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Metal accumulation by fungi: applications in environmental biotechnology

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SUMMARY

Fungi can accumulate metal and radionuclide species by physico-chemical and biological mechanisms including extracellular binding by metabolites and biopolymers, binding to specific polypeptides and metabolism-dependent accumulation. Biosorptive processes appear to have the most potential for environmental biotechnology. 'Biosorption' consists of accumulation by predominantly metabolism-independent interactions, such as adsorptive or ion-exchange processes: the biosorptive capacity of the biomass can be manipulated by a range of physical and chemical treatments. Immobilized biomass retains biosorptive properties and possesses a number of advantages for process applications. Native or immobilized biomass can be used in fixed-bed, air-lift or fluidized bed bioreactors; biosorbed metal/radionuclide species can be removed for reclamation and the biomass regenerated by simple chemical treatments.

A number of processes may contribute to the uptake of metals by fungi and yeasts. Extracellular products may remove metals from solution. These products include metabolites such as sulfide or oxalic acid and a variety of extracellular materials such as polysaccharides and extracellular melanins. Fungi and yeasts can also produce a number of proteins and polypeptides such as metallothioneins and γ-glutamyl peptides in response to the presence of toxic levels of certain metals in their environment; some of these are metal-specific and may bind a substantial proportion of the intracellular content of metals such as Cu or Cd, thereby conferring resistance to the metals. Fungi and yeasts may also mediate metal removal from aqueous systems by chemical transformations such as redox changes or alkylation which can respectively precipitate or volatilize the metals [5]. In addition to these processes, fungal biomass may take up metals by bioaccumulation and biosorption. Bioaccumulation is the uptake of metal species by means of metabolism-dependent processes which may involve both transport into the cell and partitioning into intracellular components; this has been discussed elsewhere [2].

Biosorption

To date, the most promising approach to metal removal by fungi is biosorption. Biosorption comprises binding of metals (or other solutes, colloids or suspensions) to the biomass by processes which do not involve metabolic energy or transport, although such processes may occur simultaneously where live biomass is used. It can therefore occur in either living or dead biomass. Several chemical processes may be involved in biosorption, including adsorption, ion exchange, co-ordination and covalent bonding. More than one process may contribute to uptake in any one system. The main chemical groups in biomass which are able to partake in biosorption are electronegative groups such as hydroxyl or sulfhydryl groups, anionic groups such as carboxyl or phosphate groups and nitrogen-containing groups such as amino groups. Carboxyl and phosphate groups are considered to be important binding sites for many toxic metals [18] while the amino groups of chitin were found to be a major site of thorium uptake in *Rhizopus arrhizus*. The initial thorium uptake comprised a rapid adsorption/ion exchange component which was followed by a slow covalent component [22]. The three-dimensional structure of binding sites also appears to be significant as the ionic radius affected the biosorption of ions by *R. arrhizus* [15]. The cell walls of filamentous fungi and yeasts appear to have the major role in biosorption due to possession of numerous uptake sites of the types mentioned above. Nonetheless, the interior of the cell also contains many components which bind metals so that treatments which permeabilize the cell, such as grinding [15], detergents [3] or HCHO and $HgCl_2$ [14], increase the uptake of a number of metals and radionuclides. These methods may also expose active sites in the cell wall which could contribute to increased uptake. Unlike many of the other processes for metal removal using microbes, fungal biosorption can remove suspended solids as well as solutes. *Aspergillus niger* biomass took up copper, lead and zinc sulfides onto mycelial surfaces and several fungi could adsorb solid metal compounds from acid mine waters [27].

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Uptake of metals from solution by fungi is subject to interference from a number of substances and chemical conditions. Bioaccumulation and biosorption of metals can be inhibited by the presence of anions such as phosphates which complex or precipitate them and render them unavailable to the cellular binding sites for metals [16]. Cations, including H^+, can also interfere by blocking potential binding sites. Both bioaccumulation and biosorption of cations such as Cu^{2+}, Cd^{2+}, Zn^{2+}, Mn^{2+} and Co^{2+} are decreased by a low pH [2]. The presence of other cations may also exert a similar effect through competition for binding sites [17]. However, the pH effect is dependent on the chemistry of the metal species and binding sites. For example, thorium biosorption by *R. arrhizus* biomass was higher at pH 4 than 2, apparently due to the shift of prevalent ion to hydrolyzed species from free Th^{4+}, and the distribution in the cell-wall was also altered [22].

