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Mechanism of metal ion biosorption by fungal biomass

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Alkali extracted mycelial biomass from *Aspergillus niger*, referred to as Biosorb, was found to sequester metal ions (Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+} and Co^{2+}) efficiently both from dilute and concentrated solutions upto 10% of its weight (w/w). Sequestration of metal ions from a mixture was also efficient but with attendant antagonisms. The kinetics of metal binding by Biosorb indicated that it is a rapid process and about 70–80% of the metal is removed from solution in 5 min followed by a slower rate. The mechanism of metal binding is shown to be due to exchange of calcium and magnesium ions of the Biosorb during which equimolar concentrations of both the ions were released into the medium. Following this an efficient procedure for the regeneration and reuse of Biosorb was standardized by washing the biosorbent with calcium and magnesium solution (0.1 M). Biosorbents prepared from *Neurospora*, *Fusarium* and *Penicillium* also exhibited similar mechanisms for metal ion binding, though they had a lower metal binding capacity when compared with Biosorb. Chemical modification of carboxylic acid functional groups of the Biosorb resulted in loss of 90% of metal binding capacity which could not be restored even on regeneration. The significance of this finding on the metal sequestration mechanisms of microbial biosorbents is discussed.

Keywords: biosorption, decontamination, cadmium, calcium, magnesium

Introduction

Microorganisms have a pronounced ability to bind and accumulate a variety of metal ions (Venkateswerlu & Sivarama Sastry 1979, Subramanyam *et al.* 1983, Maruthi Mohan & Sivarama Sastry 1983, 1984, Maruthi Mohan *et al.* 1984, Kumar *et al.* 1992). Some investigators have examined the utility of dried, non-living microorganisms for the removal of metal ions from aqueous solutions (Shumate *et al.* 1978, 1981, Tsezos & Volesky 1981, Nakajima *et al.* 1982, Galun *et al.* 1983a, Volesky 1987, Kuyucak & Voleskey 1989a). Microbial biomass has been sought to be used in its 'natural state' or modified, e.g. by alkali treatment, to improve biosorption capacity (Gadd 1988, Brierley 1990). Although the chitin and chitosan of fungi have been considered as likely metal sequestering components of fungal cell walls (Muzzarelli *et al.* 1980), many other structural components have been implicated as well (Beveridge & Murray 1980, Muraleedharan & Venkobachar 1990).

In the context of the perception that cheap and efficient means of removal of metal ions from waters polluted by the discharge of industrial effluents is an urgent necessity, increasing emphasis is being laid on the harnessing of microbial biomass to this end. However, in most cases, no selectivity or even marked specificity for certain toxic ions is discernible especially during removal of metal ions from dilute solutions; neither are the mechanisms of metal ion sorption unequivocally established (Beveridge 1978, Doyle *et al.* 1980, Tsezos & Volesky 1981, Kurek *et al.* 1982, Galun *et al.* 1983a,b, Tobin *et al.* 1984, Azab *et al.* 1990).

In the present study, we examine divalent metal ion binding characteristics of fungal biosorbents, extracted and processed from *Neurospora crassa*, *Fusarium oxysporum*, *Penicillium* spp. and *Aspergillus niger*. Also, it will be shown that the mechanism of metal binding by the biosorbent derived from *A. niger* is, primarily, by exchange of divalent ions with resident Ca^{2+} and Mg^{2+} of mycelial biomass. Examination of IR spectra and metal sorptive ability following chemical modification of the biosorbent with a water soluble carbodiimide and ethylene diamine has, for the first time, established carboxylic groupings as the major determinants of metal binding by processed biomass.

From a technological point of view, the biosorbent developed herein will be shown to possess the advantage of ease of regeneration (with Ca^{2+}/Mg^{2+} solutions) and with near total regain of metal sorptive capacity permitting reuse for effective sequestration of metal ions from dilute solutions.

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Materials and methods

Fungal strains and media

N. crassa (FGSC # 4200), *F. oxysporum* (FGSC # 6710) obtained from Fungal Genetics Stock Centre (Kansas City, KS), a *Penicillium* strain isolated from soil, and a lab isolate of *A. niger* were grown and maintained on a basal medium containing (g 100 ml^{-1}): glucose, 5; ammonium nitrate, 0.2; ammonium tartrate, 0.1; KH_2PO_4, 0.3; NaCl, 0.01; $CaCl_2$, 0.01; $MgSO_4$, 0.05. Trace elements included were (μg 100 ml^{-1}): Zn, 20; Mn, 20; Fe, 10; Cu, 8; Mo 2. Biotin was added to a final concentration of 0.5 μg 100 ml^{-1}. The organisms were maintained by weekly subcultures on 2% agar slants, and generally grown for 72 h at 30°C in 1 l flasks containing 500 ml medium at 100 r.p.m. in a Labline Environmental shaker. The growth conditions were essentially the same as described by Kumar *et al.* (1992).

