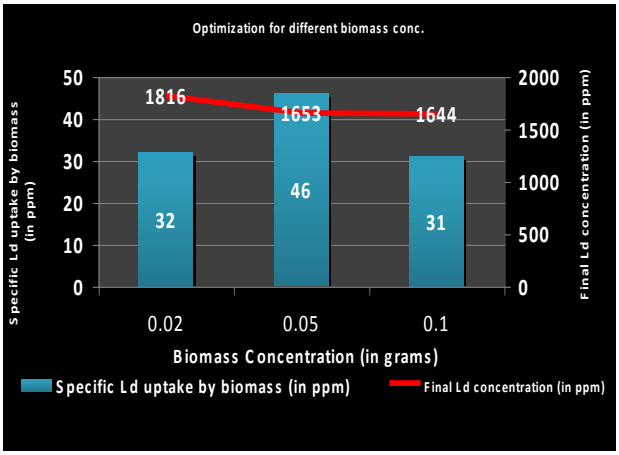


Fig 2-Optimization for biomass concentration

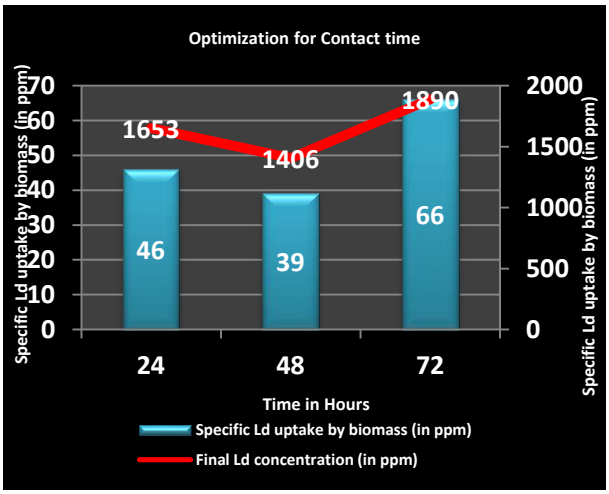


Fig 3- Optimization for contact time

Removal of lead by dead biomass of *Aspergillus flavus* showed maximum removal at pH 4-5.The results indicated that maximum adsorption of different metal species occur at different pH. However, from practical point of view pH of 5-6 was adequate.Temperature also played an important role in Pb biosorption. The temperature ranges used were 28°C, 35°C and 40ºC. Efficient Pb removal was observed at 28ºC, although Pb removal was also remarkable at the other two temperatures, but the Pb biosorption per gram of the biomass was reduced.

For the same metal different adsorbents had different removal rates. The adsorption of metal ions was greatest at 48 hrs at the specific pH and room temperature by a specific amount of powdered biomass in 20ml of metal solution with a continuous agitation at 120 rpm. This experiment showed that the removal rate is maximum after 24 hrs of contact time.Thus the current study suggests that these fungal strains may be used in future for bioremediation of wastewater and heavy metal contaminated soils.

Conclusion

The present study thus focuses on ability of pre-treated and dead biomass of fungi to bind to metal like lead which was analyzed using atomic absorption spectrophotometer. Thus we can conclude that dead cells can be preferred over live cells as it has advantages with regards to no toxicity, nutrient requirements and other maintenance conditions. Biosorption appears to be suitable as secondary or polishing application for metal removal from dilute waste streams, which would be competitive with ion-exchange resins based on cost-effectiveness.

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