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Removal of uranium from solution using residual brewery yeast: combined biosorption and precipitation

C. Riordan, M. Bustard, R. Putt† and A.P. McHale*

Biotechnology Research Group, School of Applied Biological and Chemical Sciences, University of Ulster, Coleraine, Co. Londonderry BT52 1SA, Northern Ireland. †Bass Ireland Ltd., Glen Rd., Belfast, Northern Ireland

Whilst unwashed preparations of biomass from a local brewery had an apparent maximum biosorption capacity for uranium of 360 mg/g (dry weight biomass) washing reduced this maximum to 150 mg/g. Homogenization of both biomass preparations and recovery of cellular debris had no significant effect on the maximum biosorption capacities although at lower equilibrium concentrations of uranium differences in the biosorption capacities were detected. When unwashed biomass was retained by a semi-permeable membrane 40% of uranium used in the experiments precipitated outside that membrane. Therefore a significant proportion of the uranium removed from solution, and previously attributed to biosorption by the yeast biomass, resulted from precipitation brought about by interaction with low molecular weight components loosely associated with the biomass.

Introduction

Biosorption of heavy metals and radionuclides by microbial biomass is a relatively rapid and efficient process and the phenomenon may be exploited in biotechnological processes concerned with bioremediation of metal-bearing waste-water streams (Gadd and White, 1993). Although the majority of studies in this area have involved the use of laboratory strains of microorganisms (Norris and Kelly, 1977; Strandberg *et al.*, 1981), more recent studies have involved the examination of either residual living or non-living biomass derived from industrial processes (Omar *et al.*, 1996; Volesky and May Phillips, 1995). Although the exact mechanism by which non-living biomass removes metals from solution is unclear it has primarily been attributed to interactions between the metal and the cell wall (Gadd and White, 1993).

The term biosorption implies a direct interaction between the biosorbent and the metal sorbate. In some cases precipitation of metals may occur in the presence of living microbial biomass and some bacteria and fungi exploit this phenomenon in maintaining viability in the presence of certain toxic metals (Cervantes and Gutierrezcorona, 1994). In the majority of studies biosorption of metals is demonstrated by exposing the biomass to the relevant solution of the cation and separation of the biosorbent and the sorbate by either centrifugation or filtration (McHale and McHale, 1994). In this study we demonstrate removal of uranium from solution using a variety of preparations of biomass derived from a local brewery. Using studies involving dialysis we demonstrate that removal of the uranium from solution by some of those preparations results from a combination of biosorption by the biomass and precipitation by low molecular weight, membrane-permeable materials loosely associated with the biomass.

Materials and methods

Biomass preparations

Spent brewery yeast was obtained from Bass Ireland Ltd., Ulster Brewery, Glen Rd., Belfast, Northern Ireland. The yeast slurry was untreated following fermentation. The yeast was recovered and harvested by centrifugation at 5,000 g for 20 min. and subsequently lyophilised for storage. Preparations of yeast were non-viable following lyophilization. In addition, preparations of yeast were washed twice in distilled water by centrifugation and lyophilized. In some cases the washings were harvested also and utilized in experiments as described below. Homogenized preparations of yeast were obtained by placing 25 g (wet wt; approximate dry wt equivalency = 5 g) quantities of unwashed or washed cells, suspended in 25 ml distilled water in a Braun homogenizer together with an equal volume of glass beads. The cells were homogenized for 1 min. and preparations were cooled using CO_2 throughout that period. The suspended solids in the resulting homogenate were recovered by centrifugation at 20,000 g for 30 min. and these preparations were lyophilized.

Biosorption reactions

Biosorption reactions were carried out in 10 ml volumes containing biomass at a concentration of 2 g dry wt/l and various concentrations of uranyl acetate solution in distilled deionized water. Reactions were allowed to continue for a period of 1 hour and the biomass was subsequently separated from the solutions using filtration through 0.2 μm filtration units. The concentration of uranium remaining in solution was determined using the arsenazo III method described previously (Savvin, 1961). The biosorption capacity (q) (mg uranium/g dry wt biomass) was calculated from the equilibrium concentration (Ce) remaining in solution as described previously (Holan *et al.*, 1993).

Dialysis experiments

Biomass (0.25 g dry wt) was suspended in 10 ml distilled water and placed inside Visking tubing (diam. = 0.5 cm). This was suspended in 240 ml 1 mM uranyl acetate. Dialysis was allowed to proceed for a period of 12 h prior to analysis of uranium removal from solution.

Results and discussion

Non-living biomass derived from brewery residual yeast can remove metals and radionuclides from waste-water streams (Volesky and May-Phillips, 1995; Omar *et al.*, 1996). The biosorption maximum for uranium reported in the former reference was approximately 150 mg/g biomass whereas in the latter, values in excess of 500 mg/g biomass have been quoted. In our laboratories yeast from distillery spent wash, although significantly modified by the adverse conditions during distillation, exhibited biosorption maxima in the region of 180 mg/g biomass (Bustard *et al.*, 1996). Omar *et al.* (1996) found that washing brewery yeast prior to its use in biosorption contact reactions reduced biosorption capacity maxima. Since precipitation may account for significant metal removal from solution using other forms of microbial biomass (Gadd and White, 1993) it was decided to examine the effects of washing on the biosorptive capacity of yeast from a local brewery. To this end, both washed and unwashed non-viable spent yeast preparations were added to solutions of uranium. The amount of uranium removed from solution was calculated from the quantity of uranium remaining in solution under equilibrium binding conditions. The results in Fig. 1 show that a very significant difference in the biosorption capacities for both preparations existed. The maximum biosorption capacity for the washed material was found to be 150 mg/g whereas the observed maximum biosorption capacity for the unwashed material was approximately 360 mg/g dry wt biomass.