The chemical composition of the fungal wall is strongly dependent on the culture conditions and this may in turn affect biosorptive properties. For example, thorium biosorption by inactive *R. arrhizus* biomass was greater when cells were grown on simple than on complex medium [3]. The culture phase at harvest may exert similar effects, with uptake by biosorption and bioaccumulation being generally less at later growth phases as a result of chemical changes in the mycelium. The presence of toxic metals in the growth medium can also alter the cell wall composition, sometimes resulting in production of melanins and increased metal-binding capacity [2].

Biomass immobilization

A number of investigations of fungal biosorption have focussed on biomass in its native state and numerous studies have reported the metal uptake characteristics of a variety of freely suspended organisms under varying solution conditions [1,3,6,14,15]. However, immobilized or pelleted biomass

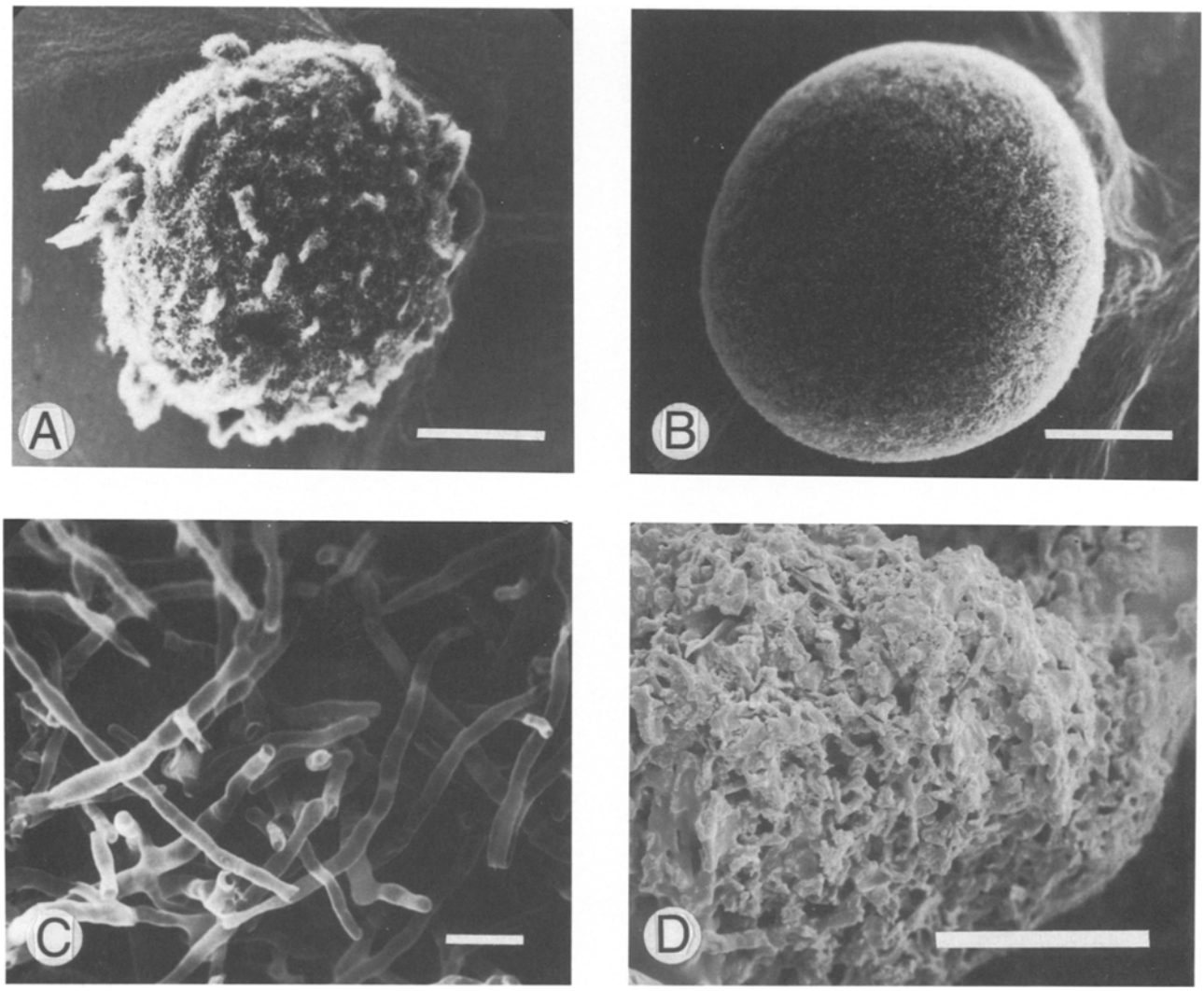

Fig. 1. Scanning electron micrographs of (A) *Cladosporium cladosporoides* mycelial pellet (bar = 1000 μm); (B) *Aspergillus nidulans* mycelial pellet (bar = 1000 μm); (C) mycelial network of *A. nidulans* pellet (bar = 10 μm); (D) immobilized *Rhizopus arrhizus* in polyvinyl formate (biomass loading 50%) (bar = 100 μm). Fig. 1(D) courtesy of Dr J.C. Roux, CEREM-DEM-SECM Lab., Centre d'Etudes Nucleaires, Grenoble, France.

offers considerable advantages in terms of handling, solid–liquid separation and ease of scale-up. Several studies have described the production of biomass pellets (Fig. 1 (A–C)) during growth of several filamentous fungal cultures [12,13,19] and the use of immobilizing support matrices ranging from natural polysaccharide gels to coal, sand, foam and a range of synthetic polymers [7–9,23,30]. Along with mechanical strength and durability, key considerations in using immobilizing matrices are the biomass loading attainable and the degree of blocking of the binding sites by the immobilizing material.

For *R. arrhizus* immobilized in polyvinyl formate (Fig. 1(D)), biomass loadings of up to 88% were reported but with a concomitant reduction in overall metal-binding capacity of 30%, both calculated on a dry weight basis [23]. Alginate entrapment techniques are simple, non-toxic, and are widely used for cell immobilization. Cell loadings of up to 70% of the dry weight have been achieved without reported loss in metal binding due to the inherent porosity of the gel beads [13]. Under certain conditions the alginate matrix itself may