Poll Res. 18 (3) : 211-216 (1999)

STUDIES ON LEAD SORPTION BY *FUSARIUM SOLANI*

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Key words : *Fusarium solani*, Biosorption, Lead, Regeneration, Storage.

ABSTRACT

The biomass of Fusarium solani (Mart.) Sacc. can effectively sorb lead from a solution of lead nitrate maximally within 5 seconds. Alkali treatment of mycelial biomass shows only a marginal increase in metal binding capacity, while homogenisation of mycelium results in an increase in sorption of lead and the biomass can be effectively regenerated by treating with 0.1M $CaCl_2$ and $MgSO_4$ solution, for recycling of the mycelia. Storage of mycelia is not deleterious to lead sorption; mycelia stored for a week can be effectively used for sorption of lead even after 5 cycles of regeneration tested.

INTRODUCTION

Metallic elements are indispensable for cellular growth and for the maintenance of metabolic functions. In trace amounts, they constitute an essential requirement for almost all living organisms, as metal-dependent processes play a determinant role in normal cellular functioning. Metals, when available in either high or very low quantities, exert an inhibitory influence on all life forms and may also arrest cellular multiplication. Owing to acid rain and industrial wastes, high levels of bioavailable forms of metals have become a major concern. This situation has further accentuated the already alarming concentration of fuel combustion-induced lead in the biosphere (Ala Al-Aoukatty *et al.*, 1991).

Metals like lead may mediate their toxic properties by displacing native metals from the normal binding sites, inducing conformational changes in proteins and nucleic acids, and by disturbing membrane permeability (Ala Al-Aoukatty *et al.*, 1991). In humans, lead tends to accumulate in the tissues of man and other animals. It results in irreversible damage of the brain in children. The major toxic effects of lead include anemia, neurological dysfunction and renal impairement. High level of exposure produces severe neurologic damage, often manifested by encephalopathy and convulsions. Such cases frequently are fatal (Flanagan *et al.*, 1995; Plunkett, 1987; Fleming *et al.*, 1997; Loomis and Hayes, 1996; Wohl *et al.*, 1996; Goyer, 1996).

Micro-organisms have a pronounced ability to bind and accumulate a variety of metal ions and may be used to remediate waters contaminated with heavy metals. Sorption and/or complexation of dissolved metals based on the chemical activity of microbial biomass, known as bio-sorption, is at the base of the recent biosorption technology for metal removal and recovery (Volesky, 1994).

Fungal cultures are known to show higher tolerance towards heavy metals (Paknikar *et al.*, 1993), and hence are potential candidates for efficient metal sorption.

The fungal isolate *Fusarium solani* (Nazareth and Mavinkurve, 1987) was found to be capable of sorbing various metals. The experimental data presented, indicates the potential of the fungal biomass of Fusarium solani to sorb lead from aqueous solutions.

MATERIALS AND METHODS

Fungal strain and culture conditions

The culture *Fusarium solani* (Mart) Sacc. was maintained on Sucrose nutrient agar (SNA) and Potato dextrose agar (PDA) slants. SNA contained (l^{-1}), 1g KH_2PO_4, 1g KNO_3, 0.5 g $MgSO_4$, $7H_2O$, 0.5 g KCl, 0.2

g glucose, 0.2 g sucrose and 20 g agar. The culture grown in potato dextrose broth was used for preparation of spore suspension.

Growth of the culture was obtained in Mineral salts medium containing (l^{-1}) 0.01 g $FeSO_4$, 0.2 g $MgSO_4$. $7H_2O$, 0.1 g $CaCl_2.2H_2O$, 0.05 g NaCl, 0.7 g KH_2PO_4, 0.1 g Na_2HPO_4. 0.5 g $(NH_4)_2SO_4$ and 1 ml of micronutrient solution [(%) : 0.7 mg $CuSO_4$, 0.5 mg H_3BO_3, 5 mg $CoSO_4$, 0.2 g Na_2MoO_4, 0.5 g $MnSO_4$, H_2O, 1.1 g $ZnSO_4$] supplemented with malt extract (0.05%) and glucose (0.5%), designated as MMG, incubated at room temperature on a rotary shaker at 150 rpm for 2d.

