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Microbial Cells as Biosorbents for Heavy Metals: Accumulation of Uranium by *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*

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Uranium accumulated extracellularly on the surfaces of *Saccharomyces cerevisiae* cells. The rate and extent of accumulation were subject to environmental parameters, such as pH, temperature, and interference by certain anions and cations. Uranium accumulation by *Pseudomonas aeruginosa* occurred intracellularly and was extremely rapid (<10 s), and no response to environmental parameters could be detected. Metabolism was not required for metal uptake by either organism. Cell-bound uranium reached a concentration of 10 to 15% of the dry cell weight, but only 32% of the *S. cerevisiae* cells and 44% of the *P. aeruginosa* cells within a given population possessed visible uranium deposits when examined by electron microscopy. Rates of uranium uptake by *S. cerevisiae* were increased by chemical pretreatment of the cells. Uranium could be removed chemically from *S. cerevisiae* cells, and the cells could then be reused as a biosorbent.

The use of microbial cells as biosorbents for heavy metals offers a potential alternative to existing methods for decontamination or recovery or both of heavy metals from a variety of industrial aqueous process streams (3, 14, 20, 21, 24). Biosorptive extraction of uranium from natural waters has been proposed as well (18; E. Heide, M. Paschke, K. Wagener, and M. Wald, German patent 2,345,430, 1975). Metal accumulation by biosorption is generally considered to be a rapid physical/chemical phenomenon which uses preexisting biomass. Two principle sites of uranium complexation in biological systems have been proposed. Rothstein and Meier (17) suggested that on the surfaces of yeast cells there are reactive groups which are chemically similar to high-molecular-weight polyphosphates and are responsible for uranium complexation. Dounce and Flagg (7) provided evidence that the carboxyl groups of proteins effectively complex uranium. Recently, Beveridge and Murray (4) and Matthews and Doyle (T. H. Matthews and R. J. Doyle, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, K86, p. 159) reported that the carboxyl groups of the peptidoglycans in the cell walls of *Bacillus subtilis* are the primary sites of divalent metal complexation.

Our investigations have been directed toward the use of microbial biosorption of metals for the removal of radionuclides (e.g., uranium) from waste streams generated by nuclear fuel-processing plants (20, 21). Although our emphasis has been on process development, we also want to understand the mechanism of uranium uptake to determine whether it can be enhanced through environmental or genetic manipulation of microbial cells. Two organisms, *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*, were selected from an earlier survey (20) for a detailed characterization of uranium uptake. Both had been used in previous metal uptake studies (11, 14, 17; H. R. Meyer, Ph.D. thesis, Colorado State University, Fort Collins, 1977), and many aspects of their physiology, structure, etc., were documented in the literature. The selection of these two organisms was fortuitous in that the evidence which we report below suggests two separate biosorption mechanisms.

MATERIALS AND METHODS

Uranium uptake experiments. The organisms used in this study were *S. cerevisiae* NRRL Y-2574 (Agricultural Research Service Culture Collection, Peoria, Ill.) and *P. aeruginosa* CSU (obtained from H. R. Meyer and S. Johnson, Colorado State University). Details of the methods for culture maintenance, cell production, and uranium assay were described previously by Shumate et al. (21). Both organisms were routinely cultured in a rich medium containing the following (in grams per liter): glucose, 10.0; yeast extract, 3.0; malt extract, 3.0; and peptone, 5.0 (22). The uranium uptake ability of *S. cerevisiae* cultured in the sucrose mineral salts vitamin medium described by Winge and Roberts (23) was also examined. Briefly, to determine uranium uptake, a suspension of washed cells from a 24-h culture was added to a uranyl nitrate hexahydrate solution (~100 g of uranium per m^3) equilibrated to the desired temperature (40°C unless noted otherwise). This cell suspension was prepared to provide about 0.4 mg (dry weight) of cells per ml in

a uranyl nitrate cell mixture. Dry cell weights were obtained by air-drying (100°C) known volumes of cell suspensions before they were exposed to uranium. Additions or adjustments to the uranyl nitrate solution were made before cells were added. In those instances where the cells were treated chemically, the washed cells were exposed to the chemical agent at room temperature for a given time and then rewashed three times with deionized, distilled water before contact (or in some cases, recontact) with uranyl nitrate. The uranyl nitrate cell mixture was shaken at 100 rpm (2.54-cm stroke) on a rotary shaker. At the desired time, cells were removed from samples of the primary suspension by centrifugation at ~2,500 × *g* for 3 to 5 min. The remaining soluble uranium was assayed spectrophotometrically by using Arsenazo III reagent (16, 19, 21). Cell-free controls were run concurrently in all experiments.

