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BIOSORPTION OF METALS BY *FUSARIUM SOLANI*

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Abstract—Micoroorganisms play an important role in biosorption of metals and in the abatement of metal pollution Sorption of metals by *Fusarium solani* is reported in this study.
Metal biosorption by the culture is shown to occur both during growth as well as by grown cells. The amount of metal sorbed increased with increasing concentration of the mycelial mass used. Most of the metal was removed immediately within 1-2 minutes after addition of the biomass, and the metal was found to be cell bound and also accumulated intracellularly. Alkali treatment of the mycelium increases the sorptive capacity.

INTRODUCTION

Man is becoming increasingly aware and concerned of the deleterious ecological effects of toxic heavy metals. Metallic pollutants released in the environment tend to remain indefinitely, circulating and eventually accumulating in the food chain (Portmann, 1980). All these metals eventually find their way into the marine environment. The problem of environmental pollution through industrial effluents and contaminated wastes has been well recognised and various methods for their treatment are being worked out.

Conventional methods for removal ot metals from aqueous solutions before they are disposed off , include chemical precipitation and sludge separation, chemical oxidation or reduction, ion exchange, reverse osmosis, electrochemical treatment and evaporation (Timmis *et al.*, 1994).

While the cheaper of these are becoming inadequate with progressively stringent regulatory limits. more effective methods are prohibitively costly. The use of micro-organisms and other bioadsorbents to remove metals is a potential alternative to the existing methods of metal detoxification from industrial and other effluents, and for the recovery of toxic and valuable metals (Shivaparvati et at, 1 989)

Microorganisms such as fungi, bacteria and algae are the commonly used agents of biosorption. The mechanisms of metal uptake include extracellular and intracellular accumulation, cell surface adsorption and extracellular precipitation. (Shumate and Strandberg, 1985).

The mechanisms responsible for biosorption (Voleski, 1994) include- (*a*) Van der Waal's forces wherein uncharged atoms or molecules are loosely bound in the matrix by electrostatic attraction, (*b*) ionic bonds between a metal cation, and an anionic reactive group of the biosorbent. (*c*) crystallisation of metals at the surface of the cell which is a slower process but one that often produces higher rate of enrichment, (*d*) electrostatic attraction or matrix entrapment, which can result in adsorption of precipitates at the cell envelopes.

All these biosorption methods have in common an independence of metabolic processes. Thus although biosorption does not require cell metabolism, it is possible that a cell mediated micro-environment such as change in pH, enhances the deposition of metals. The physiological conditions of the cell, the chemical state of the reactive sites and the metals are strongly influenced by this environment (Voleski, 1994).

MATERIALS AND METHODS

Organism and culture conditions

The culture *Fusarium solani* Mart Sacc, (Nazareth and Mavinkurve, 1987) was maintained and cultured routinely as described by Fernandes and Nazareth (1999).

Sorption during growth of culture

Five sets of four flasks each, containing MG

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medium and 3 mM $CuSO_4$. $5H_20$ or 5 mM $Pb(NO_3)_2$/ $ZnSO_4$/ $MnSO_4$. H_20/ $FeCl_3$ individually per set, were inoculated and incubated at room temperature for 2 days on a rotary shaker, at 150 rpm. One flask per set was withdrawn on each day from 0 to 3 days; the mycelial mass was filtered off and dried to constant weight by heating at 45°C. The metal remaining in solution was estimated (Vogel, 1978).

Sorption by grown cells

MG & SN grown mycelia were washed with 0.05 M Citrate Phosphate buffer, pH 5.0 centrifuged to obtain packed cell volume (pcv) and added to 5 mM metal solutions (2 : 100, pcv/v). The flasks were incubated on a shaker at 150 rpm for 30 min and the contents were filtered. The amount of metal in the filtrate was estimated as above. Metal controls were maintained.

Sorption with respect to mycelial mass

Increasing concentrations of mycelial mass, 0%, 1 %, 2.5%, 5%, 7.5%, 1 0% (pcv/v) were added to 5 mM $Pb(NO_3)_2$ solution, and incubated at 150 rpm for 30 min. The contents were then filtered and the amount of metal in the filtrate was estimated.