Preparation of Biosorbent

The mycelia were harvested by filteration through a double layer of Muslin cloth. This biomass, designated as Fusorb, was washed with sterile saline and then used as the biosorbent to study its sorptive capacity.

Lead sorption and metalion estimation

Fusorb was added to 5 m M $PbNO_3$ solution (5% pcv/v) and incubated on a rotary shaker at 150 rpm for 1 min. Aliquotes were withdrawn every 5 sec, filtered, and the residual lead in the filterate was estimated colorimetrically (Vogel, 1978), and percent sorption calculated.

Mycelium treatment for lead sorption

Alkali Treatment : The washed biomass was boiled with 5% KOH (w/v) for 5 min. The contents were then filtered and the mycelial mass washed with distilled water till neutral.

Homogenization : The biomass was ground in a mortar and pestle for 5 min, till it formed a smooth paste.

Dessication : The washed biomass was air-dried at room temperature (RT) for 1 h and then ground.

Heat Treatment : The mycelial mass was subjected to dry heat at 100°C for 30 min.

Regeneration of Fusorb

The mycelial mass (5% pcv/v) was first allowed to sorb Pb^{2+} from the lead solution for 5 mins on a rotary shaker at 150 rpm. The bound metal was desorbed with 0.1 N HCL on the rotary shaker, for 15 mins. The biomass was filtered and washed with distilled water till neutral. This biomass was then treated with;

(*i*) 5% KOH (w/v), (a) at room temperature for 30 mins, (b) boiling water bath, 15 mins. A control was maintained without KOH treatment.

(*ii*) $CaCl_2$ and $MgSO_4$ (0.1 M each) for 15 mins on a rotary shaker at 150 rpm. The Fusorb was filtered, washed till neutral and tested for Pb^{2+} sorption. This was repeated for 5 cycles.

Effect of storage of mycelium on sorption and regeneration

Alkali treated biomass was stored at room temperature (28-30°C) and at refrigeration temperature (15°C). Biomass was tested for lead sorption and effectivity of regeneration by treatment of mycelia with 0.1M solution of $CaCl_2$ and $MgSO_4$ on 3rd, 6th, 9th 12th and 15th day of storage.

RESULTS

Metal sorption capacity of Fusorb

The biomass of *Fusarium solani* is seen to sorb Pb^{2+} ions maximally within 5 sec (Fig. 1). The sorption capacity is seen to vary with the different mycelial treatments as seen. The metal binding capacity shows a marginal increase with alkali treatment of the biosorbent, (A), but is enhanced to more then double, by homogenization of both untreated and alkali treated mycelium (B). Dessication by air drying of the mycelium increases the metal binding potential to a

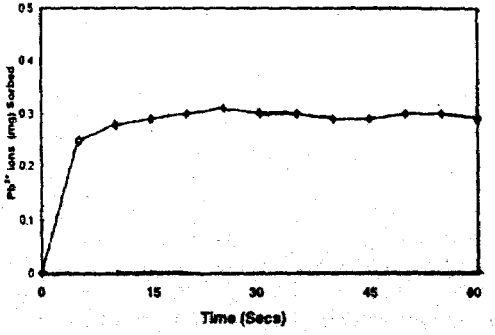

Fig 1. Sorption of Pb^{2+} ions by Fusorb from $PbNO_3$ solution.

lesser degree than homogenization (C), while a dry heat treatment causes loss of almost all capacity to sorb metal (D).

Regeneration of Fusorb

Initial alkali treatment of mycelium shows a marginal increase as indicated. Re-treatment with alkali, of the mycelia after desorption of lead, had little or no effect on the sorptive capacity.

In contrast, treatment of desorbed mycelium with 0.1M Ca^{2+} and Mg^{2+} ions, restores lead sorbing capacity of mycelium to almost 100% for the 5 regeneration cycles tested.

