Removal of Heavy Metals from Aqueous Solution Using *Rhizopus delemar* Mycelia in Free and Polyurethane-Bound Form

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This study assesses the ability of mycelia of *Rhizopus delemar* (both free and immobilized on polyurethane foam) to remove heavy metals from single-ion solutions as well as from a mixture of them. All experiments were conducted using 0.5–5 mM solutions of $CuSO_4 \cdot 5H_2O$, $CoCl_2 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$. Mycelia immobilized on polyurethane foam cells showed some times increase in uptake compared with that of free cells. Metal ions accumulation from a mixed solution was decreased slightly for cobalt and iron and considerable for copper ions. Heavy metal uptake was examined in the immobilized column experiments and more than 92% heavy metal removal (mg heavy metals removed/mg heavy metals added) from a mixed solution was achieved during the 5 cycles. During these experiments, the dry weight of the immobilized cells was decreased by only 2%. These results showed that immobilized mycelia of *Rhizopus delemar* can be used repeatedly for removal of heavy metals from aqueous solutions.

Introduction

Removal of metals from industrial waste water has conventionally been accomplished mainly by precipitation, ion exchange, and electrolytic technologies (Blanco *et al.*, 1999). More recently, biosorption of metals by immobilized cell systems has been used effectively for removal of metals from industrial effluent (Gadd and White, 1993). This technology exploits the natural tendency of cells to accumulate elements or their innate ability to degrade recalcitrant organic compounds. Cells with such abilities are immobilized either as entrapped biomass or as a biofilm to form a system for treating waste water known as a bioreactor (Qureslii *et al.,* 2001).

In the present study, the heavy metal binding capacity of free *Rhizopus delemar* mycelia is compared with that of mycelia immobilized on polyurethane foam.

Materials and Methods

Microorganisms, medium and cultivation

The fungus used in this study, *Rhizopus delemar,* is deposited at the Collection of the Institute of Microbiology at the Bulgarian Academy of Sciences. Spores of 6–7 days old culture incubated on potato-glucose agar slants at 30 °C were used for inoculation (concentration of spore suspension 1×10^6/ml). The growth medium contained: hydrol with 55–60% reducing sugar, diluted to $40\ g \cdot l^{-1}$ of reducing sugar, and $60\ g \cdot l^{-1}$ of corn steep liquor (obtained from the G. Milev state company, Sofia, Bulgaria, producing glucose from corn starch hydrolysate). The pH was adjusted to 5.0 before sterilization.

Cultivation of *Rhizopus delemar* was carried out in 500-ml Erlenmeyer flasks with 100 ml growth medium on a rotary shaker at 30 °C. After 48 h cultivation the mycelium was centrifuged (3000 × *g*, 15 min), washed with bidistilled water and used as a biosorbent.

The polyurethane foam cubes (0.5 × 0.5 × 0.5 cm in size) used for the immobilization of fungus had an average pore size of 0.6–0.8 mm and was obtained from the G. Karamintchev state company for polymers (Ruse, Bulgaria). Prewashed foam material was submerged in 100 ml growth medium in 500 ml Erlenmeyer flasks and autoclaved for 20 min at 120 °C for sterilization. The carrier cubes inoculated with 6-day-old spores of *Rhizopus de-*

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lemar (5.10^6/ml) from one slant were cultivated as described above.

Metal ion uptake

Classically, biosorption experiments were carried out in batches as follow: 1–5 g wet mass biosorbent was added to 100 ml metal ions solution, containing 1–5 mM of $CoCl_2 \cdot 6H_2O$ or $CuSO_4 \cdot 5H_2O$ or $FeSO_4 \cdot 7H_2O$ in bidistilled water (pH 4.5) in Erlenmeyer flasks, which were then agitated at 220 rpm on a rotary shaker for 1 h at 30 °C. In a second set of experiments, for the uptake of heavy metals, the biosorbent was suspended in 100 ml of mixed solution (pH 4.5) containing equal concentrations of $CuSO_4 \cdot 5H_2O$, $CoCl_2 \cdot 6H_2O$ and $FeSO_4 \cdot 7H_2O$. The suspension was shaken for 1 h at 30 °C. Then, the content of the flasks was separated by filtration, using a Whatman No. 1 filter paper. The concentration of the metal ions in the filtrates was determined using an atomic absorption spectrophotometer with an air/acetylene flame (model 2380; Perkin Elmer, Überlingen, Germany).

Copper removal trials

The immobilized biomass (3 g dry mass) was subsequently placed in bidistilled water and then poured as a slurry into a chromatography column. The flow rate was 1 ml/min. the fraction volume 10 ml, the column height 15 cm, the column volume 30 ml and the temperature 30 °C. The metal concentration was 0.5 mM of each cobalt, copper and iron ions, pH 4.5.

The column was washed with bidistilled water and desorbed with 1 mM solution of H_2SO_4. The adsorption-desorption test was repeated 5 times.

Other methods

To calculate the uptake, the dry weights of free and immobilized biomass were determined as follows. The dry weight of free cells was obtained by means of tared aluminium foil cups, dried at 105 °C for 48 h. The dry weight of immobilized cells was determined by subtraction of an average predetermined dry weight of foam cubes from the weight of foam cubes plus mycelium after drying at 105 °C for 48 h.

