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Studies on the biosorption of uranium by *Talaromyces emersonii CBS* 814.70 biomass

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Abstract Residual biomass, produced by the thermophilic fungus, *Talaromyces emersonii* CBS 814.70, following growth on glucose-containing media, was examined for its ability to take up uranium from aqueous solution. It was found that the biomass had a relatively high observed biosorption capacity for the uranium (280 mg/g dry weight biomass). The calculated maximum biosorption capacity obtained by fitting the data to a Langmuir model was calculated to be 323 mg uranium/g dry weight biomass. Pretreatment of the biomass with either dilute HCl or NaOH brought about a significant decrease in biosorptive capacity for uranium. Studies on the effects of variation in temperature on the biosorptive capacity demonstrated no significant change in binding between 20°C and 60°C. However, a significant decrease in biosorptive capacity was observed at 5°C. Binding of uranium to the biomass at all temperatures reached equilibrium within 2 min. While the routine binding assays were performed at pH 5.0, adjustment of the pH to 3.0 gave rise to a significant decrease in biosorption capacity by the biomass. The biosorptive capacity of the biomass for uranium was increased when extraction from solution in sea-water was examined.

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Introduction

The existence of heavy metals and/or radionuclides in the environment, whether they derive from natural or anthropological activities, represents a significant environmental hazard. Increasing public awareness of the detrimental consequences associated with the existence of such substances in the environment has led to significant changes in legislation governing the disposal of such materials. While the major source of such pollutants may be considered to be industrial, the quantities contained in waste materials from both agricultural and domestic sources can not be overlooked (Gadd and White 1993).

When heavy metals or radionuclides enter the ecosystem it has been found that biological activity plays an important natural role in immobilizing or detoxifying those materials (Beveridge 1989; Lovely et al. 1991). In the past it has been demonstrated that microorganisms have the ability to take up heavy metals and/or radionuclides with varying degrees of efficiency and it has been proposed that this phenomenon might be exploited in applications such as detoxification of metal-bearing waste-waters, decontamination of radioactive waste-waters, recovery of metals from ore-processing solutions and the concentration/recovery of strategic/rare metals from sea-water (Gadd and White 1993).

Whilst the exact mechanism by which microorganisms take up metals is relatively unclear it has been demonstrated that both living and non-living fungal biomass may be utilised in biosorptive processes (Volesky et al. 1993). The potential of non-living fungal biomass residues as biosorbants for heavy metals and/or radionuclides has received considerable attention since this material represents a significant by-product from several major industrial fermentation processes (Volesky et al. 1993; Norris and Kelly 1977). Fungal biomass residues from *Aspergillus niger* citric

acid fermentation waste may be used in the removal of zinc dust, magnetite and metal sulphides from wastewater streams (Gadd and White 1993). Non-living fungal biomass from *Rhizopus arrhizus* and *Penicillium digitatum* fermentations has been shown to be capable of significant uptake of uranium from dilute solutions (Tsezos and Volesky, 1982; Galun et al. 1987).

The thermophilic fungus *Talaromyces emersonii* has the ability to produce a number of commercial enzyme systems including cellulases, amylases and chitinases (McHale and Morrison 1986; Bunni et al. 1989; Irhuma et al. 1991) and as such, it represents a potential source of fungal biomass for use in biosorptive technology. In addition, increasing the value of residual biomass from *T. emersonii* fermentations would contribute sinificantly to reducing the costs of enzyme production by this organism. Here we report on the ability of *T. emersonii*-derived biomass to take up uranium from aqueous solution and the effects of various conditions on its biosorptive capacity.

Materials and methods

Microorganism

The thermophilic fungus, *Talaromyces emersonii* CBS 814.70 was maintained on malt extract agar plates supplemented with 5% (w/v) glucose and 1% (w/v) mycopeptone (Oxoid) at 45°C as described previously (McHale and Morrison 1986). Biomass was produced following growth in submerged-culture fermentation at 45°C on media containing 4% (w/v) glucose, 1% (w/v) NH_4NO_3, 0.5% (w/v) corn steep liquor (Quest/Biocon Ltd., Cork, Ireland) and mineral salts as described previously (McHale and Morrison 1986). Aliquots of 400 ml were dispensed into 2-l conical flasks and fermentations were initiated by addition of a 2% (v/v) inoculum from a liquid starter culture. Flasks were incubated in an orbital shaker at 150 rpm. Following 48–50 h growth, biomass was harvested by filtration through gauze and subsequent washing with 0.1 M NaCl. This was followed by washing in distilled water and the material was dried using lyophilisation.