Effect of storage of Fusorb on sorption and regeneration

The capacity of Pb^2 ion sorption from $PbNO_3$ solution by mycelia was lowered by the less than 5%

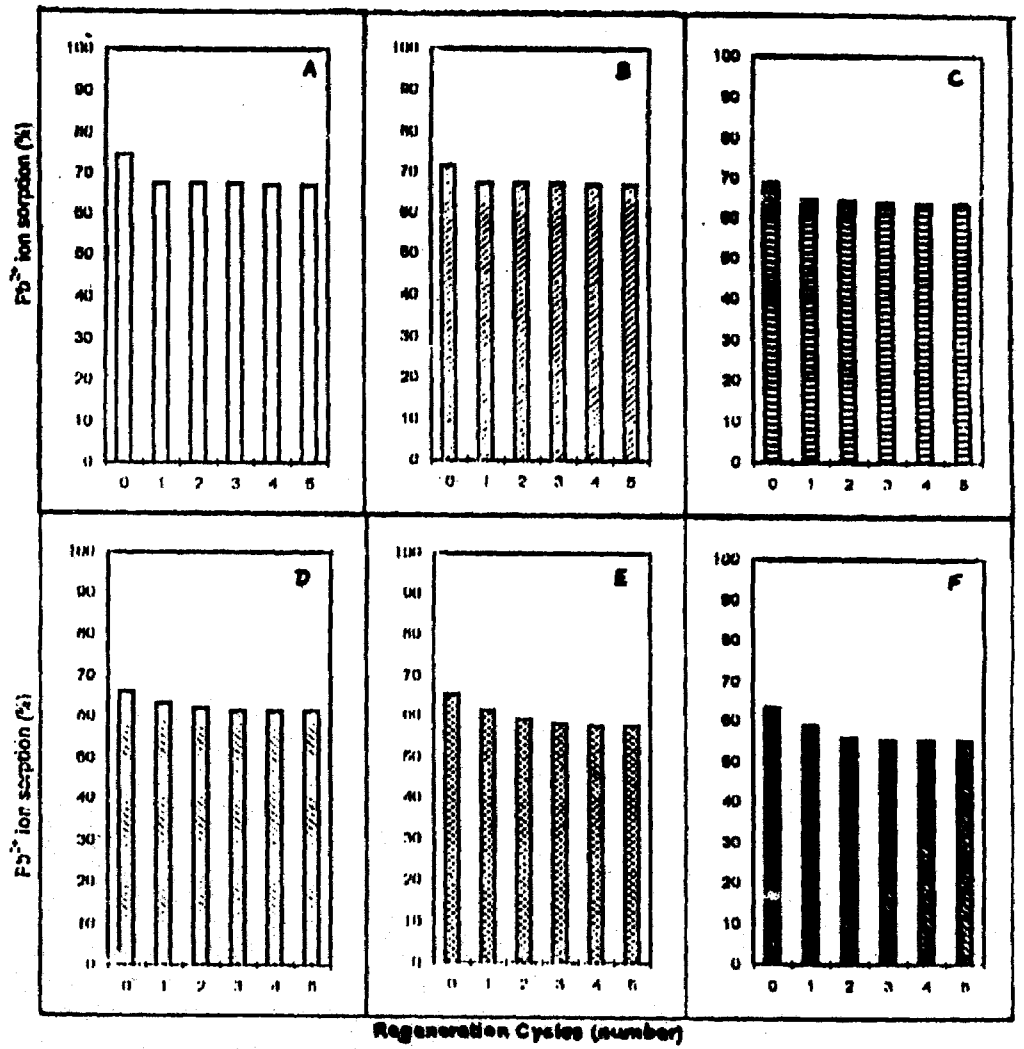

Fig 6. Effect of storage of biomass at room temperature, A : 0d, B : 3d, C : 6d, D : 9d, E : 12d, F : 15d on regeneration of Fusorb for sorption of lead.

when stored at refrigeration and at RT upto 6d, and by barely 10% upto 15d. Lead ion sorption by the stored biomass of both samples for 5 regeneration cycles was almost unchanged from the initial sorption capacity over the period 0 : 0 6d (Fig. 2, 3); the decrease in sorption capacity thereafter was 20-25%, this decrease being less at refrigeration temperature.