We tried methods other than centrifugation to obtain a more rapid separation of *P. aeruginosa* cells from the uranium solution. Filtration under positive or negative pressure through 0.22-, 0.45-, and 1.2-μm membrane filters (Millipore Corp., Bedford, Mass.) and a variety of sintered glass filters was unsatisfactory because the cells rapidly plugged the filters and the filters themselves adsorbed some uranium. Some success was obtained in preliminary experiments in which the cells were passed rapidly (under air pressure of ~1 to 2 lb/in^2) through a small glass column (inside diameter, ~1 cm) containing a 2-cm bed of Bio-Rad AG 50W-X12 ion-exchange resin (200 to 400 mesh; hydrogen form; Bio-Rad Laboratories, Richmond, Calif.) to adsorb the remaining soluble uranium. The resin bed was then washed extensively with water to remove the remaining cells. Uranium was eluted from the resin bed with 1.0 M nitric acid and analyzed by the standard Arsenazo III assay after pH adjustment (to pH 4.0 with NaOH) of the eluate.

Electron microscopy. Transmission electron photomicrographs and energy-dispersive X-ray data were obtained by L. K. West (Department of Biochemistry, University of Tennessee, Knoxville) and J. Bently (Metals and Ceramics Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.). The general procedure involved fixation of uranium-exposed cells with 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2) for 3.5 h, dehydration in a graded series of acetone solutions, and embedment in plastic (L. K. West, Ph.D. thesis, University of Tennessee, Knoxville, 1977). Energy-dispersive X-ray analyses with JEM-100CX and Phillips EM 400T/FEG analytical electron microscopes (10- to 100-nm variable beam size) of thin (100-nm) and thick (250-nm) sections were used to verify the location of uranium.

Calcium and potassium effects. To determine the effect of calcium on uranium uptake, the uranyl nitrate solution (100 g of uranium per m^3) was supplemented with 8×10^{-2} Ci of $^{45}CaCl_2$ (New England Nuclear Corp., Boston, Mass.) per m^3 and 100 g of unlabeled $CaCl_2$ per m^3. After contact with the cells, soluble uranium was measured by using the standard Arsenazo III assay, except that 8×10^{-4} M ethylenediaminetetraacetic acid was included in the assay mixture to complex the calcium (16). Soluble calcium and cell-bound calcium (sulfuric acid digest) were measured by standard liquid scintillation counting techniques.

In the case of potassium, 100 g of K^+ (as KCl) per m^3 was added to the uranyl nitrate solution before cells were added. Soluble uranium was measured by the standard Arsenazo III assay.

Complexation of uranium by amino acids. Amino acids (0.1%, wt/vol) were added to the uranyl nitrate solution, and the pH was adjusted to 4.0 with NaOH. Uranium uptake was followed as described previously.

Phosphomannan. Phosphomannans from *Hansenula holstii* NRRL Y-2449 and *Hansenula capsulata* NRRL Y-1842 (kindly supplied by M. E. Slodki, Northern Regional Research Center, Peoria, Ill.) were dissolved in deionized, distilled water and then mixed individually with uranyl nitrate hexahydrate solutions to give a final uranium concentration of 478 or 1,044 g/m^3 and a final phosphomannan concentration of 0.2% (wt/vol). After the solutions were stirred for 1 h at room temperature, the phosphomannans were precipitated by adding 1.5 volumes of 95% ethanol–0.1% (wt/vol) KCl. The precipitates were removed by centrifugation at 7,000 × *g* for 20 min, and soluble uranium in the supernatant was measured by the Arsenazo III assay. A uranyl nitrate hexahydrate solution was treated in the same manner, as a control.