Rate of sorption

Mycelial biomass was incubated, with metals as above, and aliquotes removed at 0, 1, 2, 3, 4, 5, 10, 15, 20 and 25 minutes. The amount of metal in each sample was estimated.

Quantitation of sorbed metal

The mycelial biomass was incubated with metals as above. The mycelia were then filtered and treated with 0.1 N HCI to release the cell bound metal and the metal concentration estimated. The biomass was further acid digested with sulphuric acid, and the metal content of the digest was determined to obtain the amount of intracellular metal.

Effect of alkali treatment of mycelia on sorption

The MG grown mycelial mass was washed, then treated with 5% KOH at 100°C for 15'. After alkali treatment, the biomass was washed extensively with water to neutral pH. Amount of metal sorbed by

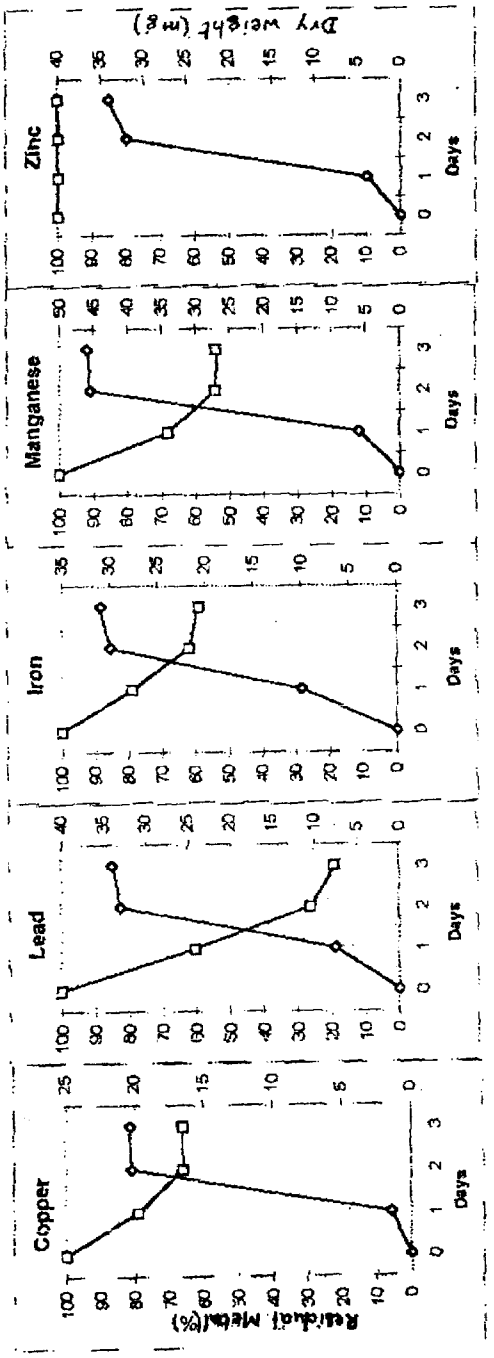

Fig 1. Sorption of metal (□) during growth of culture (◇)

mycelia, before and after alkali treatment, was determined as above.

RESULTS

Sorption during growth of culture

The sorption of metals during growth of culture is shown in Figure 1. It is seen that there is a decrease in metal salt concentation with increase in growth of the culture, with lead being sorbed the maximum followed by manganese, iron and copper. The only exception is zinc, where the concentration of metal in solution remains the same even after the maximum growth of the culture is attained.

Sorption by grown cells

1 ml pcv of MG grown myceli:a gave a sorption of 0.625 mmoles Cu^{+2}, 1.35 mmoles Pb^{+2}, 1.52 mmoles Fe^{+3} and 1.38 mmoles Mn, while sorption by SN grown cells was slightly lower at 0.615, 1.27, 1.405 and 1.33 mmoles for Cu^{+2}, Pb^{+2}, Fe^{+3} and Mn^{+2} respectively. Zn^{+2} was not sorbed at all by either MG or SN grown culture.

Sorption with respect to mycelial mass

The sorption of metals with respect to mycelial mass is shown in Fig 2. The amount of Pb^{+2} removed from solution increased with increase in concentration of mycelial biomass added; 5% (pcv/v) is the optimal amount of biomass required for 100 ml of 5mM metal salt solution.