DISCUSSION

Micro-organisms respond to heavy metals in different ways depending on the nature of the microorganism and on the concentration of the heavy metal in the environment. They accumulate metals by a number of different processes such as uptake by transport,

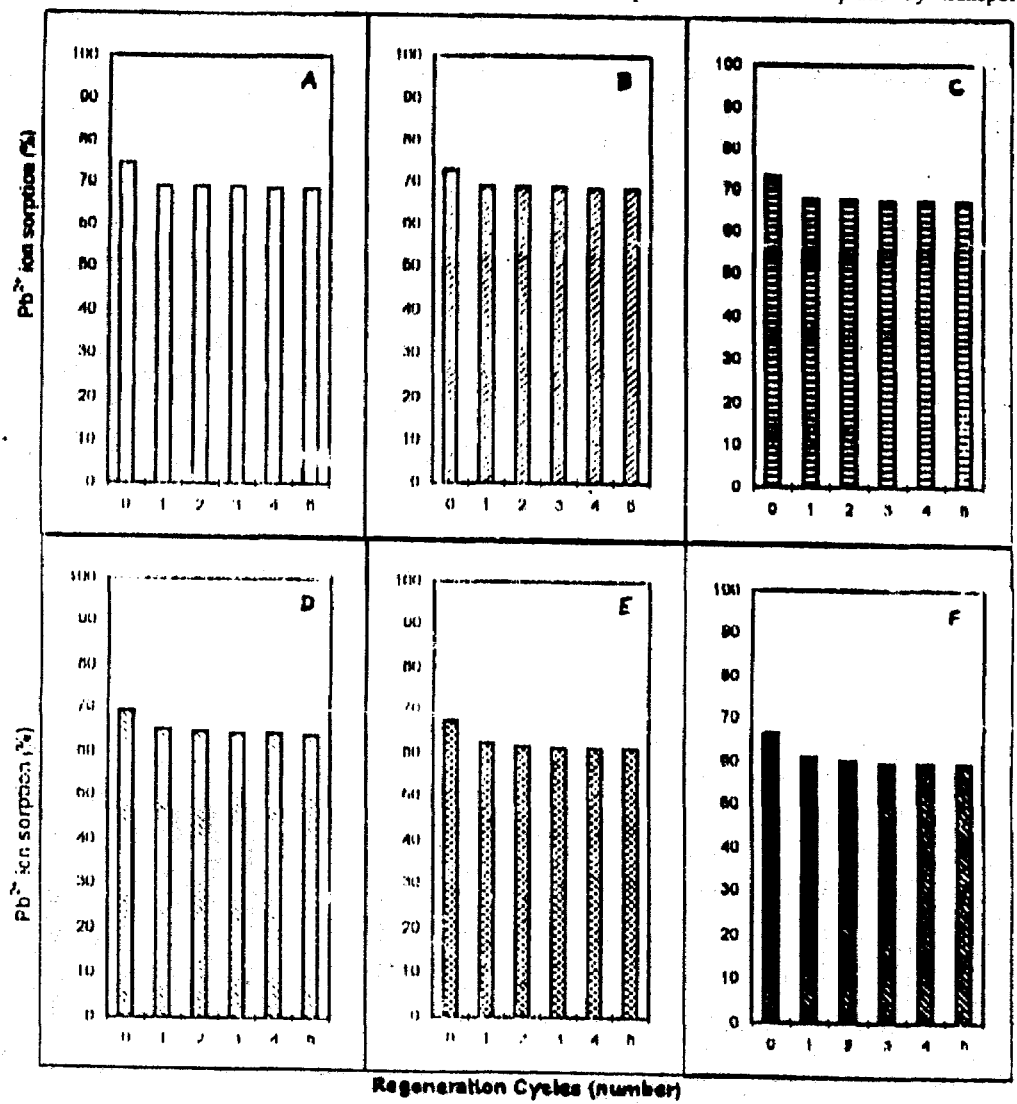

Fig 7. Effect of storage of biomass at refrigeration temperature, A : 0d, B : 3d, C : 6d, D : 9d, E : 12d, F: 15d on regeneration of Fusorb, for sorption of lead