RESULTS

The microbial cells used in these experiments were cultured in the absence of uranium. In addition, they were washed free of extraneous nutrients before exposure to uranium. Therefore, the measured uranium uptake was considered to be an inherent property of the cells and not associated with cell growth. This was further emphasized by the rapid rates of metal uptake by *S. cerevisiae* and *P. aeruginosa* (Fig. 1). The rate of uptake by *P. aeruginosa* was extremely rapid. Although attractive for process application, this rapid rate has hampered studies of the effects of environmental conditions on uranium uptake by this organism. Although used routinely in our experiments, centrifugation to separate the cells from the metal solution significantly extends the time of exposure. Throughout this study, we examined alternative separation techniques. Filtration proved to be impractical since the cells rapidly plugged membrane and glass filters and the filter materials themselves adsorbed some uranium. Using an ion-exchange separation technique (see above), we recently obtained preliminary evidence that uranium is associated with *P. aeruginosa* cells within 5 to 10 s after the cells contact uranium. Thus, as a consequence of this rapid uptake rate, the apparent lack of any effect of the conditions imposed on uranium uptake by *P. aeruginosa* in the experiments described below may have been due to our inability to detect it within the time frame of the measurements. However, uranium

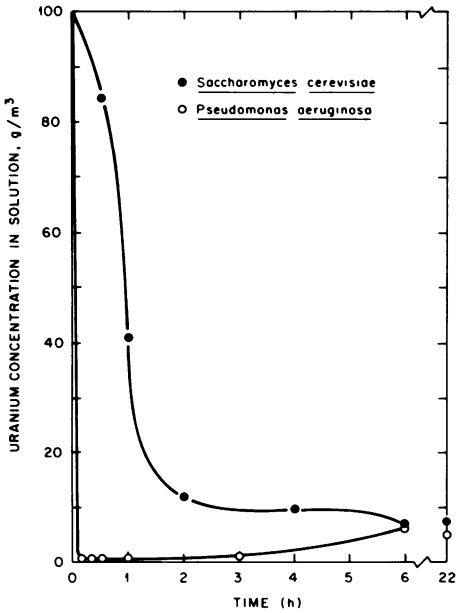

FIG. 1. *Removal of uranium from aqueous solutions by S. cerevisiae and P. aeruginosa.*

accumulation by *S. cerevisiae* was sufficiently slow so that the time required for centrifugation (3 to 5 min) was a less significant fraction of the total time before equilibrium was reached between soluble uranium and cell-bound uranium.

Despite differences in the rates of uranium uptake, the total capacity for metal accumulation (10 to 15% of the original dry cell weight), was the same for both species. Although generally the uranium concentration on the cells was inferred by the measured loss of soluble uranium, our results were confirmed by an independent analysis of the exposed cells by the Analytical Chemistry Division, Oak Ridge National Laboratory.

Electron microscopic examinations and energy-dispersive X-ray analyses showed that uranium accumulated as needle-like fibrils in a layer approximately 0.2 μm thick on the surfaces of *S. cerevisiae* cells (Fig. 2). Little or no uranium was found inside these cells or inside cells without visible uranium deposits. In contrast, uranium formed dense intracellular deposits in *P. aeruginosa* (Fig. 3). This is surprising in view of the rapid rate of metal uptake. The cells shown in Fig. 3 were exposed to uranium for 2 h. Because the rate of uranium uptake was so rapid, we did not attempt an electron microscopic examination of these cells after shorter exposure times. Although we lack visual evidence of immediate intracellular deposition of uranium, we do know that uranium is firmly associated with the cells within a few seconds (see above).

It is evident in Fig. 2 and 3 that not all of the cells possessed visible uranium deposits. Those cells which had uranium deposits (32 and 44% of all *S. cerevisiae* and *P. aeruginosa* cells, respectively [averages from several fields of view having 20 to 30 cells of *S. cerevisiae* and ~100 cells of *P. aeruginosa*]) showed no apparent structural differences from those cells which did not have such deposits; both budding and nonbudding *S. cerevisiae* cells had deposits. R. McKracken (University of Vermont) developed a technique whereby uranium-exposed cells of both species could be separated into two bands by light centrifugation (2,000 to 3,000 × *g*, 5 to 10 min) after layering on 40% (wt/vol) CsCl. As indicated by its reaction with the Arsenazo III reagent, the heavier cell band contained the bulk of the sorbed uranium. We were able to culture a few viable cells from the separated bands on streak plates, but there was no difference in uranium uptake by isolates of either species compared with the parent cultures.

The conditions used to culture cells can affect uranium uptake. *S. cerevisiae* cultures grown on a synthetic medium (23) had a faster rate of uptake by a factor of 2.5 than cultures grown on a rich organic medium (22). Although not quantitated, the growth rate and cell yield were reduced in the synthetic medium. On the other hand, there was no difference in metal uptake between aerobically and anaerobically grown yeast cells.