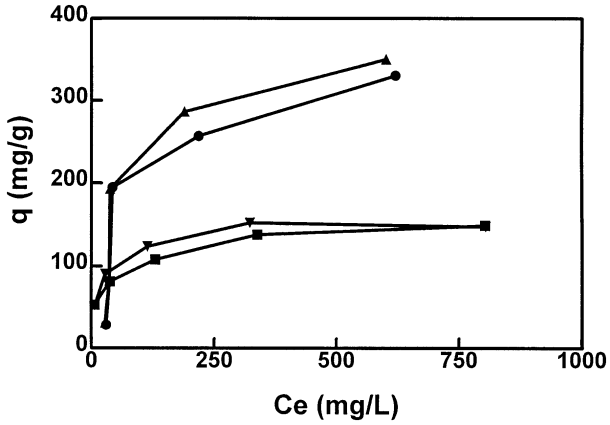

Figure 1 Biosorption of uranium by washed (■) and unwashed (●) non-living brewery spent yeast. Reactions were carried out at 15°C for 1 h and the biosorption capacity (q) and the equilibrium concentration of uranium (Ce) were determined. Studies on biosorption of uranium by homogenized preparations of washed (▼) and unwashed (▲) biomass were also carried out. The homegenates were prepared as described in the Methods section. The results represent the mean values derived from eight experiments.

These results suggested that some component associated with the washings from the biomass contributed significantly to removal of uranium from solution. It has already been suggested that the increased degree of biosorption may be attributed to fermentation products adsorbed onto the yeast cells (Omar *et al.*, 1996).

Since disruption of yeast cells contributes to an increase in biosorption capacity by those preparations (Omar *et al.*, 1996) the preparations used in the current studies were homogenized to determine whether or not this would contribute to increased uptake of uranium from solution. In these studies the biomass was either washed prior to homogenization or left unwashed and the preparations were lyophilized following homogenization. These preparations were then used in biosorption assays and the degree of uranium removal was determined as described above. The results obtained are shown in Fig. 1 and although no significant increase in the maximum biosorption capacities was detected, in both cases a slight but statistically significant increase in biosorption capacity was observed at lower equilibrium concentrations. These observations were not unexpected since the disruption of the material would result in an increased binding surface area. It was however surprising to discover that the unwashed, homogenized material resulted in removal of larger quantities of uranium from solution since the biosorbent material used in these experiments was recovered by centrifugation. These results suggested interaction of the material originally lost by washing with cellular debris.

Table 1 Removal of uranium from solution by samples of non-living brewers yeast

Sample	U_{IN}	U_{OUT}	U_{PPT}
Unwashed	7	26.7	25.3
Washed	35	24	1.3
Wash	1	40.5	17.5

The amounts of uranium are expressed as mg in each fraction. U_{IN} refers to uranium inside the tubing, U_{OUT} refers to uranium outside the tubing and U_{PPT} refers to uranium precipitated outside the membrane. The data reflect the mean values derived from eight experiments.

Since some material, associated with the cells and removed during washing, was responsible for a significant degree of removal of uranium from solution it was decided to establish whether or not that removal resulted from biosorption or a combination of true biosorption and precipitation. To this end the behaviour of washed and unwashed biosorbent in equilibrium dialysis experiments was examined. Both washed and unwashed preparations of the biomass were placed inside dialysis tubing. The tubing was placed in contact with uranium solutions and the systems were incubated overnight at room temperature. The amount of uranium remaining in solution inside and outside the membrane was measured. In performing those studies it was noted that, in the case of unwashed biomass enclosed within the tubing, a significant precipitate formed in the solution outside the tubing. By removing the precipitate from suspension prior to determining uranium concentration, the amount of uranium in solution and in the precipitate could be determined. The results obtained from these studies are summarized in Table 1. When unwashed biomass was enclosed in the tubing 40% of the uranium precipitated outside the membrane. When washed biomass was enclosed in the dialysis tubing only 2% of the total uranium precipitated outside the membrane. When the biomass was washed and the washing from that material was also placed inside dialysis tubing 67% of the uranium was found to precipitate outside the membrane. It was also interesting to note that when the washed biomass was placed inside the tubing a significant amount of uranium was found inside the tubing and it should be noted that most of this uranium was bound to the biomass. Conversely when the unwashed biomass was placed inside the tubing, a lower amount of the uranium was found inside the tubing although again much of this was bound to the biomass. The results suggest that some low molecular weight component(s) associated with the biomass result in the formation of a uranium precipitate outside the membrane. Therefore in conventional biosorption analyses using unwashed material, this removal of uranium from solution would appear as true biosorption and suggest binding of uranium to the biomass. The data however demonstrate that much of the uranium removed from solution by the unwashed biomass occurs as a result of precipitation. Indeed by looking at the bound uranium within the tubing in the unwashed and washed samples in Table 1, this precipitation event would seem to inhibit true biosorption. Although our results suggest that much of the uranium removed from solution by unwashed brewery yeast used in this study is brought about by precipitation, the overall degree of uranium removal is significantly higher than that reported previously by living cultures of yeast (Strandberg *et al.*, 1981). The results presented here suggest that it is important to interpret metal removal from solution by microbial biomass in the context of either removal by precipitation or by direct biosorptive interaction with that microbial biomass.

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