Preparation of biosorbents

The mycelia were harvested by filtration through cheese cloth, washed with distilled water and processed by treatment with 5% KOH at 100°C for 15 min. After alkali treatment, the biomass was washed extensively with water to neutral pH and stored in 0.1% sodium azide at 4°C until further use. Typically 5 g fresh weight of biosorbents was found to yield about 1 g dry weight.

Metal ion analysis

Biomass was acid digested (Sivarama Sastry *et al.* 1962), and its Ca^{2+} and Mg^{2+} contents were determined by atomic absorption spectrophotometry (AAS; Perkin Elmer Model 2380). In sorption experiments, the bound metal ions of biosorbents were in most cases eluted (desorbed) with 0.1 N HCl and their concentrations determined by AAS. In all cases, metal ion uptake was invariably examined with solutions made up in distilled water and at 28°C. Metal ion uptake was quantitated either by desorption of metal ions following termination of uptake or by analysis of a centrifuged supernatant free of biosorbent. The mass balance, compared with the original metal ions in the loading solution, was closed in all experiments. Sodium and potassium contents were analyzed by flame photometry in acid digests (or eluates) as required.

Determination of sorptive characteristics of biosorbents

To determine the sorptive characteristics of different biosorbents, the procedures described below were adopted and metal uptake invariably expressed on the basis of either fresh or dry weight as indicated.

The biosorbents (2 g each derived from *A. niger*, *N. crassa*, *Fusarium* and *Penicillium*) were suspended in 50 mM solutions of Cu^{2+} with constant shaking at 100 r.p.m. at room temperature. After 1 h of incubation, the leftover Cu^{2+} and the released Ca^{2+} and Mg^{2+} were analyzed by AAS.

In the experiments concerned with the kinetics of metal ion uptake by the *A. niger* biosorbent designated herein as Biosorb; 2 g Biosorb was suspended in a 100 ml conical flask containing 25 ml of the chosen metal ion solution (10 mM). The contents of the flask were stirred at 100 r.p.m. to ensure equilibration, aliquots removed at different time intervals and the metal ion content remaining was analyzed by AAS.

In some experiments Biosorb (2 g) was tied into a cheese cloth bag and dipped into a beaker containing 500 ml of 10 μg Cd^{2+} ml^{-1} solution. The solution was magnetically stirred and aliquots were removed at different intervals of time to check for the removal of Cd^{2+} by Biosorb.

Regeneration of Biosorb

To regenerate Biosorb, following metal ion sequestration, several protocols were examined. The first of these involved allowing Biosorb (2 g) to adsorb Cd^{2+} from 25 ml solution (10 mM) for 30 min. The bound metal ion was desorbed with 0.1 N HCl, and the Biosorb washed again and resuspended in 5% KOH for 30 min at room temperature. Biosorb was then washed to neutrality and Cd^{2+} binding ability was re-examined. This procedure was repeated for five cycles.

Following the finding that metal ion sorption by Biosorb was by exchange of Ca^{2+} and Mg^{2+} of the sorbent (see Results) a Ca^{2+}/Mg^{2+}-based regeneration procedure was evolved. In the second method, which was finally adopted, Biosorb (2 g) was allowed to bind Cd^{2+} from 25 ml solution (10 mM) for 30 min. The metal ion was then desorbed with 0.1 N HCl at room temperature and the Biosorb washed exhaustively with deionized water. It was then resuspended in a solution containing 0.1 M $CaCl_2$ and 0.1 M $MgSO_4$ (100 ml, 15 min), the Biosorb was filtered, washed thoroughly with water and its ability to bind Cd^{2+} examined again. This cycle was also repeated five times.

Identification of carboxyl groups involved in metal sorption

As metal ion binding was found to be due to the exchange of resident Ca^{2+} and Mg^{2+} of the biosorbent, the groups involved were considered to be, most likely, carboxyls. Thus an experiment was designed to modify the carboxylic group(s) with a water soluble carbodiimide and ethylene diamine as described by Beveridge & Murray (1980). An aqueous suspension of 1 g Biosorb was taken in 10 ml containing 0.5 M ethylenediamine and 0.2 M 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (Sigma, Chemical Co., St. Louis). The reaction mixture was continuously stirred for 6 h at 22°C at a pH of 4.75. After the reaction, Biosorb was washed extensively with water and then FTIR spectra were recorded to determine the extent of -COOH modification. Binding capacity for Cu^{2+} after such modification was subsequently analyzed.