Published reports of metal biosorption and our own experience with *S. cerevisiae* indicated that uranium uptake did not require cellular metabolism. However, the rapid intracellular uptake of uranium by *P. aeruginosa* suggested the possible involvement of metabolically mediated active transport. Cells of both species were exposed separately to uranium in the presence of the metabolic inhibitor 2,4-dinitrophenol (5×10^{-3} M) or sodium azide (1×10^{-4} M), or they were pretreated for 5 to 10 min with $HgCl_2$ (1.0% solution) or formaldehyde (10% solution) before exposure to uranium. These latter two compounds were lethal for both species (no cells could be cultured from the treated cell preparations). Uranium uptake by both species was not affected by either metabolic inhibitor. Although formaldehyde and $HgCl_2$ treatments had no apparent effect on *P. aeruginosa*, the rate of uranium uptake by *S. cerevisiae* (Fig. 4) was increased by these treatments. The cellular Hg concentrations before and after exposure to uranium were not determined.

Temperature and the initial solution pH had a dramatic effect on metal uptake by *S. cerevi-*

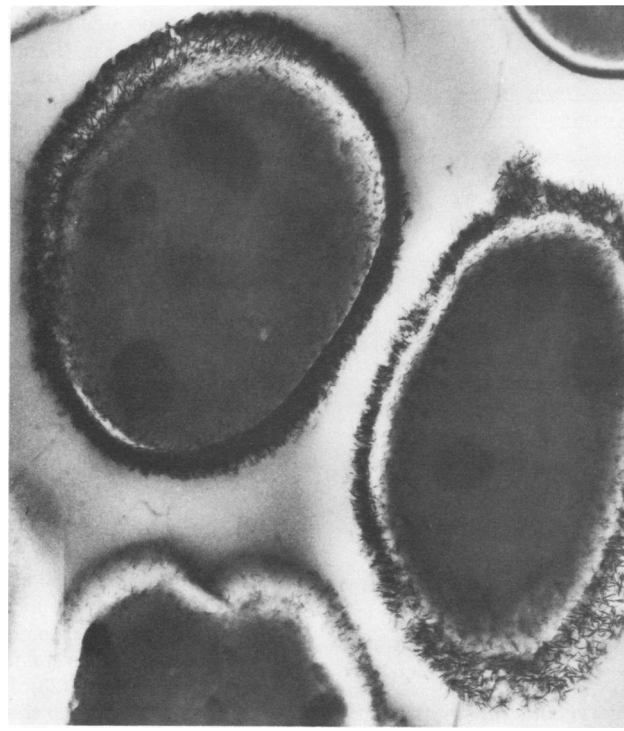

FIG. 2. *Electron micrograph of S. cerevisiae showing surface accumulation of uranium.* ×35,000.

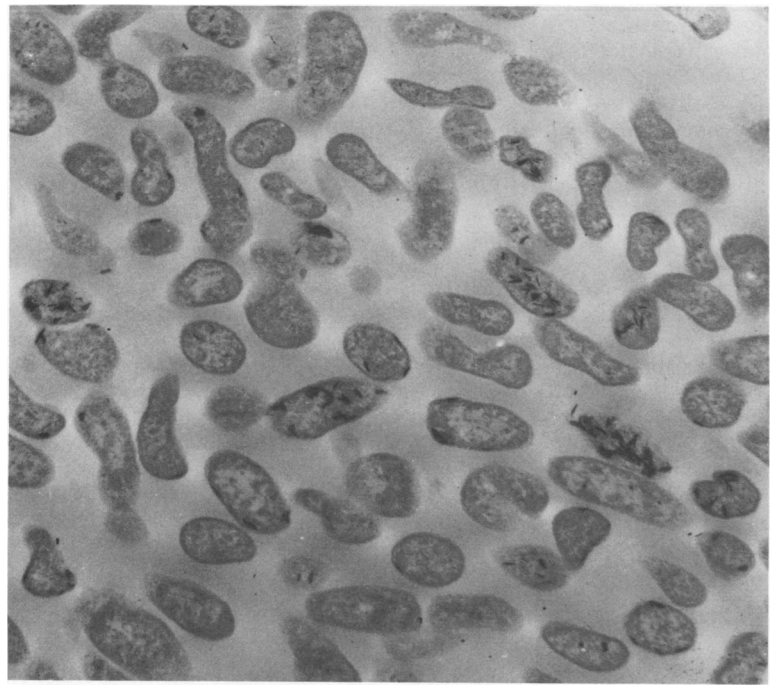

FIG. 3. *Electron micrograph of P. aeruginosa showing intracellular accumulation of uranium.* ×27,000.

