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Use of pelleted and immobilized yeast and fungal biomass for heavy metal and radionuclide recovery

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SUMMARY

The biosorption of uranium, strontium and caesium by pelleted mycelium of two species of fungi, *Rhizopus arrhizus* and *Penicillium chrysogenum* and immobilized *Saccharomyces cerevisiae* was evaluated in both batch and continuous flow systems where the presence of competing cations affected accumulation. The uptake mechanism for the pelleted fungal biomass differed from that of the immobilized yeast, the former being metabolism-independent biosorption of the metals while, in the presence of glucose, uptake in the latter organism was biphasic, surface biosorption being followed by energy-dependent influx. Removal of surface-bound metals was achieved by eluting with mineral acids or carbonate/bicarbonate solutions; a high degree of metal recovery was observed for uranium.

INTRODUCTION

Recent world events have focussed attention on the disposal of heavy metals and radionuclides and prompted research into novel processes for accumulation and recovery of such elements. Removal of radionuclides and heavy metals at their source before discharge into receiving waters currently depends on physical or chemical means and includes such methods as ion exchange and precipitation. Studies with fungal and yeast biomass have shown effective uptake for a range of metal ions including copper, cobalt, caesium, strontium and uranium with a variety of mechanisms being implicated in their uptake [12,13,23,29]. Results using batch techniques have shown that certain fungi, notably *Rhizopus arrhizus* and *Penicillium chrysogenum*, may be more effective at binding uranium than activated charcoal and certain cation-exchange resins [31,32]. Attempts have been made to exploit this ability, both to reduce environmental damage from toxic metals and to recover those with a commercial value [4,5,9,10,27]. For metal removal and recovery, dead fungal biomasss seems to offer several advantages in that it may be obtained cheaply from several industrial sources, is not subject to metal toxicity or adverse operating conditions, needs no nutrient supply and recovery of surface-bound metals may be by relatively simple non-destructive treatments [9,10,31].

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Energy-dependent uptake of divalent cations by *Saccharomyces cerevisiae* is well known [8,25] with influx being dependent on the electrochemical proton gradient across the plasma membrane [3,12,15]. For metals such as cobalt, competition for uptake between cobalt and nickel, zinc or manganese suggests that these metals share a common cation uptake system of low specificity [20]. The surface binding capacity of *S. cerevisiae* appears to be relatively low on an equal dry weight basis with the binding capacities of a range of algae, bacteria and filamentous fungi [21]. The quantity of surface bound cadmium, cobalt and zinc, for example, was greatly exceeded by the amounts subsequently accumulated by energy-dependent influx [20,35]. However, uranium uptake was found to be a surface association only in *S. cerevisiae* and biosorption occurred by the complexation of positively charged uranyl ions with the negatively charged reactive sites on the cell surface [24,28]. "Adsorption" is a term frequently used to describe metabolism-independent uptake or binding of heavy metals to fungal biomass although as it is generally difficult to separate physical and chemical processes in such interactions, the term "biosorption" is now often used to describe this non-directed binding that occurs between metals and cellular components [10,27,32].

For use in industrial or technical operations, freely dispersed biomass has several disadvantages in that it may cause problems in the operation of reactors by blocking flow lines and clogging filters, while separation of biomass and effluent can be difficult and therefore

expensive [31]. Recent interest has centred on the use of living, non-growing biomass either in the form of pelleted mycelium in the case of filamentous fungi or as immobilized preparations for yeasts and other microbes. Several industrial processes use mycelial pellets as a source of enzymes, for example, the production of inulase from *Aspergillus niger* [22] and a variety of other purposes including the production of citric acid and penicillin [34]. The use of fungal pellets to adsorb uranium has been demonstrated with *A. niger*, where adsorption by the biomass was 14-times more efficient than adsorption onto a commercial ion exchange resin [36]. Microbial biomass that is immobilized in substances such as alginic acid or polyacrylamide gels is also of potential for metal recovery [18]. Many methods of immobilizing cells have been developed but entrapment within calcium alginate is one of the simplest and has found widespread use in laboratory and pilot scale studies, including the construction of reactors for waste degradation [6]. Such work demonstrated the ability of immobilized yeast to withstand extended periods of operation. In the case of immobilized *Candida tropicalis*, used in the degradation of phenol, a half life of between 20 and 40 days was observed, whereas free cells could only be used for 20 h [16]. *Streptomyces albus* was immobilized in polyacrylamide gel, crushed to 50–100 mesh size and used in batch and column experiments [19]. Removal of uranium from solution by the immobilized *S. albus* in the absence of an energy source was demonstrated with adsorption being selective and greater amounts of uranium being adsorbed than either cobalt or copper.