Results

Metal sorption capacity of Biosorb

The potential sorption capacity of Biosorb was first studied by allowing Biosorb (2 g) to take up metal ions from 50 mM solutions of Cu^{2+}, Cd^{2+}, Co^{2+}, Zn^{2+} and Ni^{2+} for 1 h. The sorption capacity of Biosorb is seen to be as follows (mg g^{-1} dry weight of Biosorb): Cu^{2+}, 108; Cd^{2+}, 93; Zn^{2+}, 67; Co^{2+}, 53; and Ni^{2+}, 41 (Table 1). The most striking feature that emerges from the data (Table 1) is that Biosorb efficiently sequesters the highly toxic cations of cadmium and copper to the extent of 9 and 11%, respectively (on a dry weight basis). When such sorption is effected from a *mixture* containing a plurality of the ions (from 25 mM solution), the binding characteristics for different ions is seen to be in the order Cu > Zn > Cd > Co = Ni. In the latter kind of experiment, it may be noted that, although binding of individual ions is somewhat lower (20–30% of that from a solution containing each ion separately), the overall uptake of *total* metal ions comes out to be 11% (on dry weight basis); diminished binding is a consequence of ion antagonism during sequestration.

Table 1. Metal binding characteristics of Biosorb

	Metal ion bound (mg g^{-1} dry weight)	
Metal ion	individual metal ion[a]	from mixture[b]
Copper	108.3	38.35
Cadmium	92.8	18.10
Zinc	67.0	20.60
Cobalt	53.4	17.50
Nickel	41.0	17.60

Biosorb (2 g) was suspended in [a] solutions of individual metal ions (20 ml of 50 mM) or [b] in a mixture (20 ml) containing all five ions at individual concentrations of 25 mM. After incubation for 1 h with shaking (100 r.p.m.) at 28 C, the Biosorb was washed with distilled water and the bound metals were eluted with 20 ml of 0.1 N HCl for 5 min. Metal ion concentrations were determined by AAS. The values shown are averages ($n = 3$) with SD between 5 and 10%.

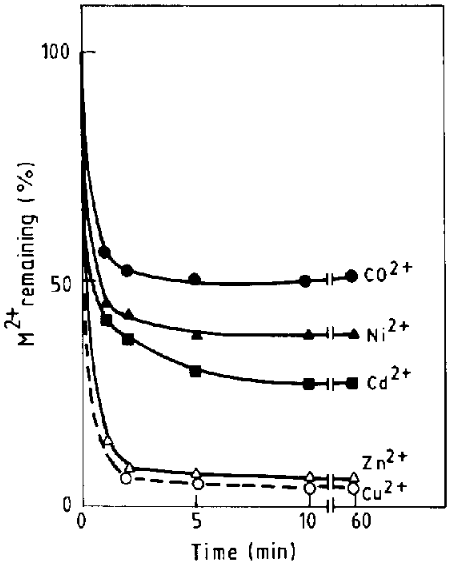

Figure 1. Kinetics of metal ion adsorption by Biosorb. Biosorb (2 g fresh weight) was suspended (with shaking, 100 r.p.m.) in 25 ml of 10 mM solution of each metal ion in separate flasks. Aliquots were withdrawn at different time intervals and metal ions leftover determined by AAS. The individual values represent means derived from three separate experiments, each involving duplicate samples (SD between 5 and 10%).

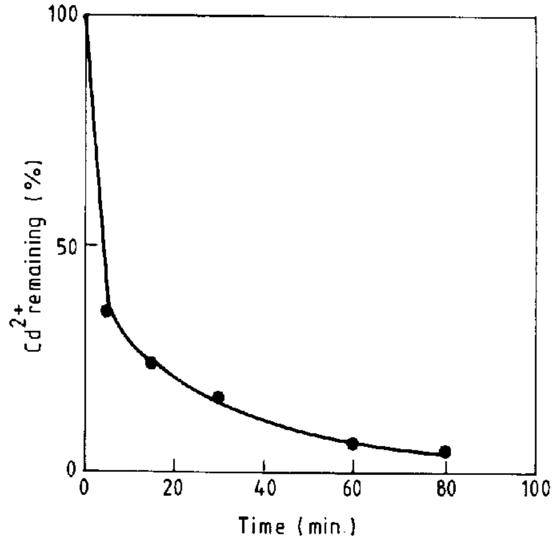

Figure 2. Concentration of cadmium from dilute solution by Biosorb. Biosorb (2 g fresh weight) was tied in a cheese cloth bag, dipped in a beaker containing 500 ml of 10 μg ml^{-1} Cd^{2+} and the contents were magnetically stirred. Aliquots were withdrawn at different time intervals to check for the removal of cadmium by Biosorb. The individual values represent means derived from three separate experiments, each involving duplicate samples (SD between 5 and 10%).