Binding studies

Unless otherwise stated, solutions of uranium were prepared by dissolving uranyl acetate in distilled, deionized water. Contact experiments were performed in flasks containing 50-ml volumes of uranium-bearing solution and the amounts of biomass added ranged between 0.05 g and 0.5 g (dry weight). Unless otherwise stated, the reactions were incubated at 20°C for 2 h. Biomass was separated from the metal-bearing solution by filtration through 0.2 µm membranes. Determination of uranium concentration was carried out using the arsenazo III method described previously (Savvin 1961) and metal uptake (q, mg uranium/g biomass) was calculated as described by Holan et al. (1993). Uranium- and biosorbent-free samples were used as controls.

Binding studies from sea-water

Sea-water samples were obtained from both the west and northwest coast of Ireland (Bertra, Westport, Co. Mayo and Portstewart, Co. Derry, respectively). Prior to preparation of uranyl solutions, samples were prefiltered using a 0.2 µm filter. The final pH of the uranium solution in sea-water was 5.7.

Results

The effect of biomass pretreatment on biosorption of uranium

Biomass obtained following growth of *T. emersonii* on glucose-containing media was harvested and dried as described above. Upon addition to biosorption reactors containing uranium it was found that this material had an observed maximum biosorptive capacity (q_{max}) of 280 mg U/g dry weight of mycelia (C_f = 300 mg/l) (Fig. 1). It is also found that the data fitted reasonably well to a Langmuir model and the calculated q_{max}, was found to be 323 mg U/g dry weight. In order to determine whether or not pretreatment, in the form of exposing mycelia to either 0.1 M NaOH or 0.1 M HCl, had any effect on the biosorptive capacity, dried biomass samples were suspended in the presence of those reagents (5 g mycelia/l) for 2 h, after which they were washed with distilled water and added to reactors. The results obtained following analysis of treated samples for uranium uptake are shown in Fig. 1 and demonstrate that, while pretreatment in HCl reduced the observed biosorptive capacity to 17 mg U/g, treatment with NaOH decreased it further to 90 mg U/g mycelia.

The effect of temperature on biosorptive capacity

In order to examine the effect of temperature on the biosorptive capacity of *T. emersonii* biomass for

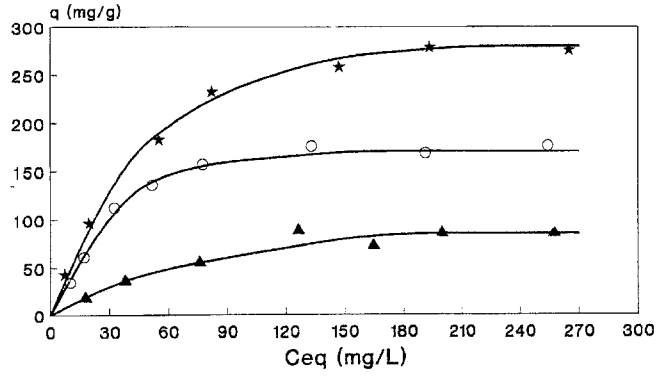

Fig. 1 The effects of biomass pretreatment on biosorption of uranium. Binding assays were carried out at 25°C and pH 5.0. Biosorption or uranium by untreated biomass (★) was compared with uptake by material pretreated by exposure to 0.1 M HCl (○) and 0.1 M NaOH (▲) for 2 h. *Ceq* the concentration of uranium remaining in solution at equilibrium, q the amount of uranium bound to the biomass (mg/g)