This paper describes the removal and recovery of caesium, strontium and uranium from aqueous solution by naturally-pelleted mycelial biomass of two species of fungi, *Penicillium chrysogenum* and *Rhizopus arrhizus* and by *Saccharomyces cerevisiae* immobilized in calcium alginate. Probable uptake mechanisms are discussed and comparisons made of metal recovery by the two forms of biomass.

MATERIALS AND METHODS

Organisms, media and culture conditions. Cultures of *Penicillium chrysogenum* (IMI 26211) and *Rhizopus arrhizus* (IMI 57412) were maintained on malt extract agar (Oxoid). For experiments they were grown in a liquid medium comprising (g l^{-1}): D-glucose, 20.0; $(NH_4)_2SO_4$, 5.0; KH_2PO_4, 0.5; $MgSO_4 \cdot 7H_2O$, 0.2; $CaCl_2 \cdot 2H_2O$, 0.05; NaCl, 0.1; $FeCl_3$, 0.0025; $ZnSO_4 \cdot 7H_2O$, 0.004; $MnSO_4 \cdot 4H_2O$, 0.004; $CuSO_4 \cdot 5H_2O$, 0.0004. Starter cultures were prepared by loop-inoculating 100 ml liquid media and incubating them for 24 h at 25 °C on an orbital shaker (100 rpm). For experimental cultures, 100 ml of medium was inoculated with 1.0 ml of the starter culture to an initial biomass concentration of approximately 0.4 mg dry wt. ml^{-1} and incubated on an orbital shaker (80 rpm) to allow the mycelium to form compact spherical pellets. The stationary-phase mycelial pellets were harvested by filtration and those approximately 5 mm in diameter were washed in distilled water and retained for experiments.

Immobilization of Saccharomyces cerevisiae. Immobilized yeast beads (alginate concentration 1.5% w/v): yeast cells 1.5% (w/v) were prepared by external gelation as follows: 300 ml of water (25 °C) was blended with 9 g of alginic acid (Sigma) and deaerated under vacuum.Pressed baker's yeast (9 g) was hydrated for 30 min in 300 ml distilled water (25 °C) and the cell suspension was mixed into the alginate solution on a magnetic stirrer, taking care to avoid excessive aeration. The alginate-yeast suspension was pumped through a capillary tube (internal diameter 1.0 mm) and dropped into a solution of 50 mM $CaCl_2 \cdot 2H_2O$ where the alginate gelled as beads. The drop height from capillary tip to the solution surface was adjusted to give spherical beads approximately 5 mm in diameter. After hardening for 2 h, the beads were transferred to a 10 mM $CaCl_2 \cdot 2H_2O$ solution and, if necessary, stored at 4 °C prior to use.

Metal uptake in batch systems. Biosorption from 100 μM solutions of CsCl, $SrCl_2$ and $UO_2(NO_3)_2$ made up in 5 mM MES (2{*n*-morpholino ethane} sulphonic acid) buffer adjusted to pH 5.0 with solid tetramethylammonium hydroxide, was achieved in an air-lift column system. The liquid to solid (v/v) ratio was 10 : 1 and air at a rate to keep the pellets or beads circulating was aspirated through a sparger. Circulation of metal solution and biomass was maintained for 3 h with 2 ml duplicate samples of the supernatant fluid being taken every 30 min for metal analyses. The effect of an energy source (50 mM glucose) on the uptake by the immobilized beads and recovery of individual metals from a combined metal solution simulating an aqueous effluent was assessed in the same way.