biosorption by cell walls and entrapment in extracellular capsules, precipitation, and oxidation-reduction reactions (Venkateswerlu *et al.*, 1970; Subramanyam *et al.*, 1983; Schumate and Strandberg, 1985; Brady and Duncan, 1993; Naseem *et al.*, 1996;) The mycelial mass of *Fusarium solani* was tested for the sorption of Pb^{2+} ions from $PbNO_3$ solution, which resulted in maximum sorption within 5 sec. It may be therefore assumed that metal sorption by the mycelium is a physio-chemical process, that takes place fast. This ease of binding of the metal, depends on the atomic weight, ionic radius and the charge of the metal. The higher the charge, faster will be the binding due to a greater ionic attraction (Volesky , 1994).

Results indicate that alkali of the mycelium, exhibits only a marginal increase in the metal binding capacity. This is comparable to the results of Naseem *et al.* (1996.) Alkali treatment of mycelium results in the deacetylation of chitin of the cell wall to chitosan, which shows an enhanced binding ability in comparison to chitin. This is due to the fact that the major part of biofixation that takes place in the fungal cell wall, specifically resides on the amine functions within this constitutive polymer (Sanuedo *et al.*, 1993). However chitin or chitosan are not necessarily the sole agents for metal ion binding sites in fungal biomass (Naseem *et al* 1996). The complex cell wall, contains high content of functional groups like amino, amide, sulphydryl and phosphate in chitin, hydroxyl groups in polysaccharides, carboxyl groups in acidic residues and phenolic groups in pigments, which have been implicated in metal binding (Naseem and Maruthi Mohan, 1995).

Homogenization of the fungal biomass results in the increase in surface area and consequently the availability of binding sites and thereby enhances metal binding capacity.

Metal sorption by dessicated, pulverised mycelia was comparable with that by undessicated mycelia, and has the advantage that dry mycelial powder can be stored for longer period for use in Pb^{2+} sorption.

The effect of dry heat on mycelial biomass results in the loss of almost all capacity to sorb metal. From this it could be inferred that metal biosorption involves certain heat labile groups. From technological point of view the biosorbent developed should posses the advantage of ease of regeneration, and with near total regain of metal sorptive capacity, permitting reuse for effective sequestration of metal ions from dilute solutions (Nassem *et al.*, 1995).

Initial alkali treatment of biomass is seen to have a marginal increase in lead sorption as mentioned earlier. But retreatment of Fusorb with alkali, after desorption of metal, shows little effect on regeneration of sorptive capacity. Regeneration of Fusorb by treatment with Ca^{2+} and Mg^{2+} ions restores sorptive capacity, thereby ensuring retention of its sorptive capacity over multiple cycles.

This regeneration of Fusorb is an advantage in recycling of biomass. These results are comparable with that of Naseem *et al.* (1996). Biosorption of metals by fungal biomass is known to be an ion exchange mechanism, thereby indicating that a simple ion exchange process predominates, constituting a reversible replacement of protons on the amino acid groups of proteins and glyco-proteins within the fungal cell wall (Tantiwach *et al.*, 1993).

Storage of mycelial biomass shows a gradual acceptable decrease in sorption of Pb^{2+} ions from $PbNO_3$ solution even upto 15 days, the decrease being more by mycelia stored at RT. This could be due to the degeneration of certain cell wall metal binding components and functional groups, which could be faster at RT. However results show that mycelia stored for a week, can still be used for lead sorption. A further point of advantage is that regeneration capacity of the Fusorb for Pb^{2+} ions sorption was unaffected by storage.

Our results indicate the high potential for the use of mycelial mass of *Fusarium solani* designated Fusorb, for sorption of lead, in abatement of pollution.

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