contribute significantly to metal binding. Cadmium removal efficiencies in excess of 70% have been observed due to alginate alone [8], although uranium binding under similar conditions was reported to be negligible [13]. In general, however, the low mechanical strength of alginate gels together with the reversibility of the crosslinking [8] may limit their range of application. Polyacrylamide type gels have been widely used in laboratory-scale biosorption studies with varying cell loadings and minimal reported losses of binding capacities but they are unsuitable for large scale processes because of the toxicity and cost of the materials and the limited mechanical strength of the resulting biosorbent [9–11].

Under suitable growth conditions certain fungi, including *Aspergillus, Rhizopus*, and *Penicillium* species, produce spherical mycelial pellets which have been used in biosorption studies (Fig. 1(A–C)) [13,19]. High metal uptake levels (exceeding those of ion exchange resins under certain conditions) and good desorption characteristics have been reported although the potential for use in multiple uptake/desorption cycles has yet to be demonstrated [13,29].

Biosorption reactor configurations

Immobilized biosorbents are analogous to ion exchange resins or carbon adsorbents and the removal of metal ions from solution by biosorption is essentially a conventional solid–liquid contacting and separation process. Conventional solid–liquid processing equipment falls into the two categories of stirred and packed bed reactors and both have been applied in laboratory and larger-scale biosorption investigations [9,15,25,28].

Stirred tank reactors may be operated in batch or continuous mode and applications to biotechnology range from classical activated sludge systems to laboratory scale batch investigations. To date, all fungal systems reported are of small scale and use suspended or immobilized fungal biomass, or biofilms. Many studies report uptake levels for different metal/biomass systems (Table 1) and adsorption isotherms are frequently used to quantify the equilibrium relationship between the uptake and solution concentration [3,15,23]. Some typical isotherms exhibiting saturation behavior are illustrated in Fig. 2. A limitation of stirred tank systems is the need for a solid/liquid separation stage prior to subsequent regeneration/metal recovery or disposal stages. In commercial full-scale processes, sedimentation is usually the method of choice whereas in smaller experimental units more expensive filtration or centrifugation methods are more common.