Rate of sorption

As shown in Fig 3, 50% or more of the total metal sorbed, occurs in the first minute, the maximum being sorbed within 1, 2, 3, 4, minutes for Mn^{+2}, Fe^{+3}, Cu^{+2} and Pb^{+2} respectively.

Quantitation of metal uptake

Metal uptake by mycelial cells was seen to be both cell bound as well as intracellularly accumulated (fig 4). In case of Cu^{+2}, not much is sorbed as compared to Fe^{+3}, Pb^{+2} and Mn^{+2}. The proportion of cell bound to intracellular metal is almost equal for Cu^{+2} but for Fe^{+3} very little is accumulated intracellularly with most of the metal sorbed, remaining cell bound. The amount of cell bound Pb^{+2} and Mn^{+2} is 4 and 3 times respectively that of metal accumulated intracellularly.

Effect of alkali treatment of mycelia on sorption

As can be seen from fig 5, alkali treatment of the mycelia is seen to enhance metal uptake. Zn^{+2} in particular although unsorbed by mycelial cells, is seen to be taken up to some extent by alkali treated cells.

DISCUSSION

The use of biological materials for heavy metal

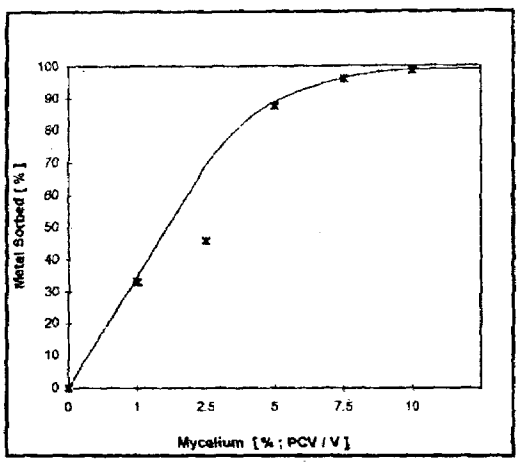

Fig 2. Sorption of lead with respect to mycelial mass.

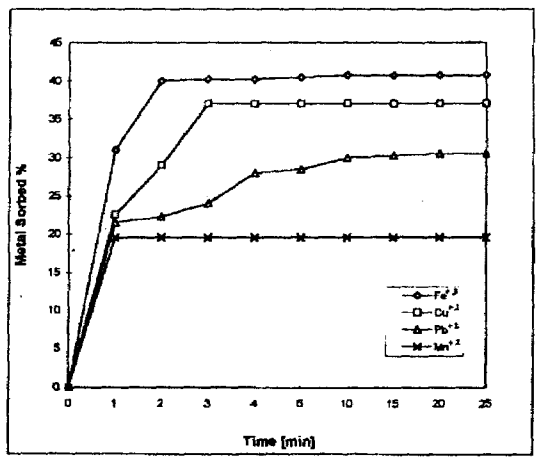

Fig 3. Rate of sorption of metals.

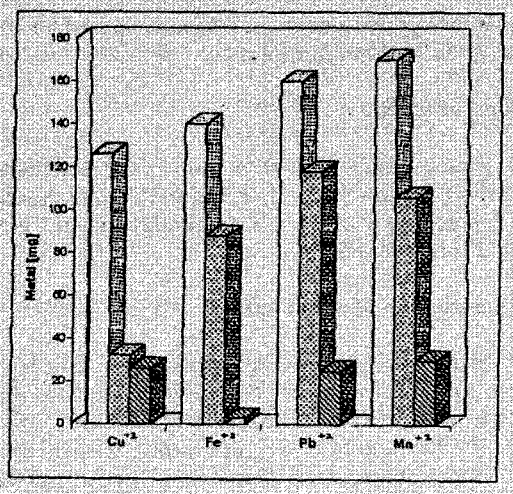

Fig 4. Uptake of metals, (□) initial concentration in solution; (▨) cell bound; (▦) intracellular.

