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URANIUM ACCUMULATION BY IMMOBILIZED CELLS OF A *CITROBACTER* SP.

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ABSTRACT

Uranium was removed from challenge flows presented to immobilized cells of a *Citrobacter* sp. In excess of 90% of the presented metal was recovered, giving high yields of accumulated metal which could be subsequently released from the immobilized cells *in situ*.

INTRODUCTION

Immobilized cells of a strain of a species of *Citrobacter* scavenge divalent heavy metal cations with high efficiency from challenge flows (Macaskie and Dean, 1984a,b,c). The optimal conditions for metal uptake have been elucidated as a combination of the pre-growth conditions of the cells (Macaskie and Dean, 1984a) and the composition of the metal solution forming the challenge (Macaskie and Dean, 1984b). Of critical importance is the presence of an organic phosphate in the flow; the activity of a phosphatase enzyme (induced during pre-growth in medium containing glycerol 2-phosphate as the sole phosphorus source) persists in the immobilized cells, cleaving the organic phosphate to yield inorganic phosphate which precipitates with the metal as insoluble metal phosphate at the cell surface (Aickin *et al*, 1979). Furthermore, when the sites on the cell surface are saturated precipitation of metal phosphate continues in the gel until it is physically impossible to pump more liquid through the column.

The metals previously tested (cadmium, copper and lead) are often present at high levels in wastes from various industrial processes, and the immobilized *Citrobacter* cells have proved successful in the treatment of metal mixtures typically found in process outflows (Macaskie and Dean 1984c). These metals were presented as simple divalent metal cations (Cd^{2+}, Cu^{2+}, Pb^{2+}) and attention is now turned towards the more complex (Baes and Mesmer, 1976) uranium. Recovery of this metal from polluted streams would have a dual benefit in the purification of uranium waste solutions and mine run-offs, and also in the recovery of the metal for re-use; in this context it has been established using Cd^{2+} that the accumulated metal could be readily recovered from the cells (Macaskie and Dean, 1984b).

The use of microbial cells as biosorbents of uranium has received attention by various workers (e.g. Tsezos and Volesky, 1981

Strandberg *et al*, 1981, Nakajima *et al*, 1982, Tsezos and Volesky, 1982, Norberg and Persson, 1984, Galun *et al*, 1984), but in these cases metal accumulation was performed in a closed, batch system. By contrast the system described by Macaskie and Dean (1984a,b,c,) and in this study operates as an open system whereby the immobilized cell columns are challenged with continuous flows for extended periods to accumulate extremely high loads of metal. Using cadmium it was found that 2·5-3·0g of Cd^{2+} was held by a gel containing 5g wet weight(corresponding to 0.5g dry weight) of organisms, representing a challenge of several weeks with a flow containing 200µg/ml of Cd^{2+}, with activity maintained at 95-96% removal of the metal, sustained throughout (Macaskie and Dean, 1984b). At the very low concentrations of metal also treated (e.g. 0·1µg/ml; Macaskie and Dean, 1984c), the lifetime of each column would be greatly extended since the duration of the experiment is limited only by the amount of metal precipitate that can be held before column blockage by the accumulated metal occurs (Macaskie and Dean, 1984b). When this occurs the columns are stripped of the metal and regenerated for re-use (Macaskie and Dean, 1984b).

Thus this approach has several distinct advantages. In addition to those described above, one important consideration is that once installed a bacterial filter of this type would run unattended and maintenance-free, an important economic consideration. This communication reports the successful application of this method to treat flows supplemented with uranyl ions, and the performance is compared to that previously obtained using divalent metal cations.

MATERIALS & METHODS

Organism The *Citrobacter* sp. was as described previously (Macaskie and Dean, 1984a,b,c).

Growth of the organisms and preparation of immobilized cell columns The *Citrobacter* sp. was grown aerobically in batch culture (31) at 30°C in minimal medium as described previously (Macaskie and Dean, 1984a,b,c). Glycerol 2-phosphate provided the sole phosphorous source while the carbon source was glycerol. Cell harvests were performed during the stationary phase of growth (48h) and 5g wet weight of cells washed with isotonic saline (8·5g NaCl/1) were immobilized in a polyacrylamide gel as described previously (Macaskie and Dean, 1984a,b,c). The gels were shredded (8 mesh/cm sieve) and packed into glass columns (10cm x 3cm), washed with isotonic saline (21) and drained. The columns and tubing were pre-equilibrated with buffer (tri-sodium citrate-citric acid, 2mM), containing 5mM glycerol 2-phosphate (B.D.H. Ltd.), pH 6·8, and similarly drained. A solution of this buffer, supplemented with uranyl nitrate hexahydrate ('Analar', B.D.H. Ltd.) equivalent to 200µg/ml of uranium was then pumped upwards through the column (60ml/h; 20°C).