Packed bed systems obviate the need for additional solid/liquid separation systems and are well suited to continuous flow adsorption and regeneration cycles. Increasingly, biosorption research is focussing on continuous systems for industrial applications. A key design feature of these systems is the bioreactor residence time and recent reports have highlighted the fact that, although biosorption is a rapid process, insufficient residence times markedly decrease metal removal levels [9,30]. Reduced uptake efficiencies ranging from 30–70% of the batch system values have been observed for continuous flow systems using fungal mycelial pellets

TABLE 1

Metal uptake capacities of selected fungi

Biomass	Metal	Uptake*		pH range	Reference
		mmol g^{-1}	%		
Saccharomyces cerevisiae	Sr^{2+}	0.24	20.9	5.5	1
	Th^{4+}	0.27	12.0	0–1.0	28
	UO_2^{2+}	0.63	15.0	4.0	14
Rhizopus arrhizus	Cr^{3+}	0.59	30.7	3.5–4.0	15
	La^{3+}	0.35	4.9	3.5–4.0	15
	Cu^{2+}	0.25	1.6	3.5–4.0	15
	Ag^{2+}	0.5	5.4	4.0	2
	Cd^{2+}	0.27	3.0	3.5–4.0	15
	Hg^{2+}	0.29	5.8	4.0	2
	Pb^{2+}	0.50	10.4	3.5–4.0	15
	UO_2^{2+}	0.82	19.5	3.5–4.0	15
	Th^{4+}	0.79	18.5	4.0	22
	Th^{4+}	0.50	11.6	0–1.0	3
Aspergillus niger	Th^{4+}	0.28	13.8	0–1.0	3
	UO_2^{2+}	0.9	21.4	5.8	29
Penicillium italicum	Th^{4+}	0.49	11.4	0–1.0	3
Penicillium chrysogenum	Th^{4+}	0.84	19.5	0–1.0	3
Penicillium C1	UO_2^{2+}	0.71	17.0	4.0	2

*The maximum uptake given in the reference cited; molar and percentage concentrations both relate to dry weight of biomass. Referral to original references is recommended for experimental details relating to metal concentrations, contact times, and biomass density.

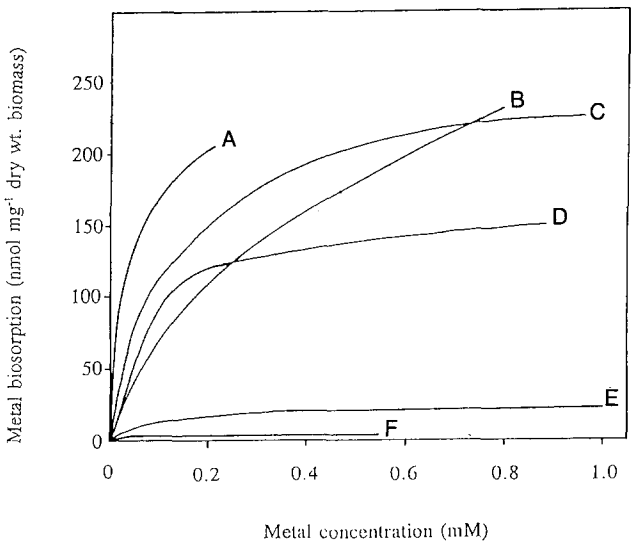

Fig. 2. Biosorption equilibria for some metal cations by fungi and yeasts. The graph shows strontium biosorption by (A) freeze-dried *R. arrhizus* biomass and (B) denatured *Saccharomyces cerevisiae* biomass [1], (C) copper biosorption by *Mucor circinelloides* biomass (Gadd and de Rome, unpublished), (D) thorium biosorption by *S. cerevisiae* biomass [6], (E) strontium biosorption by live *S. cerevisiae* [1] and (F) copper biosorption by live *S. cerevisiae* at pH 2 (White and Gadd, unpublished). Data were obtained with a biomass concentration of 1.0 mg dry wt ml^{-1} and a contact time of 1 h in (C) and (F); other conditions are given in the original publications. Curves (A)–(C) and (E) were obtained at pH 5.5 and (D) at pH <1.0 (1.0 M HNO_3). The data points have been omitted from all curves for the sake of clarity.