Metal analyses. Caesium, strontium and uranium were determined by polarographic techniques using a Metrohm 626 polarograph according to the following principles. The current arising from the reduction or oxidation of a chemical species under diffusion controlled conditions is recorded as a function of the applied potential. The current is continually recorded while a linearly increasing negative potential is applied to a hanging drop mercury electrode. In the resulting polarogram the diffusion current is proportional to the concentration of the species in solution while the half-wave potential ($E_{1/2}$) is related to the standard potential of the redox reaction and can be used to identify the species [17]. The detection limit of the

polarograph is in the range 10^{-7} to 10^{-8} M [2] and caesium, strontium and uranium were all easily detectable at the concentrations used in this study. The supporting electrolytes were 0.1 M tetraethylammonium iodide, 0.1 M tetraethylammonium iodide + 20% (v/v) dimethylformamide and 2 M acetic acid/ammonium acetate for caesium, strontium and uranium, respectively, and characteristic half-wave potentials were −2.03 V, −1.84 V and −0.41 V.

Metal desorption. Following initial biosorption of the metals by either the mycelial pellets or the immobilized yeast, desorbing agents were introduced to the column. Either 1 M nitric, sulphuric or hydrochloric acids or a 1 M sodium carbonate/bicarbonate solution (1 : 1, pH 10) were tested for their effectiveness in removing surface-bound metals. Desorption was tested over 3 h with duplicate supernatant samples being taken every 30 min.

Continuous flow systems. With the addition of a pumping system, the batch bioreactor was converted to a continual flow system and the uptake of caesium, strontium or uranium from the combined metal solution (100 μM for each metal) was assessed as before. A liquid to solid ratio of 10 : 1 was maintained as was a flow rate of 50 ml h^{-1}. Desorption of the bound metals with 1 M sodium carbonate/bicarbonate solution was also examined.

RESULTS AND DISCUSSION

Removal of caesium, strontium and uranium from solution by the fungal mycelial pellets and immobilized yeast beads is shown in Fig. 1. For the mycelial pellets, uptake appeared to be virtually complete by 2 h with the majority of metal being taken up after 30 min. A wide variety of ligands may be involved in biosorption of metals and these include carboxyl, hydroxyl, sulphydryl, amine and phosphate groups, although the relative importance of each is difficult to determine [29]. Since many metals have complex solution chemistries, it is not always easy to determine which particular metal species are involved and there may be differences in affinities between ionic species for the various ligands encountered in the biomass [29]. It follows that the efficiency of biosorption can vary considerably between fungal species of differing wall compositions [7,11]. Uptake of caesium (Fig. 1a) was greatest in *P. chrysogenum* with a value of 50% removal and least with *R. arrhizus* with a value of 41%, while for uranium, *R. arrhizus* was the most efficient, being able to remove over 90% of the metal from solution (Fig. 1c). The value for *P. chrysogenum* removal of uranium (62%) compared favourably with that determined by Zajic and Chiu [37] who used a coarse pelleted form of a *Penicillium* species which could remove up to 70% of the uranium supplied from solution. Strontium removal was similar in both *R. arrhizus* and *P. chrysogenum* and was 44% and 39%, respectively (Fig. 1b). Differences in cell wall structure and pellet morphology may account for some of the variation in the uptake values. It may be surmised that the metal ions remaining in solution after initial saturation of