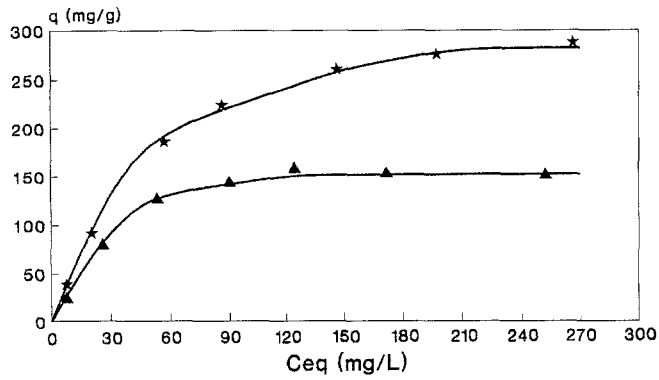

Fig. 2 The effect of temperature on biosorption of uranium by *T. emersonii*-derived biomass. Binding assays were carried out at 60°C, 40°C, 20°C (★) and 5°C (▲)

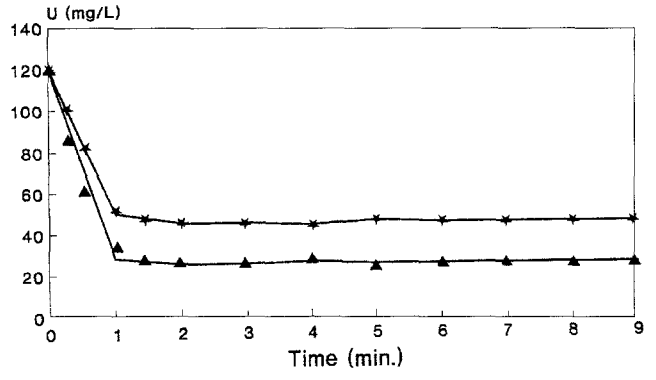

Fig. 3 The effect of temperature on kinetics of uranium biosorption by *T. emersonii* biomass. The amount of uranium remaining in solution was determined following spearation of biomass and sorbate at the indicated times. Binding reactions were performed at 50°C (★) and 5°C (▲)

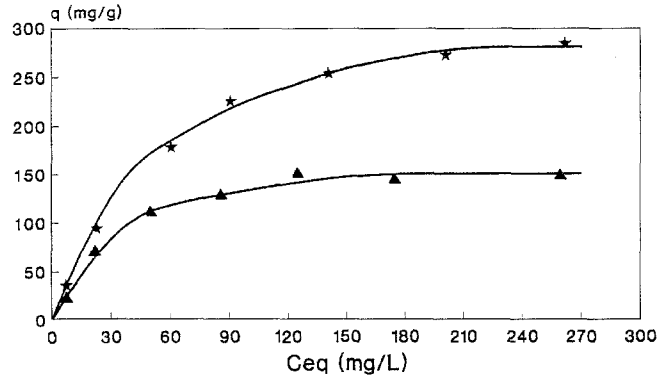

Fig. 4 The effect of pH on uranium uptake by *T. emersonii* biomass. Assays were carried out at pH 5.0 (★) and pH 3.0 (▲) at 20°C

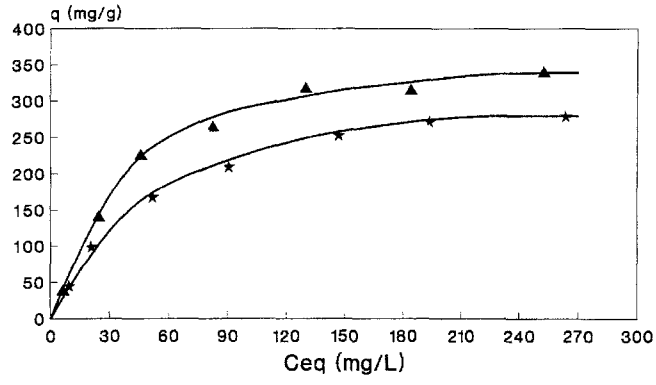

Fig. 5 Direct bisorption of uranium from sea-water using *T. emersonii* biomass. Biosorption of uranium from solutions prepared in distilled, de-ionized water (★) and in seawater (▲). Sea-water samples were obtained from two separate locations (see Materials and methods)

uranium, binding studies were carried out at a variety of temperatures and the results obtained are summarized in Fig. 2. The results indicate that, at temperatures ranging from 20°C to 60°C, no significant change in biosorptive capacity was detected. However, when binding studies were performed at 5°C the biosorptive capacity was reduced to 140 mg U/g dry weight mycellia. Subsequent studies designed in order to examine the time required for binding to reach equilibrium at 5°C and 50°C demonstrated that binding of uranium to the fungal biomass is an extremely rapid event, with equilibrium being reached within 2 min (Fig. 3) at both temperatures.

