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Biosorption of zinc by fungal mycelial wastes

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Summary. Waste mycelia from several industrial fermentation plants (*Aspergillus niger*, *Penicillium chrysogenum*, *Claviceps paspali*) were used as a biosorbent for zinc ions from aqueous environments, both batchwise as well as in a column mode. With all mycelia tested, biosorption per biomass dry weight was a function of pH (increasing with increasing pH between 1.0 and 9.0), biomass concentration (decreasing at high biomass concentrations) and the zinc concentration. Under optimized conditions, *A. niger* and *C. paspali* were superior to *P. chrysogenum*. Treatment of *A. niger* biomass with NaOH further increased its biosorbent capacity. Desorption of biosorbed zinc was achieved by elution with 0.1 M HCl, best results being obtained with NaOH-treated *A. niger*. Such treatment did not affect the capacity for biosorption in repeated experiments. NaOH-treated *A. niger* mycelia were also successfully used in removal of zinc from polluted waters in Austria, thereby showing that the simultaneous presence of other naturally occurring ions does not affect biosorption.

Introduction

Biosorption of heavy metals is receiving increasing attention as a potential method of decontaminating or recovering heavy metals from various environments (Bosecker 1986; Muzzarelli et al. 1980; Venkateswerlu and Stotzky 1989; Gadd and White 1989; Kuyucak and Volesky 1988; de Rome and Gadd 1987; Friis and Myers-Keith 1986; Tsezos and Volesky 1981, 1982; Schinner and Burgstaller 1989), hence being an alternative to existing technologies. Studies on the mechanism of removal of metal ions by microorganisms assessed that the cell wall is the primary site of metal ion accumulation (Zamani et al. 1985; Tobin et al. 1984). It has also been shown that the uptake of heavy metal cations is not mediated by metabolic processes, and can take place in dead as well as living cells. These findings offered the possibility of using dead waste mycelia for biosorption.

The biomass of filamentous fungi contains a relatively high percentage of cell wall material (Rosenberger 1975). Since most of the fungal biomass produced in industrial fermentations is currently disposed of either by landfilling or incineration, employment as a biosorbent would offer an attractive potential use of this waste material. Muzzarelli et al. (1980) and Tsezos and Volsesky (1981) have already reported success in this area.

In the present paper we report results from an investigation on the ability of three fungal mycelial wastes (citric-acid-producing *Aspergillus niger*, penicillin-producing *Penicillium chrysogenum*, and ergotamine-producing *Claviceps paspali*) to biosorb zinc. Zinc ions were chosen as a model cation for these investigations, since it is a major pollutant due to its occurrence as a waste from galvanizing processes and sewage sludge.

Materials and methods

Mycelial wastes. Waste mycelia from industrial plants producing citric acid with *A. niger*, penicillin with *P. chrysogenum* and ergotamine with *Claviceps paspali* were obtained from Lachema (Kacnejov, Plsen, Czechoslovakia) and Biochemie (Kundl, Tyrol, Austria). The material obtained was identical to that otherwise disposed of by the company. It was stored in sealed bags at room temperature until use.

Zinc biosorption in shake flasks. The ability of different fungal waste mycelia to remove zinc ions from aqueous solutions was tested as follows: 10–50 mg mycelial material was weighed into 100-ml erlenmeyer flasks. Aliquots (50 ml) of appropriately concentrated solutions of $ZnSO_4 \cdot 7\,H_2O$ in distilled water were added, mixed well by shaking, and the suspensions incubated on a rotary shaker at 90 rpm for 3 h at 30° C. The pH was adjusted to the desired value by adding the required amount of appropriately concentrated HCl at the beginning of the experiment, and then not further controlled.

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Determination of zinc removal. Removal of zinc ions from the aqueous phase was carried out as follows: the contents of one flask was filtered through G1 sintered funnels, previously cleaned by chromosulphuric acid treatment followed by extensive rinsing with double distilled water. The first 20 ml of filtrate were discarded, since they were usually somewhat turbid. To desorb zinc from the biosorbents, the columns, after loading with zinc as indicated in the Results, were eluted with appropriately diluted HCl (0.1–0.001 M), and 5-ml fractions were collected and analysed for their zinc content as described below.