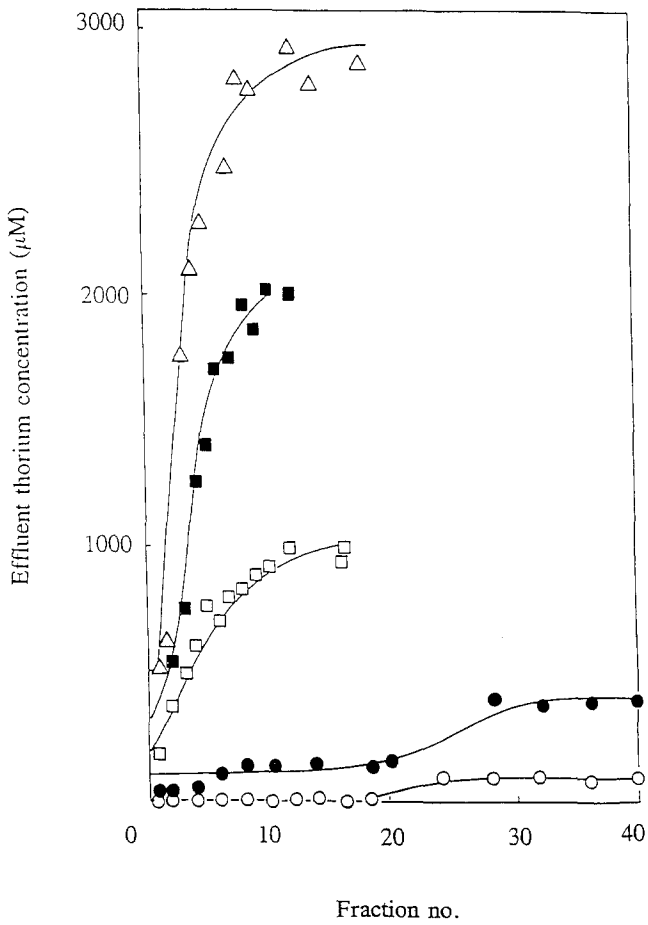

Fig. 3. Breakthrough curve for a laboratory-scale air-lift bioreactor of volume 500 ml (working volume 200 ml) containing 1.9 g dry wt of *R. arrhizus* biomass; 10-ml samples were taken for analysis. The influent thorium concentrations were (○) 100; (●) 400; (□) 1000; (■) 2000; (△) 3000 μM thorium nitrate in 1.0 M HNO_3 with a flow rate of 200 ml h^{-1} (adapted from [28]).

[13] although residence times of the order of minutes are generally reported to be acceptable [7,9,24].

The conventional packed bed system is the fixed bed or column reactor in which stationary biosorbent particles are contacted with metal-bearing solution pumped through the column in an upflow or downflow arrangement. Initially, the effluent metal concentration is low as fresh biosorbent effectively sequesters the incoming metal ions. This concentration rises as the biosorbent becomes loaded until, at the point of saturation, the effluent concentration equals that of the inlet stream. Breakthrough curves are commonly used to describe the change in effluent metal concentration with respect to time or the liquid volume treated [7,28,30]. Typical breakthrough curves for a laboratory-scale airlift system removing thorium from an acid solution are presented in Fig. 3. As two or more packed bed columns are usually operated in parallel, sharp or steep breakthrough curves are desirable to facilitate switching from one column to another as saturation is approached [28,29].

Well known variants of the fixed bed system include fluidized bed and air-lift reactors. In both cases the biosorbent particles are mobilized by upflow of effluent through the column induced by pumping or a rising air stream introduced at the column base. Disadvantages of greater space requirements, particle attrition and increased capital and operating costs are offset by reduced pressure drops and increased removal efficiencies and retention times [9,28].

Biosorbent regeneration and metal recovery

Once the metal-loaded biomass is separated, biosorbed metals can potentially be desorbed in order to regenerate the biosorbent materials and reclaim valuable metals. The methods for biomass regeneration which have been demonstrated comprise washing with reagents such as dilute mineral acids, e.g. for Ra or Zn [20], or carbonate or bicarbonate solutions for actinide elements [4,26]. In general the number of times that unaltered biomass can be reused is limited so that recycling biomass is uneconomic but this can be improved by immobilization of the biomass [26]. For the process to be useful, it is also important to maximize the degree of concentration between the original effluent and the final eluate. This is achieved by maximizing the metal loading of biomass and minimizing the eluant volume to produce the greatest possible solid:liquid ratio in the eluant slurry [21] although gains can also be made by reuse of

unsaturated desorbent [4]. As the capacity of fungal biomass for metals is strongly dependent on the biomass concentration during biosorption, increasing the biomass concentration may increase the total removal at the cost of reduced loading per unit of biomass [2] and reduce the concentration of an eluted metal product. This emphasizes a significant point that, in any practical application, the development of metal biosorption must be considered as part of an overall metal-removal or metal-reclamation process.

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