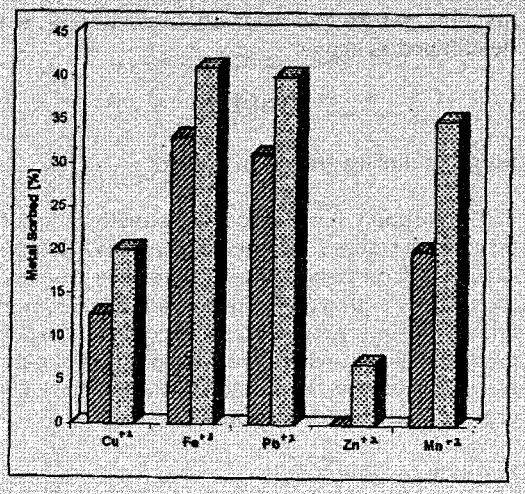

Fig 5. Sorption of metal by, (▨) untreated; and (▦) alkali treated mycelia.

removal and recovery has gained credibility in recent years, for which a number of algal, bacterial and fungal biosorbents have been studied.

Most of the studies on metal removal are done using grown mycelium. Moharl *et al.* (1 996) have used grown cells of *Neurospora erassa* for studies on uptake of Co^{+2}, Cd^{+2}, CU^{+2}, Ni^{+2} and Zn^{+2}. Fourest *et al.* (1994) have used dead biomasses of filamentous fungi for sequestering metal ions solutions. Whenever studies are carried out using growing cells, (Gadd, 1987) the very low concentration of metal tolerated during growth leads to the difficulty in calculating amount of metal sequestered. But in case of *Fusaritim solani*, its tolerance to metal salts is fairly high, and hence the amount of metal sorbed during growth could be determined. It has been shown to remove copper, iron, manganese, and lead during growth but is not able to remove zinc from the growth medium.

In addition to removal of metals from solution during growth, the grown cells can also sorb metals from solution. Especially where tolerance to metals during growth is fairly low, the grown cells can sorb the metals, such as copper, at a higher concentration. In such instances, it is advantageous to use grown cells for metal sorption.

The amount of metal sorbed by mycelia grown in MG with ammonium salts as nitrogen source, and in SN with nitrate as nitrogen source was almost equal, although tolerance to certain metals such as copper, manganese and zinc was decreased during growth in SN medium as compared to that in MG medium. This is in contrast to the results of Karna et al (l996), which indicate that *Neurospora crassa* was more efficient in Co^{+2} removal when cells were grown in nitrate containing medium than in ammonium salt containing media.

Sorption of metal was observed to be proportional to the amount of mycelial mass used. This is because increase in mycelial biomass increases the number of reactive sites for binding of metals. Thus if sorption is applied for scavenging of metal ions from aqueous environments, where the amount of metal ions generated periodically is known, then the amount of mycelium required can be standardised.

When the rate of sorption of mycelium was determined it was observed that the maximum sorption is achieved within one minute for all the metals. Therefore it can be assumed that metal sorption by the mycelium is a physical process, and takes place fast. This ease of binding of the metals, depends on the atomic weight, ionic radius and the charge of the metal- higher the charge, faster will be the binding due to a greater ionic attraction (Voleski, 1994).

Alkali treatment of the mycelia has an enhanced effect on metal uptake by the mycelium. This may be due to the effect of the OH^- groups on the mycelial mass which creates additional binding sites. The use of alkali treated cells has an additional advantage in

that alkali treatment causes cell death. The use of non viable cells for sorption therefore limits the introduction of viable microbial contamination. Also, toxicity of metallic contaminations and the highly variable conditions prevalent in many wastes, preclude the use of living organisms and necessitate the utilisation of non living systems for metal removal.

Inactive biomass, in general, is not selective in the metal sorbed, rather it simultaneously removes several different toxic and heavy metals from solutions regardless of their concentrations. (Mohan et al, 1995). The dead mass can also be regenerated and reused for sequestering more metal ions.

Biological material can thus be developed into a very good system for the removal of metallic pollutants. It can be very cost effective and seems to be a very promising field of research. It has been shown that *Fusarium solani* can be used as an efficient system for sequestering of metals from polluted environments.

ACKNOWLEDGEMENT

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