siae. As Fig. 5 shows, the rate of uptake increased with temperature between 20 and 50°C. Although the initial rate of uptake increased as the pH was raised from pH 2.5 to 5.5, maximal equilibrium distributions were obtained between pH 3.0 and 4.0 (Fig. 6). There was no discernible

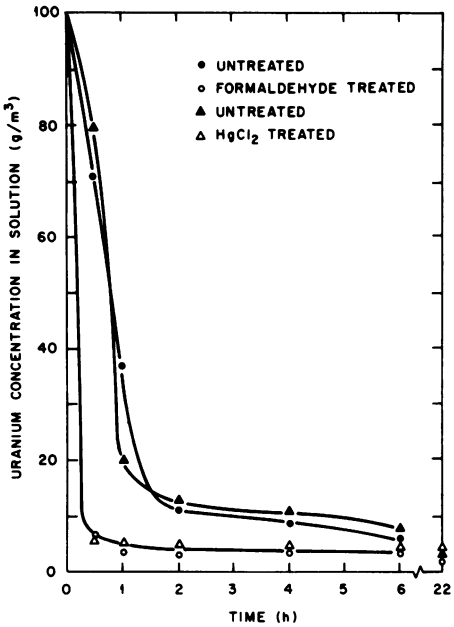

FIG. 4. *Effects of $HgCl_2$ and formaldehyde pretreatments on uranium uptake by S. cerevisiae.*

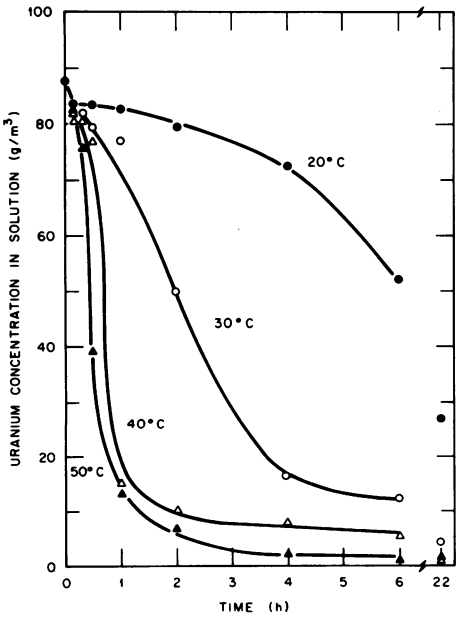

FIG. 5. *Influence of temperature on uranium uptake by S. cerevisiae.*

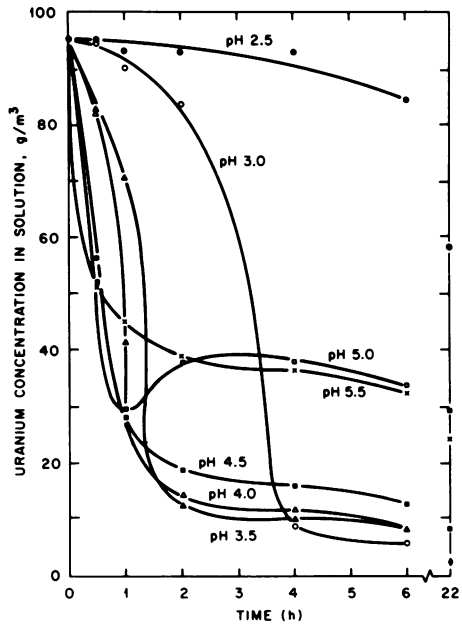

FIG. 6. *Effect of initial pH on uranium uptake by S. cerevisiae.*

response of *P. aeruginosa* to temperature or pH, although it must be remembered that we were unable to observe the transition between the initial and equilibrium stages. Since the normal pH of the uranyl nitrate solution was about 4.0, no routine pH adjustment was necessary.