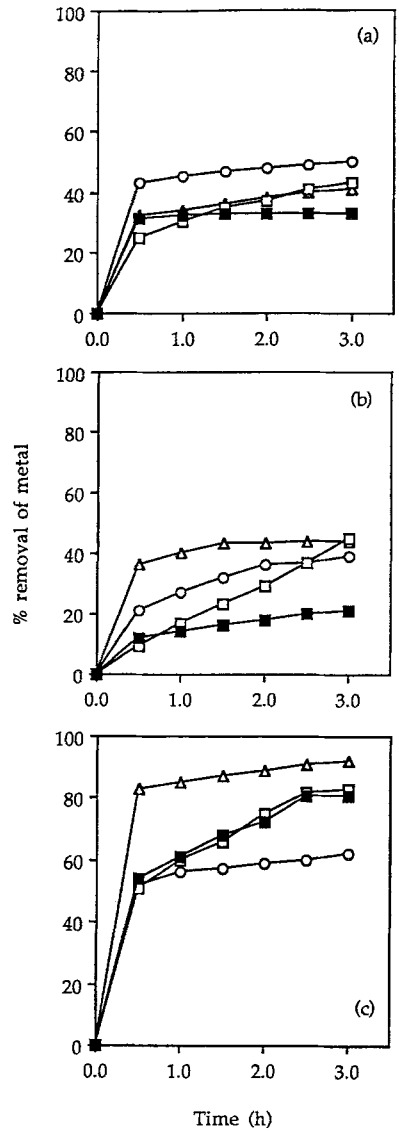

Fig. 1. The percent removal of (a) caesium, (b) strontium and (c) uranium from an initial concentration of 100 μM by mycelial pellets of (△) *R. arrhizus* and (○) *P. chrysogenum* and by immobilized *S. cerevisiae* in the (□) presence or (■) absence of 50 mM glucose. Each point is the mean of two determinations and typical results are shown from one of three experiments. Uptake values for Cs were 82, 119, 179 and 166 nmol (mg dry wt)$^{-1}$, for Sr were 88, 92, 187 and 41 nmol (mg dry wt)$^{-1}$ and for U were 180, 147, 345 and 345 nmol (mg dry wt)$^{-1}$ by *R. arrhizus*, *P. chrysogenum* and *S. cerevisiae* (in the presence and absence of glucose), respectively.

the biomass could be completely removed by subsequent treatment in a separate bioreactor.

The accumulation of caesium, strontium and uranium from their individual metal solutions by alginate-immobilized *S. cerevisiae* in the presence of 50 mM glucose is also shown in Fig. 1, where the greatest uptake was demonstrated for uranium, with 83% of the total available being removed from solution. The least uptake occurred for caesium with 43% removal, while for strontium the value was 45%. The accumulation of caesium and uranium by *S. cerevisiae* appeared to be biphasic with initial surface binding or biosorption being followed by a slower phase of uptake, presumably intracellular. Strontium uptake in the presence of glucose however, exhibited a steady influx, and no surface binding phase was discernible. Comparison of these results with those obtained in the absence of 50 mM glucose allowed an estimate of the contribution of surface binding to total uptake to be made. For caesium this value was 76%, while for strontium only 46% of the total uptake appeared to result from energy-independent biosorption. Only uranium displayed similar kinetics of uptake in the absence as in the presence of glucose, indicating that energy-independent mechanisms were solely involved in the accumulation. Strandberg et al. [28] found that uranium uptake by free dead biomass of *S. cerevisiae* was surface-associated biosorption, with positively charged metal ions being complexed by negatively charged surface binding sites such as $R\text{-}COO^-$. The relatively high value of uptake found by Strandberg et al. [28] suggested that uranium complexed with existing reactive sites on the cell surface and that additional metal crystallized on these bound molecules. Electron microscopic examination showed that uranium accumulated as needle-like fibrils in a layer approximately 0.2 μm thick on the surface of the *S. cerevisiae* with little or no uranium being found inside the cells [28]. It appears therefore that energy-independent biosorption is the main uptake mechanism utilized in the accumulation of uranium by immobilized *S. cerevisiae*. However for caesium, two methods of uptake were demonstrated with some energy-dependent influx being observed (Fig. 1a). It should be noted however, that the pH value of the effluent (pH 5) may not have been favourable to intracellular uptake. The external pH can markedly influence uptake and accumulation of heavy metal and radionuclides and there may be an optimum pH for maximal rates below or above which a decrease occurs [12,13]. Strontium uptake in *S. cerevisiae* was maximal at pH 6.96 [23].