Determination of the activity of the immobilized cell columns The uranium content of the column outflow was determined by the method of Moeken and Van Neste (1967) using a rhodamine B reagent. This gave a red complex with uranium, which was estimated at A 555nm (Pye Unicam SP 600 spectrophotometer), versus a freshly prepared solution of uranyl nitrate in 2mM citrate buffer at the uranium concentration anticipated (by prior trial experiments) to be present in the column outflow. Since the input concentration of uranium was 0·84mM, the citrate in the column buffer was present at a 2·5-fold excess, an important consideration in the preparation of calibration solutions

as uranium complexes strongly with carboxyl groups (Dounce and Flagg, 1949). Since the input and output concentrations were known, the uranium loads of the columns could be calculated by difference.

Recovery of the loaded uranium from the immobilized cells When the columns had accumulated heavy loads of uranium, the column feed tube was removed and the column was allowed to drain. The column was then eluted with 1M citrate buffer, pH 4·0 at a flow rate of 60ml/h. Under these conditions uranium was eluted from the columns as an intense yellow solution which could be read directly at A 435nm versus a calibration of 5mg/ml of uranium in 1M citrate buffer, pH 4·0. The calibration in citrate buffer was linear with respect to uranium concentration, and as confirmation was assayed (suitably diluted) also by the rhodamine B method described above. The results obtained by both methods were identical, and this more rapid method was adopted for the high concentrations of uranium in the unloaded column outflows.

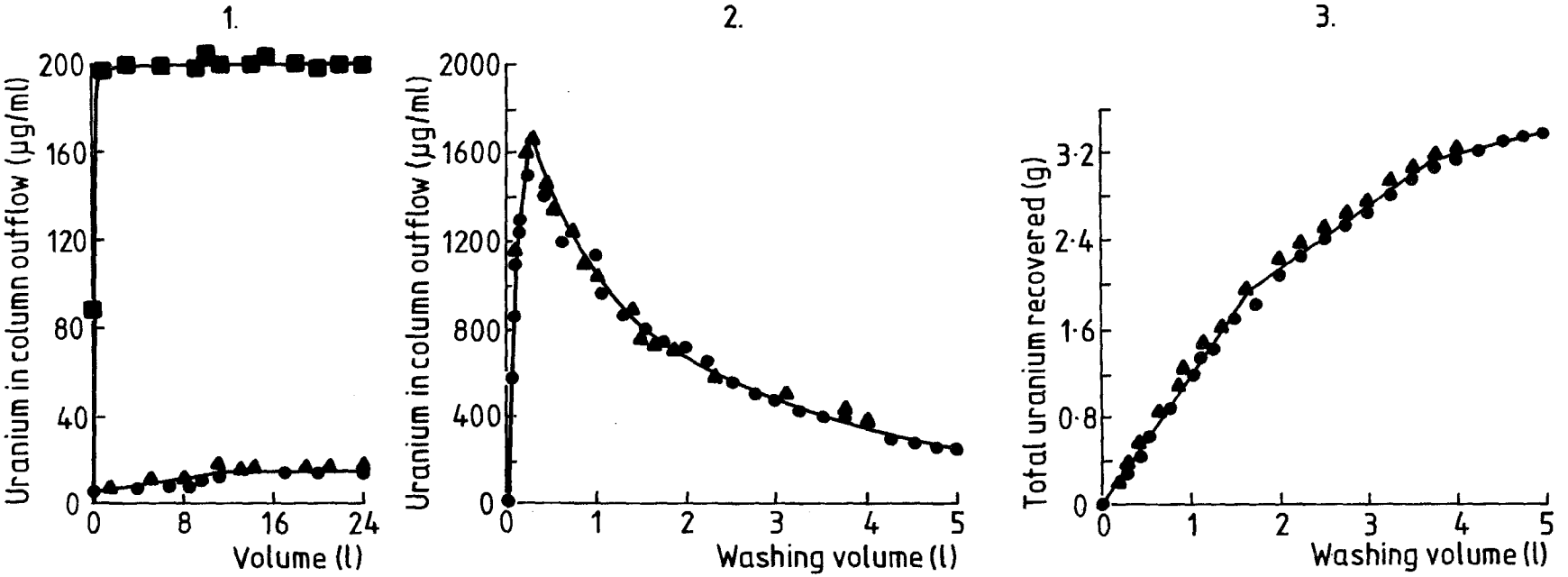
RESULTS

Activity of the column preparations against uranium When challenged with a flow containing 200µg/ml of uranium, in excess of 90% of the presented metal was removed (Fig. 1). After 8l of the uranium solution had passed through the column the efficiency of metal removal was 95-96%, which was identical to that observed previously using Cd^{2+} (Macaskie and Dean, 1984b). Thereafter the activity fell slightly, but stabilized at an efficiency of uranium removal of 91% after 14l, continuing unabated until the experiments were terminated at 24l. By contrast, no uranium uptake was observed using a cell-free column (Fig. 1), in accordance with the results reported previously for Cd^{2+} (Macaskie and Dean, 1984a,b).