Zinc analysis. Samples from the filtrates were appropriately diluted and subjected to analysis in a Perkin Elmer (Überlingen, FRG) 2380 atomic absorption spectrophotometer at 213.9 nm (slit, 0.7; hold mode; measuring time, 3 by 3 s; standards 0.1, 0.3, and 0.5 mg Zn^{2+}/l [$ZnSO_4 \cdot 7\,H_2O$ in 1% (w/v) HCl].

Zinc biosorption by the column method. Mycelial wastes (100–300 mg) were placed on a layer of glass wool in pasteur pipettes, and rinsed with distilled water. Aqueous solutions of zinc in appropriate concentrations were passed through the columns at flow rates of 10 ml/h. Fractions (10 ml) were collected and analysed for their zinc content. In order to find out the maximal biosorbent capacity under such conditions, the columns were equilibrated with zinc solution until the zinc concentration in the eluent equalled that of the original solution.

Alkali treatment of waste mycelia. This was carried out by boiling mycelia in 1 M NaOH at 120° C for 6 h, followed by filtration of the suspension and rinsing the debris with distilled water until the pH of the filtrate equalled that of the distilled water.

Results

Influence of mycelial density of the suspension on zinc biosorption

Preliminary experiments were carried out to assess the appropriate incubation time: 180 min was found to be sufficient for optimal and reproducible biosorption (data not given).

Three different types of industrial waste mycelia were examined with respect to their ability to remove zinc ions from aqueous solutions. The relationship between mycelial density, zinc concentration and zinc biosorption is given in Fig. 1a–c. Control experiments, either lacking biomass or zinc ions, were included. In these trials *C. paspali* mycelium was the best biosorbent for zinc ions, followed by *A. niger*, whereas *P. chrysogenum* was clearly inferior. With carefully optimized biomass concentrations, up to 65% zinc could be removed from the aqueous phase by biosorption. Maximal values for specific biosorbent capacities of the individual mycelia ranged from 0.5 g/g for *P. chrysogenum* up to 1.0 g/g for *C. paspali.* This means that under carefully optimized conditions, zinc may be removed from aqueous environments by using an equal weight of mycelium.

Influence of alkaline treatment of zinc biosorption by A. niger waste mycelia

Muzzarelli et al. (1980) reported a positive effect of NaOH treatment on the capacity of *A. niger* mycelia to biosorb various metal cations. As shown in Fig. 2, such a treatment also increased the capacity of the *A. niger* mycelium used in the present study. The effect was more pronounced at higher mycelial densities.

Influence of pH on zinc biosorption

Biosorption was also studied in relation to the pH of the aqueous phase: consistent results were obtained for all three types of mycelia, and an example is given for NaOH-treated *A. niger* mycelia in Fig. 3. Better biosorption was achieved at pH 4.0 > 3.0 > 2.0, and hardly any was apparent at pH 1.0 (not shown). Although also not shown, it was noted that raising the pH above 4.0 further increased the biosorbent capacity.