As immobilized yeast beads contain an equal proportion of alginate to yeast, the effect of alginate on the uptake of metals was examined. Fig. 2 shows the biosorption of uranium by immobilized *S. cerevisiae* in beads of varying alginate/yeast ratios as well as beads composed entirely of alginate. Little biosorption occurred in the alginate beads demonstrating that it is not a strong metal biosorbent. Maximal uptake values were found for beads comprising 70% yeast and as the proportion of alginate in the beads increased and that of the yeast was reduced, the value of uranium uptake was reduced.

Since industrially-produced effluents will generally contain more than one metal species, uptake of individual cations from a solution combining caesium, strontium and uranium by either pelleted fungal biomass or immobilized yeast was also examined (Fig. 3). It is evident that biosorption of metals by fungal biomass is a relatively non-specific process with each metal binding site being able to be used by any number of metal species depending on their relative concentrations and chemical properties, the nature of the ligand and external physicochemical factors [10]. Thus, competition for the binding sites limits those available for any single metal species within a mixed metal solution. Removal of individual cations by *P. chrysogenum* from a combined metal solution showed lower values than those obtained for the control for all three metals while in pellets of *R. arrhizus*, removal of caesium and strontium was reduced while that of uranium remained at the control value. Therefore, *R. arrhizus* may exhibit some specificity or selectivity toward uranium, accumulating greater concentrations than those of the other two metals. A similar specificity of *R. arrhizus* for uranium was observed by Nakajima and Sakaguchi [19] who found that uranium uptake from a solution of nine different metal species far exceeded the uptake of any one of the other eight metals. Uranium biosorption by *R. arrhizus* involves three distinct processes [33]. Coordination of uranium to the amine nitrogen of chitin and

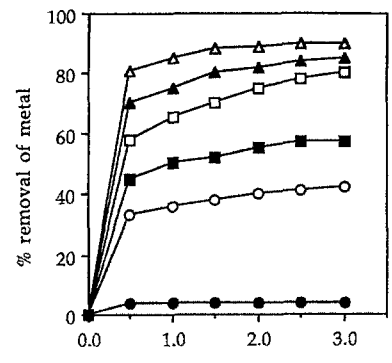

Fig. 2. The percent removal of uranium by immobilized *S. cerevisiae* from an initial concentration of 100 μM. Yeast: alginate proportions (△) 70 : 30, (▲) 60 : 40, (□) 50 : 50, (■) 40 : 60, (○) 30 : 70 and (●) 0 : 100. Each point is the mean of two determinations and typical results are shown from one of three experiments.

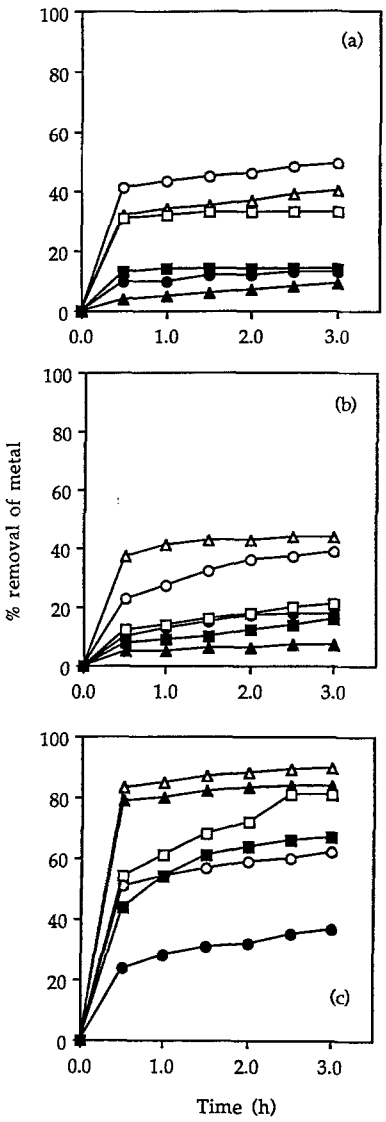

Fig. 3. The percent removal of (a) caesium, (b) strontium and (c) uranium by (△, ▲) *R. arrhizus*, (○, ●) *P. chrysogenum* and (□,■) *S. cerevisiae* from 100 μM single metal solutions (open symbols) and a combined metal solution (100 μM each; closed symbols). Each point is the mean of two determinations and typical results are shown from one of three experiments.

