Appl Microbiol Biotechnol (1993) 39:661–665

Applied and Microbiology Biotechnology

Uranium uptake by immobilized cells of *Pseudomonas* strain EPS 5028

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Received: 2 November, 1992/Accepted: 26 January, 1993

Abstract. Polyacrylamide-gel-immobilized cells of *Pseudomonas* strain EPS 5028 were effective in the removal of uranium (U) from synthetic effluents. Metal accumulation was performed in an open system in columns filled with immobilized cells that were challenged with continuous flows containing U. Possible variables of the system were studied. Uranium uptake by the immobilized cells of this microorganism was affected by pH but not by temperature or flow rate. In addition, U binding could be interpreted in terms of the Freundlich adsorption isotherm indicating single-layer adsorption. The feasibility of reusing the immobilized cells was suggested after the recovery of U with a solution of 0.1 M sodium carbonate.

Introduction

Some of the aqueous discharges emanating from industrial processes such as mining, smelting, metal-plating, ore-processing activities and energy-production processes, contain dissolved heavy metals that, due to their chemical or radiological characteristics, can generate significant environmental problems (Shumate et al. 1978). The use of microbial biomass for the detoxification of industrial effluents for environmental protection and/or recovery of valuable metals offers a potential alternative to existing technologies (chemical precipitation, chemical oxidation and reduction, ion exchange, filtration, electrochemical treatment and evaporative recovery; Norberg and Person 1984).

Preliminary studies have shown that living or dead cells of *Pseudomonas* EPS 5028, an exopolysaccharide-producing microorganism, can accumulate uranium (U) from solution (Marqués et al. 1991) and its extracellular polysaccharide has also been used for U adsorption (Marqués et al. 1990). However, free cells cannot be used for a long period owing to their mechanical instability and their susceptibility to microbial degradation. In addition, free cells are not suitable for use in a column; due to their low density and size they tend to plug the bed causing a large drop in pressure. To overcome these deficiencies in free cells, an immobilization process converts microbial biomass to a particulate form for use as a conventional adsorbent (Tsezos 1988).

The use of immobilized microbial cells as biosorbents of U has been described by various authors (Nakajima et al. 1982; Macaskie and Dean 1985, 1987, 1988; Tsezos 1988). Reclamation of U from polluted streams would be doubly beneficial, in the purification of U from industrial processes or waste-waters and also in the recovery of the metal for reuse. We have studied the factors that may affect U uptake by *Pseudomonas* EPS 5028 when whole cells are immobilized in a polyacrylamide gel and packed into columns.

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Materials and methods

Organism. A Gram-negative rod designated as *Pseudomonas* sp. strain EPS 5028 was isolated from Barcelona soil and selected on the basis of the yield of its extracellular polysaccharide (Congregado et al. 1985; Fusté et al. 1986).

Growth media. The organism was maintained on Trypticase Soy Agar by weekly transfer to fresh medium. Cells for subsequent immobilization were obtained by culturing in glucose mineral salts medium (GMS) (Marqués et al. 1991) at 30° C with vigorous forced aeration for 48 h. Bacteria were harvested during the stationary phase of growth to ensure the maximum biomass for immobilization experiments.

Immobilization of the organism in a polyacrylamide gel and determination of U uptake by immobilized cells. The biomass was harvested by centrifugation at 8000 *g* for 20 min, washed three times with deionized distilled water and resuspended in 20 ml sterile water (an aliquot of this cell suspension was dried at 105° C for determination of dry cell weight) supplemented with 2.5 g acrylamide monomer and 0.25 g *N,N'*-methylenebisacrylamide as a cross-linking agent. The polymerizing reaction was initiated by adding 2.5 ml of a 2.5% (w/v) solution of potassium persulphate, and accelerated by adding 2.5 ml of a 5% (w/v) solution of 3-dimethylaminopropionitrile. The suspension was shaken gently until the gel began to set and was then refrigerated at 4° C

throughout polymerization to prevent thermal damage to the cells. The gels were prepared in a plastic cylinder stoppered at each end. When the gel had set, these stoppers were removed and the gel was shredded by forcing it through a plastic sieve. The shredded gel was packed in a glass column (33 cm × 2.5 cm) washed with 1 l deionized distilled water and allowed to drain. A cell-free gel was prepared and similarly washed and drained. Both columns were then challenged with aqueous solutions containing uranyl nitrate hexahydrate (Merck, Darmstadt, Germany), pH 4.0–4.3, equivalent to 100 μg/ml of U, at a flow rate of 80 ml/h at 22° C. Any deviations from this standard procedure were noted. A fresh column was used for each experiment. Cell-free controls were run concurrently in all experiments.