Metal sequestration by Biosorb

To compare the kinetics of sorption of different metal ions, Biosorb (2 g) was suspended separately in 10 mM metal ion solutions and ion sequestration was followed as a function of time. The results (Figure 1) show that the kinetics of metal uptake are essentially similar in all cases and that at these concentrations, maximal uptake is attained around 5 min. Cu^{2+} and Zn^{2+} were most efficiently removed (upto 90%) while 50–70% removal was obtained for Cd^{2+}, Co^{2+} and Ni^{2+}. The order of efficiency for metals was again as indicated earlier.

One prime requirement in a biosorbent is that it should possess the ability to bind and remove metal ions effectively from a dilute environment. This aspect was therefore examined by a different procedure. Biosorb (2 g) was placed in a cheese cloth bag and dipped into 500 ml Cd^{2+} solution (10 μg ml^{-1}), kept stirred as indicated and the time course of Cd^{2+} uptake was followed. The data, graphically depicted in Figure 2, demonstrate that Biosorb can effectively sequester upto 60% Cd^{2+} in the first 5 min; this is followed by a phase of slower sorption which eventually goes upto more than 90% by 60 min.

Mechanism of metal ion sorption

During the course of studies conducted herein, it was noted that binding of metal ions to Biosorb resulted in release of Ca^{2-} and Mg^{2+} into the medium, suggesting the operation of an exchange mechanism. Consequently, Biosorb (2 g) was suspended in 50 mM Cd^{2+} (20 ml). Binding of Cd^{2+} as well as the concomitant release of Ca^{2+} and Mg^{2+} was studied. The results (Figure 3) show that the binding of Cd^{2+} by Biosorb at various time points is accompanied by a stoichiometric release of Ca^{2+} plus Mg^{2+} ions at each time point of Cd^{2+} sorption.

Similar data were obtained with other fungal biosorbents, for Cu^{2+} binding, and this is also accompanied by an equimolar release of Ca^{2+} and Mg^{2+} put together (Table 2).

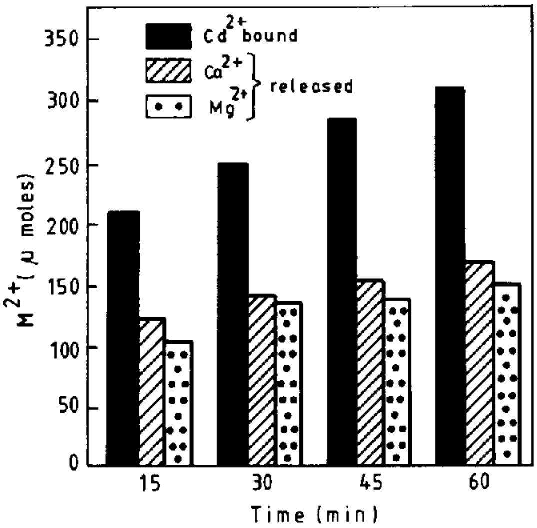

Figure 3. Release of Ca^{2+} and Mg^{2+} following Cd^{2+} binding by Biosorb. Biosorb (2 g fresh weight) was suspended (with shaking, 100 r.p.m.) in 20 ml Cd^{2+} solution (1 mmol) and incubated at 28°C. Aliquots were withdrawn at different time intervals and unbound Cd^{2+} remaining as well as Ca^{2+} and Mg^{2+} release were analyzed by AAS. The individual values represent means derived from two separate experiments, each involving duplicate samples (SD between 5 and 10%).

Table 2. Copper binding and release of Ca^{2+} and Mg^{2+} by biosorbents

Organism	μmol of Cu^{2+} bound g^{-1} fresh weight biosorbents	μmol released		
		Ca^{2+}	Mg^{2+}	$(Ca^{2+}+Mg^{2+})$
Aspergillus	308.7	146.0	155.8	301.8
Pencillium	235.7	82.6	163.4	246.0
Fusarium	154.1	42.8	90.4	133.2
Neurospora	133.0	77.5	58.0	135.5

Biosorbents (2 g fresh weight) were suspended in 20 ml of 50 mM Cu^{2+} solution and incubated at 28°C with shaking at 100 r.p.m. for 60 min. Cu^{2+} bound and ions released were analyzed by AAS. The values shown are averages ($n=3$) with SD between 5 and 10%.