adsorption in the cell wall chitin structure occur simultaneously and rapidly and this is followed by slower precipitation of uranyl hydroxide within the chitin microcrystalline cell wall structure. A free radical on the chitin molecule, possibly assigned to a hydroxyl group appears to be involved in the uranyl ion coordination to nitrogen. This crystallization of uranium onto already bound molecules allows considerable amounts of uranium to be removed from solution and may account for the preferential ability of chitinous fungi such as *R. arrhizus* to bind uranium.

The uptake of individual metals from the combined metal solution, in the absence of glucose, by immobilized *S. cerevisiae* is also shown in Fig. 3. For all three metals, the final uptake values were reduced when compared to those obtained for uptake of the cations from single metal solutions, reflecting the possible competition for binding sites. Uptake of strontium was the least affected with a reduction in uptake of only 14% compared with a reduction of uptake of 57% for caesium and 17% for uranium. For uranium, the high affinity of the surface-reactive sites, combined with the probable crystallization of the metal in the cell wall maintains the uptake of uranium at high levels even in the presence of competing cations. Since active influx is not a feature of the uranium uptake mechanism, there will be no competition for transport sites.

For any proposed industrial metal/radionuclide recovery system, uptake onto microbial biomass constitutes an initial phase of the process but this must be followed by a recovery phase. Technical applications of radionuclide accumulation will ultimately depend on the ease of element recovery either for subsequent reclamation or for further containment or concentration of the isotopes. The simplest and cheapest method of recovering surface bound metals from microbial biomass is to wash or elute the metal from the surface by means of an appropriate desorbing agent. Fig. 4 shows the effectiveness of 1 M mineral acids (only the results for nitric acid are shown for clarity) and a 1 M solution of sodium carbonate/sodium bicarbonate to desorb radionuclides from the loaded biomass. The initial uptake values for caesium, strontium or uranium were taken as 100%. Desorption is therefore expressed as percentage removal of the metal relative to this initial loading. There was little difference in the overall efficiency of each of the mineral acids to desorb the metals and it can be concluded that the efficiency of desorption depends on the H^+ concentration rather than on the anionic species present. The ability of mineral acids to act as desorption agents has been widely demonstrated [7,30]. Tsezos [30] showed recovery of uranium adsorbed onto waste biomasss of *R. arrhizus* by elution with dilute mineral acids. Yakubu and Dudeney [36] observed values of 80–90% desorption of uranium from *A. niger* with the use of mineral acids. The acids hydrochloric, nitric and sulphuric were used because of their relatively low cost and because uranium is reported to be highly soluble in acids. Furthermore, studies of uranium uptake indicated that hydrogen ions can compete effectively with uranium to produce a reduced uptake by the biomass [32]. Fig. 4a shows the desorption of caesium from loaded biomass. The greatest efficiency of desorption was observed for the mycelial pellets with values of between 74 and 90%, while for the yeast beads only 12–18% removal of the loaded