Determination of the activity of the immobilized cell columns. The column outflows were assayed for residual U content by the method of Savvin (1961), using a 0.1% solution of arsenazo III (Aldrich) prepared following the procedure of Shumate et al. (1978). Arsenazo III reagent gives a red complex with U, which was estimated at 650 nm (Kontron Uvicon 810 spectrophotometer) versus a freshly prepared solution of uranyl nitrate (standard curve). Since the inflow and outflow concentrations were known, the U loads of the column were calculated from the difference. Column activity was expressed as the percentage removal of U throughout.

Release of U from the column. Four EPS 5028 immobilized cell columns (330–340 mg bacterial dry weight) that had taken up U from 125 ml of U solution were each treated (3 h) with 50 ml of different agents that solubilize or complex U to remove it from the cells. The columns were eluted with 0.1 M sodium carbonate, 0.1 M sodium citrate, 0.1 M disodium ethylenediaminetetraacetic acid (EDTA) and 0.01 M sodium oxalate, respectively. To determine whether surface binding sites were altered by these treatments, the columns were washed with deionized distilled water and re-exposed to U twice. The activity of the recycled columns was determined as described before.

A more detailed study of sodium carbonate eluent was performed, because of the results obtained with previous experiments (data not shown). Three columns with different bacterial dry weight (177 mg, 593 mg, 773 mg) and one cell-free column were eluted with 150 ml of 0.1 M sodium carbonate after 3 l of U solution had passed through each column. The treated cells were washed with deionized distilled water and re-exposed to U.

Uranium uptake by immobilized dead cells. To study the effect of physiological state, a bacterial suspension was heated (100° C, 15 min) to kill the cells. No cell could be cultured after this treatment. Immobilized dead cells were tested for U adsorption. An immobilized living cell preparation was used as a control.

Results

Effect of cell concentration on the uptake of U

Accumulation of U by immobilized living cells of *Pseudomonas* EPS 5028 was studied at different cell concentrations from 155 mg to 1054 mg of bacterial dry weight. As the cell concentration increased, the amount of U adsorbed by each cell (specific uptake) decreased, whereas the total amount of U adsorbed increased. These results are shown in Fig. 1.

Effect of the external U concentration on the uptake

Uranium accumulation was studied over a range of 5–875 μg U/ml. As shown in Fig. 2, the total uptake was

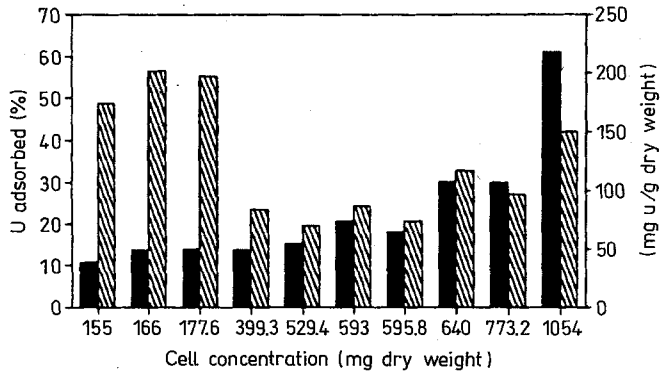

Fig. 1. Effect of cell concentration on the uptake of uranium (U). Columns of different cell dry weight were challenged with 2.5 l of U solution (100 mg/l, pH 4.0) at 80 ml/h and room temperature: ■, total U adsorbed; ▩, mg U/g dry weight