In further studies, Biosorb (1 g) was either acid digested or extracted with 0.1 N HCl, and contents of Ca^{2+}, Mg^{2+}, Na^{+} and K^{+} were quantitated in digests and eluates. The results obtained are shown in Table 3. They indicate that Biosorb contains substantial amounts of Ca^{2+} and Mg^{2+}, and somewhat lesser quantities of Na^{+} but K^{+} was not detected. Furthermore, all metal ions present in Biosorb are totally acid extractable and their content is fully adequate to account for an exchange mechanism of metal ion sorption by Biosorb.

Regeneration of Biosorb

Since Biosorb was obtained by alkali treatment of fungal biomass, it was initially thought that regeneration could be

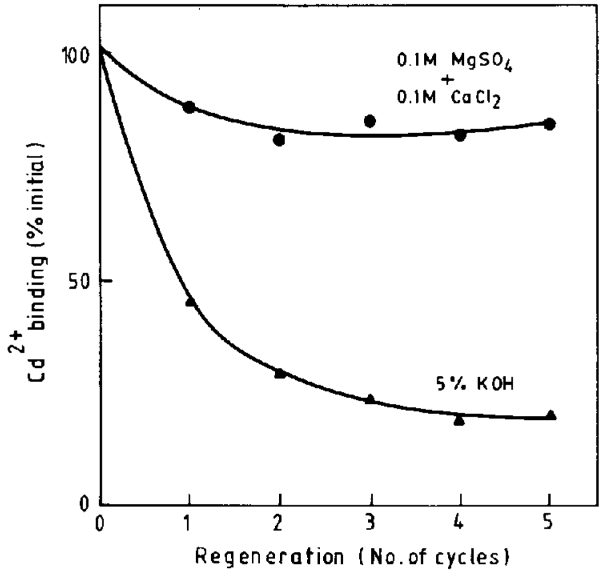

Figure 4. Regeneration of Biosorb. Following Cd^{2+} binding from 25 ml Cd^{2+} solution (10 mM) for 30 min. The bound ions were desorbed with 0.1 N HCl and reused after regeneration as indicated (see Materials and methods). Typical results are shown from at least three separate experiments (SD between 5 and 10%).

Table 3. Metal ion contents of Biosorb

Treatment of Biosorb	Metal ions present mg g^{-1} fresh weight of Biosorb			
	Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}
Acid elution[a]	9.95	4.34	1.20	ND
Acid digestion[b]	10.47	4.86		ND

ND, not detected.

[a] Biosorb (1 g) was treated with 10 ml of 0.1 N HCl and metal ions eluted were estimated.

[b] Biosorb (1 g) was digested with acid mixture (Sastry *et al.* 1962) and ions in the digest were estimated.

Ca^{2+} and Mg^{2+} were estimated by AAS and Na^{+} and K^{+} by flame photometry. The values shown are averages ($n=3$) with SD between 5 and 10%.

effected by alkali after stripping Biosorb of adsorbed metal ions with acid. However, after such a cycle, the regenerated sorbent had a significantly lower sorptive capacity.

Following the findings that metal ion sorption was by exchange of Ca^{2+} and Mg^{2+}, it was thought that regeneration could be achieved by simply resuspending Biosorb (denuded of its absorbed metal ions) in Ca^{2+}/Mg^{2+} solutions, so that its Ca^{2+}/Mg^{2+} contents could be restored. The optimal procedure found (described in Materials and methods) involves the use of 0.1 M $CaCl_2$ + 0.1 M $MgSO_4$ and was found to be remarkably efficient. The effect of several cycles of regeneration according to both procedures is represented in Figure 4. It was found subsequently that regeneration was repeatable over several cycles with retention of around 85% of the original sorptive capacity of Biosorb.

Effect of modification of carboxylic group of the Biosorb

A water soluble carbodiimide and ethylenediamine were utilized to modify carboxyl groupings of Biosorb. The reactions involved are indicated in Figure 5.

A comparison of the IR spectra of Biosorb before and after carbodiimide and ethylenediamine treatment (Figure 6) reveals the nature of the chemical modification(s) effected. The strongly enhanced absorption at 3400 cm^{-1} (-NH stretching) is a reflection of the replacement of carboxyl by amide (-CO-NH) groupings. The moderate intensity band at 1600 cm^{-1} is also, very likely, due to -NH deforming (due to reaction of ethylenediamine) or NH_3^+. In place of the carboxyl absorption (at 1661 cm^{-1}) there is a band in the region 1632 1647 cm^{-1} assignable to the carboxyl of the amide groupings.