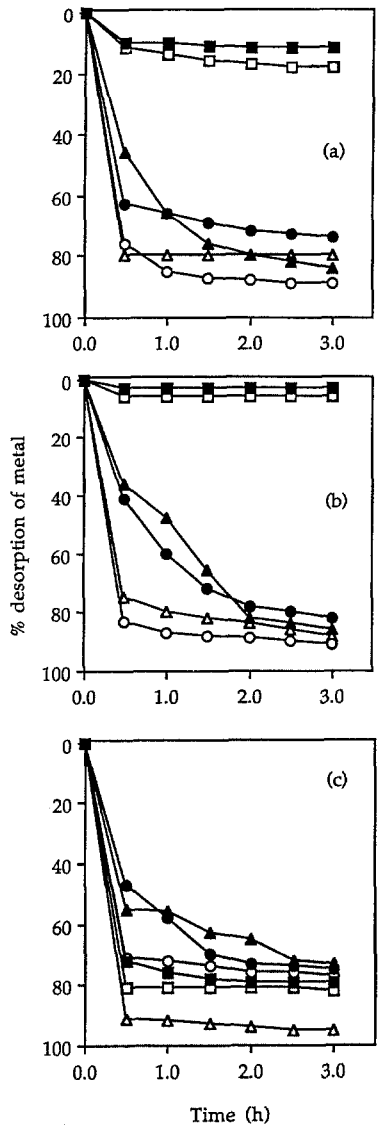

Fig. 4. The percent desorption of (a) caesium, (b) strontium and (c) uranium from loaded biomass of (△, ▲) *R. arrhizus*, (○, ●) *P. chrysogenum* and (□, ■) *S. cerevisiae* by 1 M HNO_3 (open symbols) and 1 M Na_2CO_3/$NaHCO_3$ (closed symbols). Each point is the mean of two determinations and typical results are shown from one of three experiments.

caesium was recorded. For strontium only 3–6% of the metal was removed from the yeast biomass by either mineral acids or by the carbonate/bicarbonate solution (Fig. 4b), indicating perhaps that the desorbing agent chosen was not suitable or that the metal was bound in such a way as to make desorption difficult. For the fungal biomass however, the desorption values ranged from 82 to 91%. The greatest efficiency of desorption was observed for uranium and biomass of *R. arrhizus* where a value of 95% was recorded. Uranium desorption from the immobilized *S. cerevisiae* by both desorbing agents was also high when compared to the desorption of either caesium or strontium for the same biomass, with values of 79 and 82% being recorded. Generally mineral acids were more efficient at desorption than the carbonate solution. Sodium carbonate was shown to be a very effective eluant for uranium from loaded *R. arrhizus* biomass [30]. The elution pH for Na_2CO_3 is in the alkaline range of pH 11–12 and at these pH values, there is increased formation of uranium complexes with the carbonate [1,26]. This high affinity of the carbonate ion for uranium shifts the equilibrium in favour of the liquid phase (eluent) and brings the uranium out of the biomass and into the eluate. Uranium uptake from seawater by microbial biomass is reduced by the presence of the carbonate species, mainly by keeping uranium in solution via the uranyl carbonate series of complexes [14].

This study demonstrates that both "naturally immobilized" fungal pellets or yeast cells immobilized in an alginate matrix can be used for effective removal and subsequent recovery of radionuclides in batch systems. However, for larger scale applications, the use of continual flow systems is envisaged. Uptake of caesium, strontium and uranium from a combined solution, in the absence of an energy source, was examined using a continual flow system where the effluent flow was maintained at a rate of 50 ml h^{-1} to be circulated through the biomass. For both mycelial pellets and immobilized yeast, decreased efficiency in uptake was observed (Fig. 5). Generally, the values were between 30 and 70% of those recorded for the batch experiments. Only the uptake of uranium by *S. cerevisiae* approached a similar value (75% uranium uptake, approximately 90% of the value obtained in the batch study). The efficiency of the desorbing solution to remove the metal from the loaded biomass was also reduced in the continual flow system, but not to so great an extent. The desorption values were between 60 and 100% of the batch study values although it should be noted that only the carbonate solution was used, as prolonged use of the mineral acids destroyed the integrity of the yeast beads leading to loss of biomass. Alginate beads were apparently unaffected by the mineral acids used over times ≤ 3 h. There was also an increase in the time taken for desorption (Fig. 6).