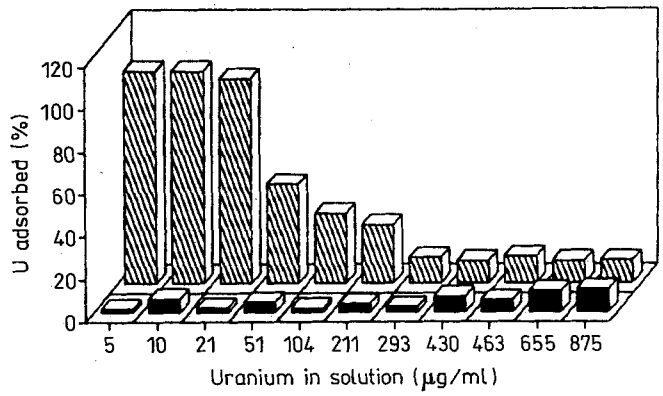

Fig. 2. Effect of external U concentration on U uptake. Immobilized cells (502–737 mg dry weight) were challenged with 2 l of U solution (pH 4.0) at 80 ml/h and room temperature: ■, cell-free gel; ▩, immobilized-cell gel

greatest at low external concentrations (5, 10, 21 μg/ml), and efficiency decreased as the U concentration in solution increased up to 21 μg/ml. Uptake stabilized at U concentrations higher than 293 μg/ml, although the specific amount of U per unit biomass increased with increasing external U concentration, from 60 mg U absorbed/g dry weight (at 21 μg/ml) to 320 mg U absorbed /g dry weight (at 875 μg/ml). It should be noted that at high concentrations (655–875 μg U/ml) the uptake observed using a cell-free column was similar to the uptake by the immobilized cells. These results are in agreement with previous results for U uptake by free cells of *Pseudomonas* EPS 5028 (Marqués et al. 1991). The relationship between the concentration of residual U in solution and the amount of U absorbed per unit biomass was determined by applying the Freundlich isotherm model, $Q_e = K_j \cdot C^{1/n}$, which can be linearized using a log-log plot (Tsezos and Volesky 1981): Q_e is the amount of U absorbed per gram of dry cells, C is the residual U in the solution per millilitre and K_j and n are constants.

As shown in Fig. 3, a straight line is obtained when $\log Q_e$ is plotted versus $\log C$, although at very low so-

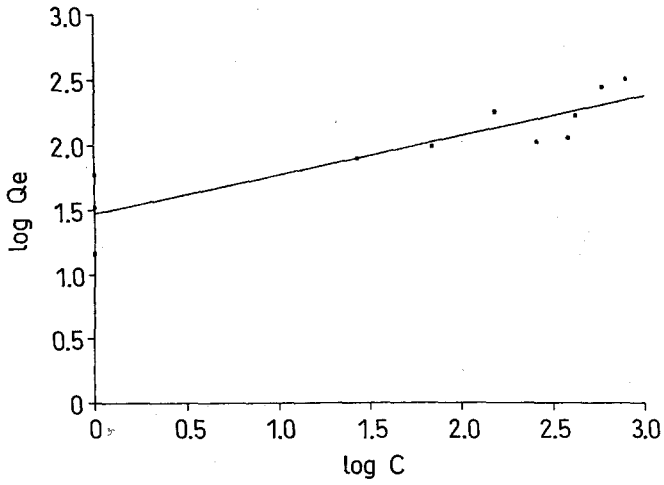

Fig. 3. Linearized Freundlich U adsorption isotherm. Log Q_e (mg U absorbed/g dry weight) is expressed as a function of log C (concentration of U remaining in solution)

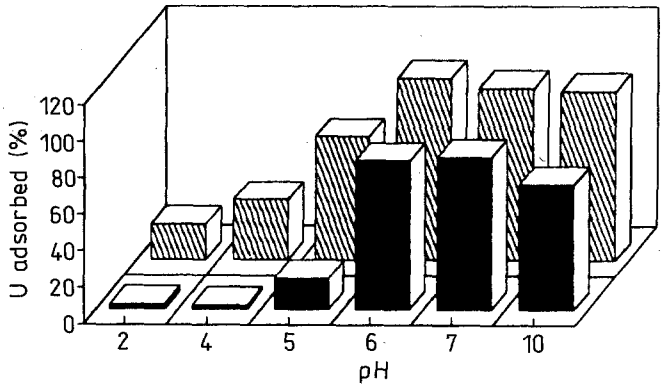

Fig. 4. Effect of solution pH on the uptake of U. Immobilized cells (500–700 mg dry weight) were challenged with 2 l of U solution (100 mg/l) at 80 ml/h and room temperature: symbols as for Fig. 2

lute concentrations, the adsorption expression was not linear. The Freundlich isotherm is related to single-layer adsorption at the cell surface.