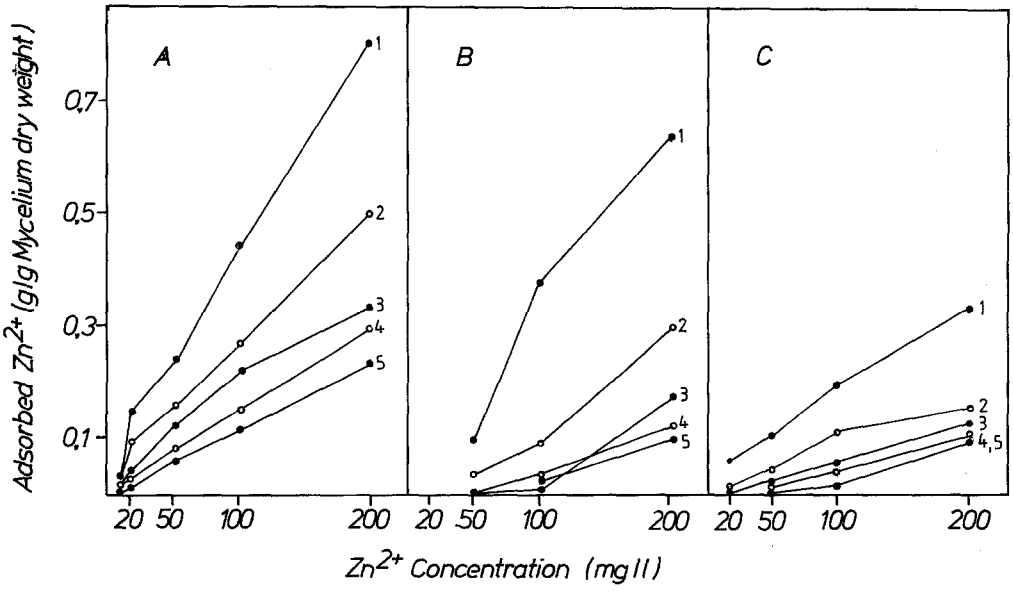

Fig. 1. Effect of zinc concentration and mycelial density on zinc biosorption by: **A**, *Claviceps paspali*; **B**, *Aspergillus niger*, and **C**, *Penicillium chrysogenum*. The pH was 4.0 for all experiments. Biomass concentrations were (mg/ml): *1*, 0.2; *2*, 0.4; *3*, 0.6; *4*, 0.8; *5*, 1.0. Results are shown from one of at least three experiments that yielded consistent results

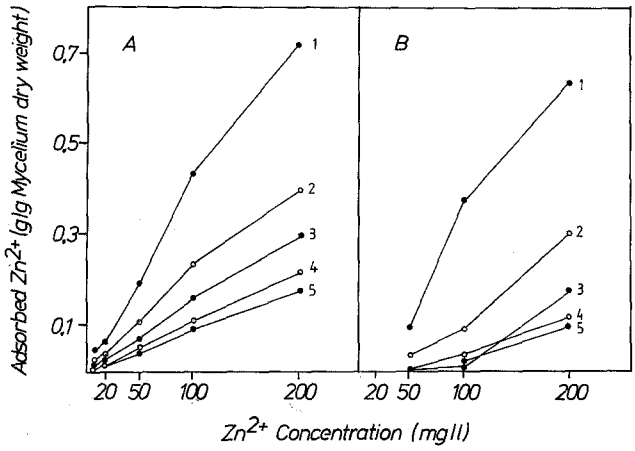

Fig. 2. Effect of NaOH treatment on the influence of zinc concentration and biomass density on zinc biosorption by *A. niger*: **A**, NaOH treated; **B**, untreated control. *Numbers* indicate biomass densities as specified in the legend to Fig. 1

Maximal adsorption capacity

The possibility of using the mycelia described to remove zinc ions from waste-waters would be facilitated if the mycelia could be used in a column. Capacities obtained thereby (at pH 4.0) are given in Table 1. It is seen that the values were not so high as in the batch process, but considerable biosorption was apparent.

There was little influence of temperature (between 15 and 30° C) and the flow rate of equilibration (below 10 and 50 ml/h) on the degree of biosorption (data not shown).

Zinc desorption

Having achieved biosorption in columns, subsequent trials were carried out to remove the bound zinc. This was carried out by rinsing the columns with 0.1 M HCl. Virtually all zinc could be desorbed from NaOH-treated *A. niger* (Table 2), whereas lower values were obtained with other mycelial preparations. The acid treatment apparently did not damage the mycelia, since they could be used repeatedly without notable change in biosorbent capacity (nine independent experiments).