Taken together, the spectral changes observed show that a major fraction of the carboxyl groups of Biosorb have been modified as per the reaction scheme of Figure 5. Thus,

Table 4. Effect of carboxyl group modification on biosorption

Treatment	Cu^{2+} binding (mg g^{-1} Biosorb)	% Control
Control	104.0	100
Carbodiimide[a]	11.5	11
Regenerated[b]	5	4.8

Biosorb (2 g), control and modified forms were suspended in 20 ml of 50 mM solutions of Cu^{2+} for 1 h as in Table 1.

[a] Carboxylic acid group modified (see Materials and methods).

[b] After one cycle of use of Biosorb (carbodiimide treated) it was regenerated by Ca^{2+} and Mg^{2+} and reused. The values shown are averages ($n = 3$) with SD between 5 and 10%.

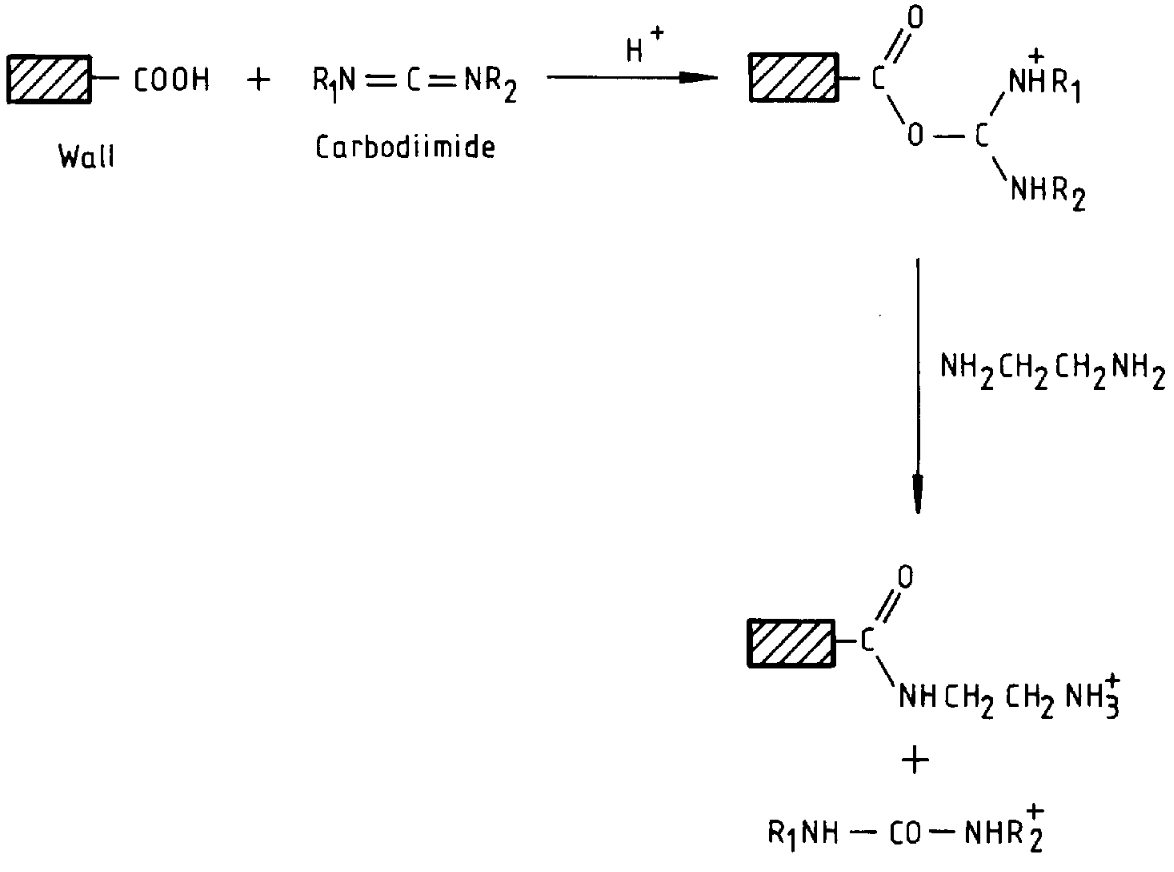

Figure 5. Schematic representation of modification of carboxylic groups by carbodiimide reaction.