At termination, it was calculated that the columns had each accumulated about 4·5g of uranium and the loaded metal was evident as a dense yellow precipitate in the gel. Since 5g wet weight of cells corresponded to about 0·5g dry weight (Macaskie, unpublished), the uptake of uranium by the cells can be expressed as 900% of the bacterial dry weight.

Recovery of the loaded uranium from the gels Previous work has shown that cadmium could be recovered from cadmium-loaded columns *in situ* using a wash of 0·5M citrate buffer, pH 5·0 (Macaskie and Dean, 1984b). However under these conditions uranium recovery was poor. The strength of the citrate wash was increased to 1M citrate buffer at pH 4·0 and the data of Figs. 2 and 3 were obtained. It was observed that the uranium was eluted as a concentrated solution only. Unlike the behaviour previously observed with cadmium recovery, (Macaskie and Dean, 1984b) no particulate slurry was observed. Otherwise the uranium recovery profile (Fig. 2) was similar to that previously obtained with Cd^{2+}, and uranium continued to be eluted as a fairly concentrated yellow solution throughout the 5l of wash tested. The profile of total uranium recovery (Fig. 3) shows that after 5l of washing 3·3g of the calculated total of 4·5g of uranium had been recovered. The uranium remaining bound represents 27% of the accumulated total. These results are in agreement with those previously observed for Cd^{2+}, but in the latter case the recalcitrant Cd^{2+} represented 15% of that accumulated, and furthermore, elution was terminated after 3l (Macaskie and Dean, 1984b). More extended elution in the case of uranium was not considered worthwhile because, and as observed also with Cd^{2+}, the

Fig. 1. Uranium accumulation by immobilized cells of a *Citrobacter* sp.
Fig. 2 and Fig. 3. Recovery of the loaded uranium from the immobilized cells.



Legend to Fig. 1. *Citrobacter* sp. was pre-grown and immobilized as described in Materials & Methods. A buffered solution of uranyl nitrate (2mM citrate buffer-5mM glycerol 2-phosphate-200µg/ml of uranium, pH 6·8) was pumped through the columns at one column volume (60ml) per hour. ●, ▲, Uranium content in the column outflow from two immobilized cell preparations. ■, Uranium content in the outflow of a cell-free preparation.
Legend to Fig. 2 and Fig. 3. The columns (●, ▲; two independent preparations) were each loaded with 4·5g of uranium as described above. They were then unloaded of their bound uranium using a citrate buffer wash (1M, pH 4.0) at one column volume (60ml) per hour and the uranium content/ml (Fig. 2) and total uranium recovered (Fig, 3) were determined as described in Materials & Methods.

rate of uranium recovery occurred in distinct phases, falling exponentially with the elution volume (Fig. 3).

DISCUSSION

The accumulation of uranium by immobilized cells of our *Citrobacter* sp. is comparable to the performance of this organism in accumulating cadmium (Macaskie and Dean, 1984a,b,c) and greatly exceeds that of other recently published methods (Table 1) relying on simple adsorption techniques. Since the current method is based on a continuous enzymic process (Macaskie and Dean, 1984a) it is an open system and is not subject to the low saturation limits governing other described batch processes (Table 1); it will run for greatly extended periods (Macaskie and Dean, 1984b) without the need for sequential adsorption and desorption cycles (c.f. Nakajima *et al*, 1982, Tsezos, 1984, Norberg and Persson, 1984), and the uranium is readily recoverable from the columns (Figs. 2 & 3). In these respects an open system of this type is superior to other published methods.

Table 1. A comparison of some of the recently published methods for the recovery of uranium using microbial cells or cell products

Organism	Method of uranium uptake	Uranium concentration	U accumul. (mg/g d.w.)	Reference
Rhizopus arrhizus	Adsorption to fungal biomass	150 µg/ml	180	Tzesos & Volesky (1981)
Acinetobacter RAG-1	Binding to extracellular emulsan	up to 400 µg/ml	>800	Zosim *et al.* (1983)
Penicillium digitatum	Adsorption to fungal biomass	100 µg/ml	5.7	Galun *et al.* (1984)
Pseudomonas aeruginosa	Binding to microbial cells	100 µg/ml	150	Strandberg *et al.* (1981)
Saccharomyces cerevisiae	Binding to microbial cells	100 µg/ml	150	Strandberg *et al.* (1981)
Zoogloea ramigera	Binding to polysaccharide matrix round cells	500-2500 µg/ml[a]	800	Norberg & Persson (1984)
Streptomyces viridochromogenes	Adsorption to immobilized whole cells	20 µg/ml[b]	312	Nakajima *et al.* (1982)
Chlorella regularis	Adsorption to immobilized whole cells	20 µg/ml[b]	159	Nakajima *et al.* (1982)
Citrobacter sp.	Binding to immobilized whole cells by enzymic process	200 µg/ml[c]	9000	This communication

U. accumul. : Uranium accumulated (total) expressed as mg Uranium/gram dry weight cells or emulsan

[a] 4 Cycles. [b] 10 Cycles (see text). [c] 1 Cycle.

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