Effect of flow rate and temperature

Uranium removal (at 100 μg/ml) was not influenced by the flow rate in the range 11–188 ml/h. The accumulation of U by EPS 5028 cells was determined at 4, 22, 33° C. Although the process was a little more efficient at 33° C, similar activities were obtained at 4 and 22° C.

Effect of solution pH

As shown in Fig. 4, the initial pH of the solution had a significant effect on metal uptake. The total U uptake by the immobilized cells increased as the pH increased from 2.0 to 10.2. The maximum U accumulation was obtained at pH 6.0 (99% of U removed). It was found that cell-free columns showed high rates of U accumulation as the pH increased from 5.0 to 10.2. The accumulated metal was evident as a yellow precipitate in the gels with or without cells. No U deposition was observed at pH 2.0 and 4.0. At these pH values U accumulation by immobilized cells was much higher than in cell-free columns.

Removal of the loaded U from the columns

Immobilized *Pseudomonas* EPS 5028 cells were studied through several adsorption-desorption cycles to test different agents as desorbents to recover U from loaded columns. As shown in Fig. 5, sodium carbonate (0.1 M) was the most effective, removing 60% of the bound U with the first washing. Because the rate of uptake in the three adsorption cycles was maximal it was not possible to see any effect on metal uptake. Sodium citrate (0.1 M) removed 24% with the first washing. A slight decrease in the metal uptake was observed after this treatment. EDTA (0.1 M) and sodium oxalate (0.01 M) removed 27% and 35%, respectively, with the first wash. In both cases the further metal uptake capacity of the cells was not influenced. For all four treatments, the amount of U recovered slightly increased with the second and the third washes.

Figure 6 illustrates the efficiency of desorption of sodium carbonate (0.1 M) and the effect on metal uptake. Three columns with immobilized cells (177 mg, 593 mg, 773 mg dry weight) and one cell-free column were challenged with fresh U flows, and eluted in situ with 0.1 M sodium carbonate to recover U from the columns as an intense yellow solution. Sodium carbonate enhanced the total U uptake from 2% (cell-free column), 12%, 18% and 26% to 24% (cell-free column), 24%, 27% and 38%, respectively. Moreover, the amount of U released was slightly increased with the

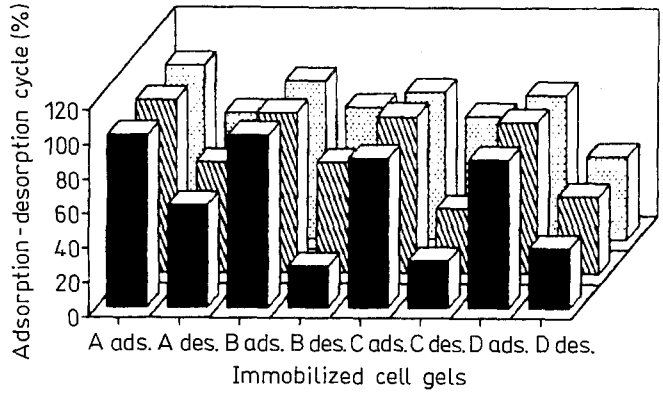

Fig. 5. Effect of desorption agents on uranium uptake: ads., adsorption; des., desorption. Immobilized cells (331–342 mg dry weight) were challenged with 125 ml of U solution (100 mg/l, pH 4.0) at room temperature: gel A (des. with 0.1 M sodium carbonate); gel B (des. with 0.1 M sodium citrate); gel C (des. with 0.1 M EDTA); gel D (des. with 0.01 M oxalate); ■, first cycle; ▨, second cycle; □, third cycle