Chemicals

All cemicals were commercial preparations of analytical grade.

Results and Discussion

Influence of the mycelial density of the suspension on cobalt biosorption

Preliminary experiments were carried out to assess the appropriate incubation time: 60 min was found to be sufficient for optimal and reproducible biosorption (data not given). It was confirmed that the metal cations were not accumulated by the polyurethane foam cubes.

Mycelia of *Rhizopus delemar* (free- and immobilized cells) were examined with respect to their ability to remove cobalt ions from aqueous solutions. The relationship between mycelial density, residual metal ions concentration and cobalt biosorption is given in Fig. 1. Control experiments, either lacking biomass or cobalt ions, were included. The immobilized mycelium showed considerable higher specific biosorbent capacities than free cells in all tested cases of mycelial density. With carefully optimized biomass concentrations, the cobalt ions could be removed completely from the aqueous phase by immobilized biosorbent. Maximal value for specific biosorbent capacity of the immobilized mycelium was calculated to be 19.6 mg/g.

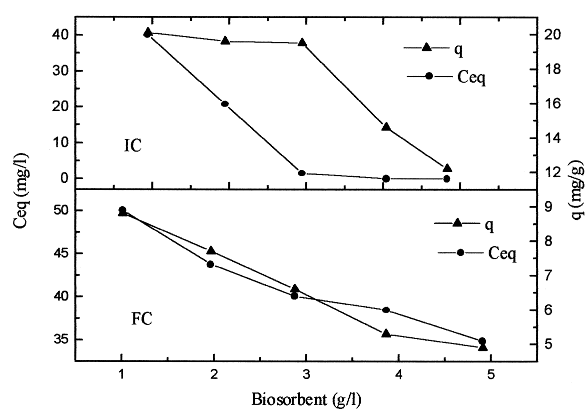

Fig. 1. Purification of cobalt solution (1 m_m) by increasing concentration of free- (FC) or immobilized cells (IC) of *Rhizopus delemar* during 60 min at 30 °C. q – cobalt ions uptake; Ceq – residual concentration of cobalt ions in solution.

At the experimental conditions used, the biomass concentration was found to have a significant effect on biosorption. In general, the amount of heavy metal adsorbed per unit weight was higher at lower biomass concentrations and decreased with increasing amounts of biomass. Reduction in biomass concentration in the suspension at a given metal concentration enhances the metal/biosorbent ratio, and thus increases metal uptake per g of biosorbent, as long as the latter is not saturated. Illustration of this behavior is given in Fig. 2, showing cobalt sorption by *Rhizopus delemar* for two types of biosorbents – free- and immobilized cells. Thus, maximal uptake 8.8 mg/g and 20.1 mg/g was achieved for both cases at initial concentration of 58.93 mg/l cobalt ions with an initial cobalt/biosorbent ratio, of 58.3 mg/g and 62.6 mg/g free and immobilized biosorbent, resp. All these data, obtained with simple experiments, can be of great interest in scale-up processes to optimize industrial effluent purification.

The results obtained in these experiments showed that using immobilized batch system of filamentous fungus enhanced the biosorption levels, as compared with native mycelia. It is quite possible that the immobilization of mycelia on this carrier generates more accessible potential ligands within the cell wall, hence allowing more cobalt ions to precipitate at these surfaces. Increased uptake of metal ions can be expected at equal weight of mycelia as result of a larger distance between cells, resp. smaller electrostatic interaction between function groups of the cell surface (Rome and Gadd, 1987).

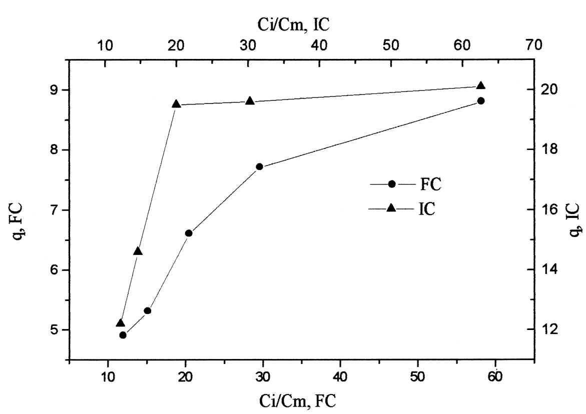

Fig. 2. Influence of initial metal/biosorbent ratio upon cobalt uptake by *Rhizopus delemar*: Ci – initial metal concentration in solution (mg/l); Cm – biosorbent concentration (g/l); q – cobalt uptake (mg/g dry wt).