Biosorption of zinc from natural aqueous solutions

NaOH-treated *A. niger* mycelia (the best material, as determined in the present study), was then used to test whether zinc biosorption can also be carried out from natural aqueous environments. For this purpose, samples from certain local rivers or creeks, known to contain zinc ions because of industrial waste contamination, were collected and the ability to remove zinc by *A. niger* was investigated in the column mode. It can be seen in Table 3 that zinc ions from all samples were removed to a high degree by a single column. By using a tandem arrangement of only a few columns, almost complete removal (>99%) could be achieved. The degree of biosorption in these experiments was significantly higher than that established in Table 2 due to the high pH of the samples, which is beneficial for biosorption.

Discussion

In this paper we have provided evidence that some – but not all – industrial waste mycelia can successfully be used for biosorption of Zn^{2+}. The best values obtained in this study are among the best reported in the literature (Muzzarelli et al. 1980; Subramanian et al. 1983; Kuyucak and Volesky 1988; Venkateswerlu and

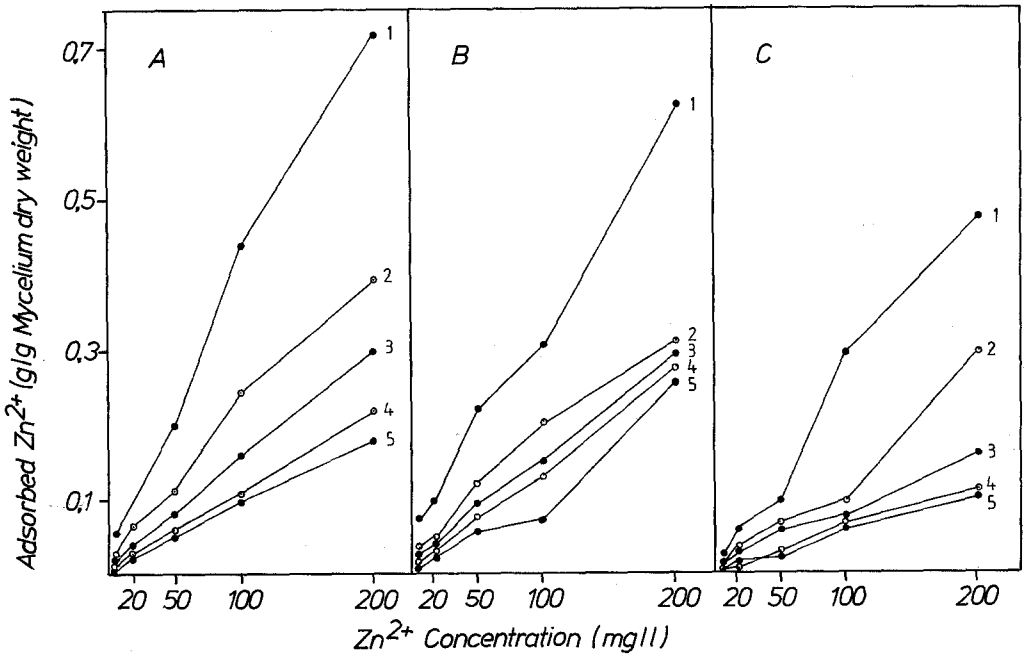

Fig. 3. Effect of pH on the influence of zinc concentration and mycelial density on zinc biosorption by NaOH-treated *A. niger*: **A**, pH 4.0; **B**, pH 3.0; **C**, pH 2.0. *Numbers* indicate biomass densities as specified in the legend to Fig. 1

Table 1. Biosorption of Zn^{2+} by industrial waste mycelia by a column method

Mycelium	Amount of mycelium (mg)	Zn^{2+} biosorption	
		(mg Zn^{2+}/g mycelium)	(% of Zn^{2+} applied)
Aspergillus niger,	100	93.6	33
	200	54.6	39
NaOH-treated	300	38.1	40
A. niger,	100	97.6	35
untreated	200	43.7	31
	300	31.9	34
Penicillium	100	85.5	31
chrysogenum	200	42.8	31
	300	19.9	37
Claviceps	100	97.6	35
paspali	200	43.7	31
	300	31.9	34

The total Zn^{2+} applied was 28 mg in 140 ml. The pH was 4.0 for all experiments. The percentage of applied Zn^{2+} is calculated as $100 \times (A/B)$, where A is the difference between 28 mg minus the total amount of unbound zinc obtained, and B is 28 mg