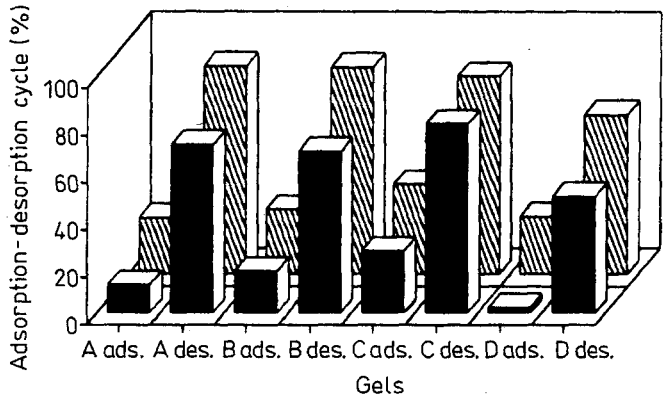

Fig. 6. Effect of 0.1 M sodium carbonate on U uptake: gels (A, 177 mg dry weight; B, 593 mg dry weight; C, 773 mg dry weight; D, cell-free gel) were challenged with 3 l of uranium solution (100 mg/l, pH 4.0) at 80 ml/h and room temperature: symbols as for Fig. 5

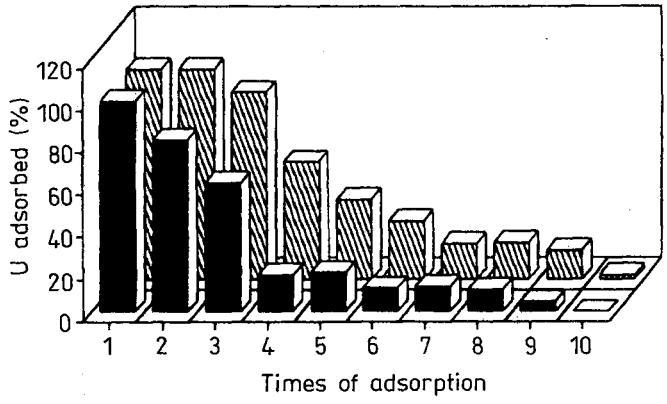

Fig. 7. Effect of the physiological state of cells on U uptake. Immobilized cells [living cells, 592 mg dry weight (■); dead cells, 642 mg dry weight (▩)] were challenged with 2.5 l of U solution (100 mg/l, pH 4.0) at 80 ml/h and room temperature

second wash, from 49% (cell-free column), 71%, 68% and 80% to 68% (cell-free column), 88%, 88% and 84%, respectively.

Uranium uptake by immobilized dead cells

As shown in Fig. 7, the uptake of U by immobilized *Pseudomonas* EPS 5028 cells was higher after heat treatment. The percentage total U adsorbed by heat-killed cells was 47% higher than in living cells.

Discussion

Surfaces of cells are usually anionic and bacterial walls are not an exception. It is to be expected that the polyanions of the wall would interact with and bind the cations of the aqueous environment. Gram-positive walls are potent metal chelators. Carboxyl groups of the D-glutamic acid residues of the peptidoglycan are the most potent metal scavengers in the wall of *Bacillus subtilis* (Beveridge and Murray 1980). Gram-negative envelopes are structurally and chemically different. Beveridge and Koval (1981) suggested that in *Escherichia coli* K-12 metals interact with the polar head groups of the phospholipids, available anionic sites of the lipopolysaccharide and the acidic groups of exposed polypeptides.

If a multiplicity of potential accumulation sites occurs in the cell wall, the accumulation of U should increase when the cell concentration is increased. Several authors have suggested that the electrostatic interactions between cells may be a significant factor in the biomass concentration dependency, increasing the adsorption when the distance between cells is great. However although the specific uptake (mg U/g dry weight) may be lower at high biomass concentration, the total removal of metal from solution is higher (Gadd et al. 1988). On the other hand, a high biomass concentration could make a "screen" effect of the dense outer layer of cells, protecting the binding sites from the metal. Furthermore some cells could be released from the gel (Nasri et al. 1987).