Comparison of heavy metals uptake by free mycelia (FC) of Rhizopus delemar and mycelia immobilized on polyurethane foam (IC)

The results obtained for the uptake of individual cobalt, copper and iron ions and of mixtures of them by free and immobilized mycelia of *Rhizopus delemar* are given in Table I. These results show us that metal accumulation by *Rhizopus delemar* is a chemical equilibrated and saturatable mechanism. Thus, adsorption increases when the initial metal concentration rises, as long as binding sites are not saturated. Heavy metal accumulation from single ion solutions decreased in the following orders: Cu > Co > Fe. On the other hand, the immobilized mycelium accumulated some times more heavy metal ions then free mycelium. Metal ions accumulation from mixed solutions was decreased slightly for cobalt and iron, but considerable for copper ions. The decreasing of total accumulation capacity in the multielement solutions indicates that there might be occur competitive inhibition of Cu^{2+} by the other cations in the solution. The uptake of heavy metals appeared to be realized via a general cation uptake system, with limited specificity related to the ionic radii of the cations or chemistry. Probably copper ion binding sites are the same as for the cobalt and iron ions, but the binding of both ions happen more easily than the copper ions. On the other hand, accumulation of cobalt, strong complexing agent, modifies the conformation of cell wall polymers and thereby mask copper binding sites. These effects are very important, because industrial effluents are very complex solutions with different metabolic ions. Previous work with *Rhizopus* biomass using different ions in direct competition studies reported similar binding behavior (Tobin *et al.,* 1987; Brady and Tobin, 1995). Nakajima and Sakaguchi (1986) reported that *Penicillium lilacinum* can accumulate a large quantity of uranium from solution containing uranium only, but selective uranium absorption from the mixed solution is comparatively low.

Table I. Comparison of heavy metal uptake by free mycelia (FC) of *Rhizopus delemar* and mycelia immobilized on polyurethane foam (IC). The data shown are mean values of three experiments.

Culture	Metal ion concentration [mM]	Metal uptake [mg/g]				Metal uptake from mixed solution [mg/g]			
		Co[a]	Cu[b]	Fe[c]	Total	Co	Cu	Fe	Total
Rhizopus delemar	1.0	7.7	17.1	6.9	31.7	6.7	1.9	6.3	14.9
FC	2.0	20.3	24.8	15.5	60.6	17.4	2.6	14.1	34.1
	3.0	21.9	32.1	15.7	69.7	18.7	3.4	14.3	36.4
	4.0	22.5	33.5	15.7	71.7	19.2	3.5	14.3	37
	5.0	22.5	34.8	15.5	72.8	19.2	3.5	14.3	37
Rhizopus delemar	1.0	18.8	21.2	10.4	49.8	16.1	2.4	12.5	31.0
IC	2.0	38.3	25.1	22.8	86.2	32.3	4.8	27.4	64.5
	3.0	38.7	33.3	22.9	94.9	32.6	5.4	27.5	65.5
	4.0	38.9	34.2	23.1	96.2	32.7	5.3	27.7	65.7
	5.0	38.9	34.3	23.1	96.3	32.7	5.3	27.7	65.7
Polyurethane foam	1.0	0	0	0	0	0	0	0	0

Precultured free or immobilized cells (about 3 g wet wt) were suspended in 100 ml of solution (pH 4.5) containing 1–5 mM cobalt (a), copper (b) or iron (c) ions only. Each suspension was shaken for 1 h at 30 °C.

Table II. Removal and subsequent recovery of heavy metals from a system of two consistently connected columns packed with immobilized cells.

Cycle	Heavy metal uptake [mg/g]			Heavy metal removed [%]			Heavy metal recovered [%]		
	Co	Fe	Cu	Co	Fe	Cu	Co	Fe	Cu
1 (fresh IS)	8.80	9.14	10.29	99	98	96	97	96	94
2 (1st recycle)	8.72	8.96	10.20	98	96	95	95	93	92
3 (2nd recycle)	8.54	8.86	10.01	95	94	93	92	92	91
4 (3rd recycle)	8.48	8.71	9.79	94	92	91	92	90	90
5 (4th recycle)	8.34	8.75	9.80	92	92	91	90	91	89

Initial heavy metal concentrations in the solution (mg/l): Cu – 32.3; Co – 26.2; Fe – 27.9.
Intial biosorbent concentration: 1st column – 2.99 g dry weight; 2nd column – 3.0127 g dry weight.

Removal of heavy metals in immobilized column experiments

Since the *Rhizopus delemar* biomass was able to effectively bind heavy metals, the next step was to examine heavy metal uptake in the immobilized column experiments. The model waste water consists of 0.5 mM of each cobalt, copper and iron ions was passed through two consistently connected columns packed with immobilized cells. This system permits to overcome the interionic competition, and to improve the uptake. The efficiency of heavy metal remove (mg heavy metal removed/mg heavy metal added) in a column system is given in Table II. Cobalt and iron were removed more than 98% by immobilized mycelia in first column, but the mainly copper removal was realized by the immobilized cells in the second column. After elution of bound metals, re-uptake of heavy metals was possible and dry weight of the immobilized cells decreased by only 2% during 5 cycles. These results show that the microbial cells are stable after immobilization and can be used repeatedly.

During this study it proved possible to use a growth medium based only on hydrol (in place of glucose) and CSL (two by – products obtained in glucose-starch industry) for obtaining of biosorbent. Using these inexpensive materials we presented the possibility for high percent of heavy metal removal by immobilized on PUF mycelia of filamentous fungus from solutions of individual ions as well as from a mixture of them.

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