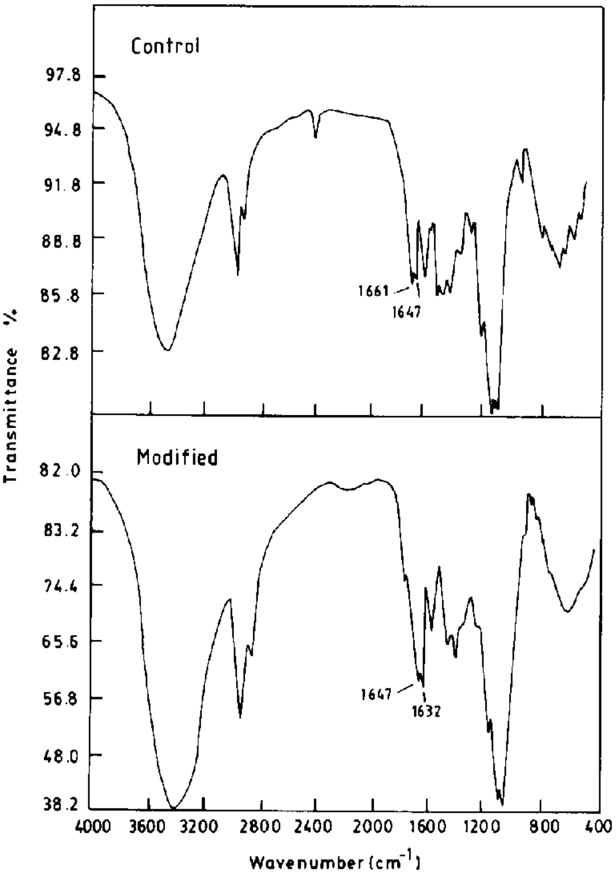

Figure 6. IR spectra of Biosorb.

the decrease in metal sorptive ability correlates quite satisfactorily with the elimination of carboxyl groupings as metal binding sites, following chemical modification (Table 4).

Discussion

Biosorption of metal ions is currently of great interest as a possibly convenient method for the scavenging of metal ions from aqueous environments ceaselessly being polluted today. As Volesky (1986) has emphasized, although the selective retentive ability of microbial biomass for metal ions in aqueous solution has been known for decades, the fact that dead microbial cells can do the same is a comparatively recent discovery. The use of killed cells also has the added advantage of not introducing viable microbial contamination.

In recent years a number of bacterial, algal and fungal biosorbents have been studied from the above standpoint. *Rhizopus arrhizus* has been employed to concentrate uranium from solutions at pH 4 (Tsezos & Volesky 1981). Zhou & Kiff (1991) have used *R. arrhizus* fungal biomass for copper sequestration at pH 7. Kuyucak & Volesky (1989b) have found that the seaweed *Ascophyllum nodosum* removes cobalt ions at pH 4.5. Indeed, a large number of such studies have been conducted at acidic pH. In most cases, although impressive metal ion removal has been reported, little selectivity for metals has been observed. Furthermore, the underlying mechanisms of metal sorption remain unclear.

The present study has explored the removal of the metal ions Cd^{2+}, Cu^{2+}, Co^{2+}, Ni^{2+} and Zn^{2+} from dilute as well as concentrated aqueous solutions by the sorbent elected, Biosorb. This sorbent, derived by alkali treatment of mycelia of *A. niger*, exhibits several novel features in metal sequestration at neutral pH, an important consideration in the removal of toxic ions from natural waters.

Firstly, at appropriate concentrations, Biosorb possesses the ability to accumulate upto around 9% of its dry weight of the extremely toxic Cd^{2+} and about 11% of Cu^{2+}. Although maximal affinity is found for Cu^{2+} and Cd^{2+}, Zn^{2+}, Co^{2+} and Ni^{2+} are also sequestered to fairly high levels. Consequently, in batch operation, Biosorb can efficiently abstract Cd^{2+} from even dilute solutions (10 p.p.m.). Metal sorption by Biosorb is in the order Cu > Cd > Zn > Co > Ni.

In this context, it is interesting that Falla & Block (1993) have found that the high-affinity binding sites of isolated envelopes of *Pseudomonas fluorescens* for divalent metal ions exhibit the order Zn > Cd > Ni > Cu.

Secondly, an interesting feature is a reciprocal antagonism between a mixture of different (like) metal ions displayed by Biosorb. Although not specifically examined herein, our results suggest that there are reciprocal antagonisms displayed for binding to Biosorb without affecting the total metal binding capacity. Such antagonisms between divalent metal ions are not uncommon during ion uptake by living microbial cells (Venkateswerlu & Sastry 1970, 1979, Maruthi Mohan *et al.* 1984, Maruthi Mohan & Sastry 1984, Srinivasa Rao *et al.* 1984) but in the case of the dead biomass herein, the competitive interaction assumes a special significance in that it is intimately associated with the mechanism of biosorption.