Both pelleted and immobilized forms of fungal biomass have proved capable of operating in continual flow systems, albeit for short residence times. However, the operating efficiency of the system needs to be improved. Containing the biomass in the form of pellets or beads makes handing and separation of the biosorbent and effluent easier and allows for a greater degree of control. However, such metal recovery systems are not yet sufficiently well developed to replace current technologies, but they could nevertheless be used as an ad-

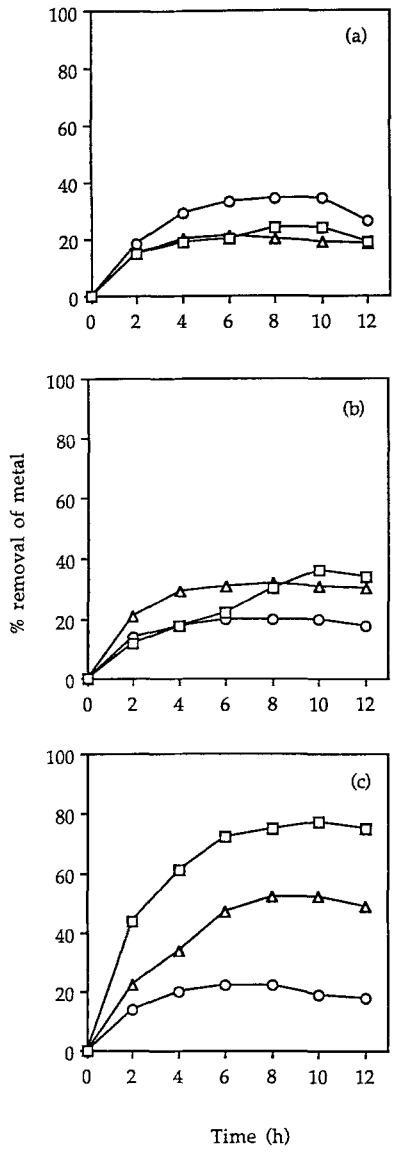

Fig. 5. The percent removal of (a) caesium, (b) strontium and (c) uranium from a combined metal solution (100 μM for each metal) by mycelial pellets of (△) *R. arrhizus*, (○) *P. chrysogenum* and by (□) immobilized *S. cerevisiae* in a continual flow system. Each point is the mean of two determinations and typical results are shown from one of three experiments.

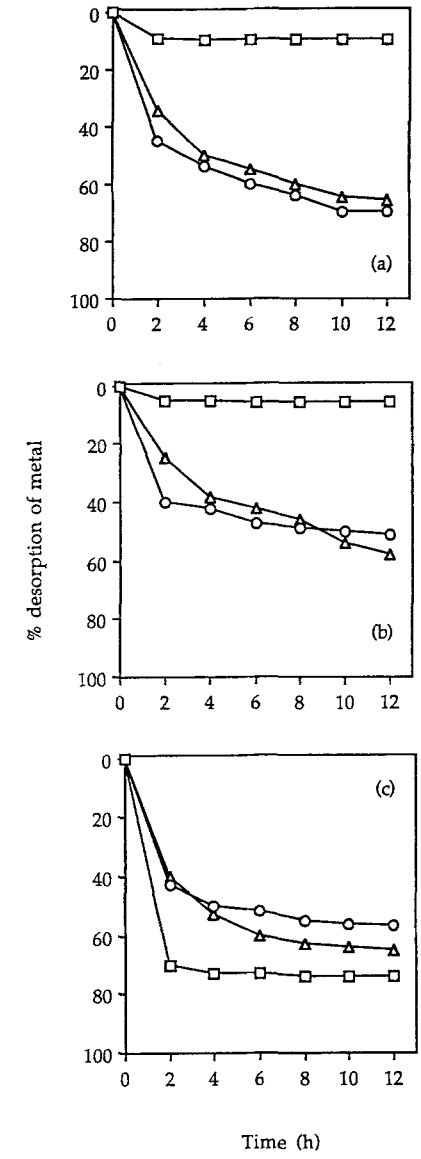

Fig. 6. The percent desorption of (a) caesium, (b) strontium and (c) uranium from loaded biomass of (△) *R. arrhizus*, (○) *P. chrysogenum* and (□) *S. cerevisiae* by 1 M $Na_2CO_3/NaHCO_3$ in a continual flow system. Each point is the mean of two determinations and typical results are shown from one of three experiments.

junct to existing processes and be utilized as a concentration stage so that recovery from very dilute solutions is a preliminary step to other conventional treatments. Alternatively, the immobilized biomass may be used as a polishing stage for removing trace concentrations of metals that ion exchange resins cannot deal with.

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