Uranium uptake decreased as the U concentration in solution increased. This corresponds with previous results for U uptake by free cells of *Pseudomonas* EPS 5028 (Marqués et al. 1991). Despite the complexity of the adsorption process, which can include cation-exchange, complexation, etc., adsorption isotherms have been used to characterise metal uptake and they appear to be of use for projected industrial applications (Tsezos and Volesky 1981). Hence, it was decided to fit the available biosorption data with one of the most widely accepted adsorption isotherm models, namely that of Freundlich. This model is a special case for heterogeneous surface energies and is related to a monolayer adsorption at the cell surface.

The uptake of U by *Pseudomonas* EPS 5028 immobilized cells conformed to the Freundlich isotherm in the range of concentrations studied. This is in accordance with the finding that metal uptake was unaffected by temperatures of 4, 22 and 33° C. It suggests that the accumulation of U by immobilized *Pseudomonas* EPS 5028 cells seems to be dependent on physicochemical adsorption at the cell surface and not on biological activity. Similarly, U uptake was not significantly affected by flow rate. These results agree with those found by other authors (Macaskie and Dean 1984).

Studies on the uptake of U by immobilized biomass were complicated by the nature of both the adsorbent particles and the metal species in aqueous solution. Environmental changes can affect reactive metal-binding sites, but also the solution chemistry of U is quite complex. When we chose polyacrylamide polymer as our immobilization matrix we were not concerned about its metal-binding capacity because other authors working this field had not referred to it (Macaskie and Dean 1984, 1985, 1987, 1988; Nakajima et al. 1982). Cell-free columns showed high rates of U accumulation as the pH increased from 5.0 to 10.2. This occurrence can be explained by the fact that polyacrylamide

polymer has many negative charges to bind U under these ionic conditions. Thus the U accumulation may be due to the combined effect of the gel and the cells entrapped in it. From pH 2.0 to 4.0 U accumulation by immobilized cells was much higher than in cell-free columns.

Hydrolysis of the uranyl ion, for a total U^{6+} concentration of 100 μg/ml at pH 4.0, gives about 80% of U^{6+} in the form of UO_2^{2+}, while at pH 5.0 only 9% is in this form and it is the only species present at pH 2.0 (Tsezos and Volesky 1981). Strandberg et al. (1981) suggested that UO_2^{2+} may be the first U species biosorbed. If we take all these findings into consideration it may be possible to understand the difference in behaviour of free cells of *Pseudomonas* EPS 5028, which showed the highest amount of U accumulated at pH 3.0 whereas the total uptake decreased as the pH increased from 3.0 to 11.0 (Marqués et al. 1991). There was no significant difference in U uptake by the cell-free and immobilized cell gel at pH 6.0. In both cases nearly 100% of the U was adsorbed. Concerning these results, it certainly seems to be much more effective to use a cell-free gel at pH 6.0 than an immobilized cell gel at pH 4.0. However, at pH values above 5.0, solubility of U is very low and precipitation of U oxides in the metal solution occurs (Tsezos and Volesky 1981; Tobin et al. 1984). This limitation imposed by the U solution must be considered to avoid practical problems in industrial applications.

The most effective agent tested to remove bound U from the gels was 0.1 M sodium carbonate. This treatment allowed reuse of the gel for U adsorption but afterwards the gel's capacity for U adsorption was higher. A similar effect is described by Strandberg et al. (1981). It has been demonstrated that dead cells accumulate heavy metals to the same or a greater extent than living cells (Tsezos and Volesky 1981; Tsezos 1988). The reasons for this include immunity from metal toxicity and other adverse operating conditions, availability and ease of manipulation (Gadd et al. 1988). The total amount of U accumulated by dead cells in the present study was also higher than that taken up by living cells. It is possible to understand this behaviour because denaturation of the cell wall and cell membrane would leave the U binding sites much exposed to the metal. This result, added to the finding that U uptake is not influenced by temperature, suggests that accumulation of U by *Pseudomonas* EPS 5028 is presumably not directly mediated by any metabolic process. It is consistent with the view that U adsorption occurs by the complexation of positively charged U ions with negatively charged reactive sites of the cell surface.

Acknowledgement. This research was funded by grant PA 86-0299 from the Comisión Asesora de Investigación Científica y Técnica.

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