Binding of Cd^{2+} (as also Cu^{2+}) by Biosorb is associated with a stoichiometric release of Ca^{2+} plus Mg^{2+}. Analysis reveals that Biosorb has a significantly high content of these two ions. It is clear, therefore, that metal ion sequestration by Biosorb involves an exchange of *resident* Ca^{2+} plus Mg^{2+} for ions abstracted from solution.

To our knowledge this is the first example of such a straightforward ion exchange mechanism. Kuyucak & Volesky (1989b) had also noted a relationship between Co^{2+} uptake and Ca^{2+} release with the algal biomass of *A. nodosum*. However, this situation, though analogous to an extent, is quite different from that obtained with Biosorb for three reasons: firstly, it involves Co^{2+} and not Cd^{2+} or Cu^{2+}; secondly, uptake/release of Co^{2+} occurs optimally between pH 2 and 3; thirdly, Ca^{2+} and H^+ ions are both involved and, for reasons not clear, the relationship, in any case, has been stated not to hold for the first biosorption cycle.

In microorganisms, in general, and in fungi, in particular, the exact nature of the groups responsible for metal binding as well as the operative mechanism are unclear. The fungal cell wall is a complex structure (Tsezos 1983) containing chitin microfibrils embedded in an amorphous layer of proteins and polysaccharides, such as mannans, glucans and galactans. Of these, chitin has attracted much attention. The increase in metal sorptive capacity on alkali treatment of fungal biomass has been linked with the deacetylation of chitin to chitosan. Also, recently, Miyoshi *et al.* (1992) have shown that copper binding by chitosans is probably also related to their higher order structures since fungal chitosans bind copper better than chitosans from *crustaceans*. In this context, it should be remembered that alkali extraction of fungal biomass can cause additional changes such as protein removal, for example, whose effects on the 'higher order structure' of cell wall constituents are not well understood.

However, chitin/chitosan are not necessarily the sole candidates for metal ion binding sites in fungal biomass. The metal sorption capacity of *R. arrhizus* is considerably more than that explicable on the basis of its chitin content (Tsezos 1983). Also, on alkali extraction of the fungal biomass from *Ganoderma lucidium* (Muraleedharan & Venkobachar 1990), although the chitin content was found to have doubled, there was no parallel enhancement in its copper binding ability nor was copper sorption diminished on leaching out all its chitin.

Thus, it is necessary to examine the roles of functional groups of cell surface constituents other than chitin/chitosan to understand metal sorption characteristics of fungal derived biosorbents. Studies with *Bacillus subtilis* (Beveridge & Murray 1980) and *A. nodosum* suggest that carboxyl groups of cell surface polymers may be important in metal binding. In *A. nodosum* wherein also (as already mentioned) a calcium–cobalt exchange takes place during cobalt binding, the carboxyl groups, especially of mannuronic acid residues, are thought to be involved in metal binding; in *B. subtilis* as well the carboxyl groups of glutamic acid residues of cell wall peptidoglycan have been implicated.

However, in none of these instances has an unequivocal identification of carboxylic groupings as the primary sites of metal binding been possible. In the present study, apart from the characteristics of metal sorption which strongly implicate carboxyls, definite evidence has been adduced to support this hypothesis.

Firstly, reaction with carbodiimide–ethylenediamine has been shown to alter carboxyl groupings as evidenced by changes in the IR spectral characteristics of chemically modified Biosorb.

Secondly, following such modification, the metal sorptive capacity is irretrievably lost; as expected, this cannot be restored by calcium and magnesium ion dependent regeneration.

Thus the data obtained are consistent with the dominant, if not nearly exclusive, role of cell surface carboxyl groups in metal ion binding by Biosorb. They also constitute the first example wherein a straightforward exchange with resident calcium and magnesium ions constitutes the sole mechanism of biosorption. Further studies (in progress) would be needed to pinpoint the exact nature of the molecules and the residues which contribute the carboxyl groups in question. In any case, it is clear that this feature of Biosorb is responsible for its efficient metal binding characteristics, as well as for the ease with which this biosorbent can be regenerated under mild conditions that ensure retention of its sorptive capacity over multiple cycles of use. Similar features of Biosorb for binding silver ions with some characteristic differences were also observed (unpublished).

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