The effect of pH on biosorption of uranium by *T. emersonii* biomass

In routine biosorption reactions carried out in this study, mixtures of fungal biomass in contact with uranium solutions had a pH of 5.0. Previous reports in the literature (Tsezos and Volesky 1982; Guibel et al. 1992) have suggested that biosorption by fungal biomass was pH-dependent. In order to determine whether or not this was the case with biomass derived from *T. emersonii* fermentations, binding studies were carried out at pH 3.0. The results obtained are shown in Fig. 4 and these demonstrate a significant decrease in biosorption capacity (150 mg U/g biomass) at the lower pH.

Biosorption of uranium from sea-water using *T. emersonii* biomass

In order to determine whether or not the biomass was capable of uranium uptake from a naturally occurring heterogeneous medium (with respect to ionic species content), the ability of the biosorbent to take up uranium from sea-water was examined. Solutions of uranium were prepared in sea-water that had been prefiltered using a 0.2 μm membrane. The results obtained following biosorption of uranium from those solutions are summarized in Fig. 5, and indicate a slight, but significant increase in the observed biosorptive

capacity of the biomass from 280 mg U/g biomass to 340 mg U/g biomass in the presence of sea-water. No significant difference in uranium uptake from sea-water, sampled from the two separate locations, was observed.

Discussion

The ability of microbial biomass to take up uranium and indeed other metals from aqueous solution has been widely reported in the literature. Whilst the range of uptake appears to be extremely broad, maximum uptake capacities have been reported to be in the region of 250 mg/g dry weight (Tsezos and Volesky 1982; Holan et al. 1993). Exceptionally high uptake has been reported for biomass derived from *Streptomyces longwoodensis* (440 mg/g dry weight) (Friis and Meyers-Kieth 1986). The values reported for uranium uptake by untreated biomass derived from the thermophilic fungus *T. emersonii* in the work presented here, 280 mg/g dry weight, suggest that this material represents a biosorbent exhibiting potential for use in the treatment of toxic-metal-bearing waste-water streams.

Although the exact mechanism of metal uptake by microbial biomass is relatively unknown, it has been suggested that it is dependent upon interactions by the metal species in solution with polysaccharides comprising the cell wall (Tsezos and Volesky 1982). In the case of biosorption by fungal biomass, the amine nitrogen of chitin and its derivatives have been suggested to be heavily involved in the formation of metal coordination complexes, thereby forming nucleation sites for further binding of uranium, or more specifically uranyl hydroxides (Tsezos 1983). Other reports suggest the involvement of phosphate residues in metal binding/uptake (Galun et al. 1983). During the studies carried out here it was found that pretreatment of the *T. emersonii* biomass with either HCl or NaOH contributed to a significant decrease in the maximum biosorption capacity. These results were somewhat unexpected since it has previously been reported that metal sorption by biomass was relatively resistant to various forms of pretreatment including boiling, exposure to ethanol, dimethylsulphoxide ad fomaldehyde (Feldstein et al. 1982). It has ben reported that pre-treatment with KOH contributed to an increase in the availability of latent metal-binding sites in biomass derived from the fungas *Penicillium digitatum* (Galun et al. 1983). Pretreatment of *Rhizopus arrhizus* biomass with NaOH resulted in higher uptake capacities for zinc (Faurest and Roux 1992). In contrast, it has been reported that polysaccharides contained in marine algae such as *Ascophyllum nodosum* are extremely sensitive to alkaline pH, resulting in β-elimination, and to acid pH at which hydrolysis may occur (Holan et al. 1993). The results obtained with the *T. emersonii* biomass following pretreatment with acid or alkaline pH may suggest removal of weakly bound extracellular, cell-wall components such as protein and protein-carbohydrate complexes from the biomass. Alternatively the results may lead to a hypothesis suggesting pretreatment-induced rigidity of groups at the binding surface by denaturation/fixation that may otherwise have been involved in a metal-binding role as a result of relative mobility in a microenvironment.