Table 2. Desorption of Zn^{2+} from various industrial waste mycelia by elution with 0.1 M HCl

Mycelium	Mycelial amount (mg)	Zn^{2+} adsorbed (mg/g mycelium)	Zn^{2+} desorbed (%)
A. niger,	100	97.4	57
untreated	200	43.7	55
	300	31.9	53
A. niger,	100	93.6	97
NaOH-treated	200	54.6	82
	300	38.1	83
C. paspali	100	97.6	12
	200	41.7	12
	300	36.7	14
P. chrysogenum	100	85.5	8
	200	42.8	10

Values were obtained using a column as described in the legend to Table 2. Sixty millilitres of 0.1 M HCl were applied for a single desorption experiment. The percentages are calculated as total amount of zinc in 60 ml of 0.1 M HCl per total amount of zinc originally bound to the column

Table 3. Adsorption of Zn^{2+} from various natural aqueous environments by *A. niger* waste mycelia (NaOH-treated) in a column

River	pH	Zn^{2+} content (µg/l)	Biosorption (%)
Schwechat (Mannswörth)	8.4	320[a]	64
		256[a]	62
Saalach (Weissbach)	9.4	383	78
Liesing (Altmannsdorf)	7.4	515	35
Leoganger (Ache)	6.9	595	38
Donau (Hainburg)	7.2	210	40

One hundred milligrams of *A. niger* mycelium (NaOH-treated) was used in a column for these experiments. *Names in brackets* indicate the town where the samples were harvested.

[a] Two separate determinations

Stotzky 1989), and have moreover been obtained in a column mode, which should facilitate practical application. It should also be stressed that the removal of zinc was very effective from samples of natural environments, indicating the absence of interference caused by other naturally occurring compounds and/or ions, such as alkali metal and ferrous ions or phosphates (unpublished data). The different suitability of different fungal mycelia for zinc biosorption may be related to the presence of different numbers of functional groups within the mycelial material involved in adsorption and/or chelating the metal ion.

Subramanian et al. (1983) reported that pregrowing the mycelia of *N. crassa* in the presence of high concentrations of a trace metal (copper) preconditions the cell wall for increased biosorbent capacity. This is particularly interesting in view of the fact that the mycelia of *A. niger*, which turned out to have the best biosorbent capacity, stem from a citric acid fermentation plant, a process carried out in the presence of high amounts of potassium hexacyanoferrate (Röhr et al. 1983).

Unfortunately, the mechanisms responsible for biosorption are still ill-defined, and appear to be multifactorial: the potential ligands within the cell wall comprise —COOH, $-NH_2$, —SH, —OH, and $-PO_4^{3-}$ groups (Tobin et al. 1984); moreover, precipitation of the metal within the cell wall has also been documented (Kuyucak and Volesky 1987). The increase in biosorption by raising the pH from 3.0 to 5.0 and beyond would indicate the involvement of negatively charged groups. However, the fact that alkali treatment, which removes most of the cell wall material containing —COOH and phosphate groups (Mahadevan and Tatum 1965), improved the biosorbent capacity, argues strongly against such an interpretation.

Muzzarelli et al. (1980) also found that alkali treatment of *A. niger* mycelia improved their capacity to chelate various metal ions. They interpreted the biosorbent ability of their mycelia as being solely due to the presence of chitosan. In fact, the *A. niger* mycelia contained the highest percentage of glucosamine (26% of dry weight), and the dependence of biosorption on pH during these results may be in accordance with this assumption. It is, however, quite possible that the removal of the amorphous polysaccharides from the cell wall by alkali treatment simply generates more accessible space within the β-glucan - chitin skeleton, hence allowing more zinc ions to precipitate at this surface. Increased precipitation of zinc hydroxide can be expected at increased pH. While this interpretation is speculative as yet, it is clear that alkali treatment appears to be an effective means of improving the biosorbent capacity of fungal mycelia. We are now examining whether this material can also be used to biosorb other heavy metal ions from aqueous solutions.

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