While uptake of uranium by *Pseudomonas* sp. EPS-5028 biomass was found to be temperature-independent (Marques et al. 1991) it has been reported that uptake of the metal by *R. arrhizus* and *S. longwoodensis* increased slightly with increasing temperature (Friis and Myers-Kieth 1986; Marques et al. 1991). Uptake by *Saccharomyces cerevisiae* showed a significant increase when the temperature was increased from 20°C to 50°C as did that by *Penicillium* biomass when the temperature was increased from 30°C to 50°C (Shumate et al. 1978; Galun et al. 1983). It has been suggested that the increase in uptake at increased temperature is due to either a higher affinity of sites for the metal or an increase in binding sites on the relevant biomass (Marques et al. 1991). Uptake of uranium by *Talaromyces* biomass remained unchanged in the region of 15–60°C although a highly significant decrease in the uptake capacity was observed at 5°C (Fig. 2). Since the reduced capacity for uranium have been result of inadqauate reaction times, the kinetics of binding of uranium to the *T. emersonii* biomass was examined at 5°C and 50°C. As with binding of uranium to biomass from many other sources (Tsezos and Volesky 1982), equilibrium of binding to the *Talaromyces* biomass was reached within 2 min (Fig. 3). Whilst a definitive reason for this decrease in uptake capacity at 5°C would be premature in the absence of further data, the result tends to support the above hypothesis involving a certain degree of mobility of groups/moieties on the biosorbent surface. A decrease in temperature would be expected to lead to a decreased mobility of potential binding groups.

The results obtained in this study demonstrate that binding of uranium to the *T. emersonii* biomass decreases significantly when the pH is decreased from 5 to 3 (Fig. 4). This is consistent with other reports in the literature concerned with binding of metal ions to fungal biomass (Faurest and Roux 1992). Decreasing pH would be expected to contribute to the solubility of uranium and hence decrease interactions between the metal and the biosorbent (Faurest and Roux 1992; Tsezos and Volesky 1981). In addition, at low pH there would be a tendency for the amine nitrogen of chitin in the cell wall to be protonated, suggesting a decreased affinity for the sorbate in solution (Niu et al. 1993). In the studies presented here, however, the pH-dependent decrease in the binding capacity of *T. emersonii* biomass for uranium may also be related to the results obtained following pretreatment of the biomass with HCl (Fig. 1), i.e. pH-dependent release of binding mediators from the mycelial surface and/or contribution of decreasing pH to a denaturation/fixation event at the binding sufrace.

It has previously been shown that the presence of other metal ions in solution, in some cases, detrimentally influences uptake of uranium by microbial biomass (Tsezos and Volesky 1982). Because of the occurrence of mixtures of metal salts in most waters the application of fungal biomass to decontamination of metal-polluted waters or to the recovery of economically valuable metals from natural or industrial waters would necessitate a degree of selectivity by the biomass. Since the degree of inhibition of biosorption of a specific ionic species by the presence of other metal ions would reflect the practical usefulness of any biosorbent, it was decided to examine biosorption of uranium by *T. emersonii* biomass from samples of sea-water to which the uranium had been added. The results, as shown in Fig. 5, demonstrate that no inhibition of uranium uptake occurs in the presence of ionic species contained in the sea-water sampled from a number of sites. Although the metal ion content of the sea-water was not determined, the results do suggest that biomass derived from *T. emersonii* fermentations is capable of uranium uptake with some degree of specificity.

On the basis of this work, it would appear that *T. emersonii* biomass represents a source of material suitable for practical use as a biosorbent for uranium from aqueous solution. However, the material may require some form of stabilization in order to retain binding sites lost upon pretreatment washing at acid and alkaline pH. Further studies involving uptake of other metal ions by the biomass are currently under investigation in our laboratories.

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