Water washing was ineffective in removing uranium from the cells. Several agents that solubilize or complex with uranium were used to treat cells of *S. cerevisiae* that had been exposed to uranium; 16-h suspensions in 0.1 M nitric acid, 0.1 M disodium ethylenediaminetetraacetic acid, and 0.1 M ammonium carbonate removed only 59.3, 72.3, and 83.5%, respectively, of the bound uranium. To determine whether surface binding sites were altered by these treatments, the treated cells were washed and reexposed to uranium. As Fig. 7 shows, all three treatments enhanced the initial rate of uranium uptake, but nitric acid and sodium ethylenediaminetetraacetic acid greatly reduced the concentration of uranium on the cells at equilibrium. Two other agents (data not shown), sodium citrate (0.1 M) and potassium oxalate (1.0 M), removed 57 and 14%, respectively, of the bound uranium and resulted in an increased rate of metal uptake similar to the increased rate observed after ammonium carbonate treatment. Whereas ammonium carbonate treatment before or after the initial exposure to uranium increased the rate of metal uptake, sodium citrate and potassium oxalate were effective only after prior exposure of

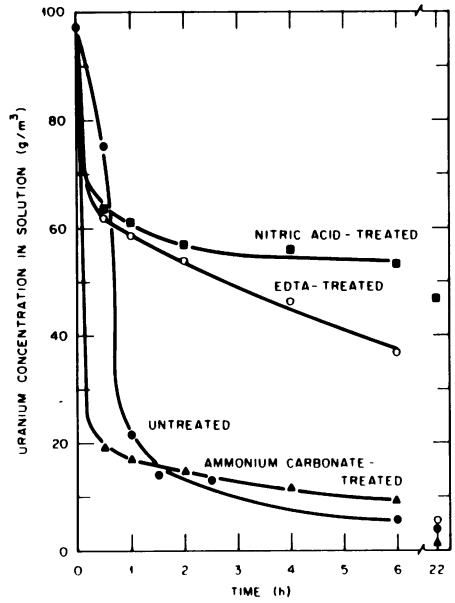

FIG. 7. *Uranium uptake by S. cerevisiae (preexposed to uranium) after treatment with nitric acid, disodium ethylenediaminetetraacetic acid (EDTA), or ammonium carbonate.*

the cells to uranium. Sodium ethylenediaminetetraacetic acid and nitric acid were not tested in this way.

It is known (7) that insoluble complexes of proteins, such as casein, and uranium occur. However, we found that soluble Casamino Acids (Difco Laboratories, Detroit, Mich.) also exhibited this phenomenon. Amino acid analyses of a Casamino Acid solution before and after exposure to uranium tentatively implicated cysteine and glutamic acid as the amino acids involved in the complexation and precipitation of uranium. To determine whether these and other amino acids were strong enough complexing agents to compete with cells for uranium, they were incorporated individually (0.1%, wt/vol) in the uranyl nitrate solution (pH 4.0) before cells were added. The presence of several amino acids had no effect on uranium uptake by *P. aeruginosa*. Whereas glutamic acid and aspartic acid (dicarboxylic amino acids) strongly inhibited uranium uptake by *S. cerevisiae* (Fig. 8), none of the monocarboxylic amino acids, including the sulfur-containing amino acids cysteine and methionine, had any effect. Substitutions on the amino group (e.g., *N*-methylglycine, *N,N*-dimethylglycine, and glycylglycine) of a monocarboxylic amino acid did not result in interference, whereas the corresponding organic acids (e.g., acetic acid) did interfere with metal uptake.

Rothstein and Meier (17) observed that certain divalent cations (Ba^{2+}, Ca^{2+}) interfered with uranium uptake by *S. cerevisiae*, whereas monovalent cations had no effect. We wanted to verify this with our yeast strain and to determine whether there was a similar effect on internal uranium accumulation in *P. aeruginosa*. Also, potentially interfering metals would be of concern in any biological treatment process for uranium removal. Potassium (100 g of K^+ per m^3, as KCl) had no effect on uranium uptake by either organism, nor did Ca^{2+} (100 g/m^3, as $CaCl_2$) interfere with uptake by *P. aeruginosa*. ^{45}Ca was taken up concomitantly with uranium in this organism. Both the initial rate of uranium uptake and the ultimate equilibrium distribution in *S. cerevisiae* were altered by Ca^{2+} (Fig. 9). Calcium was bound at a slightly slower rate than uranium during the first 2 h, but uranium became displaced from the cells as calcium uptake continued. The presence of uranium enhanced calcium uptake.

Phosphate groups have been implicated as sites of uranium complexation by *S. cerevisiae* (17). Phosphorylated polysaccharides (phosphomannans) comprise a portion of the cell wall in several yeast species, including *S. cerevisiae* (9). We were able to obtain samples of purified and well-characterized (particularly with regard to the phosphate/mannose ratios) yeast phosphomannans from two species of *Hansenula*. As Table 1 shows, uranium complexation by these

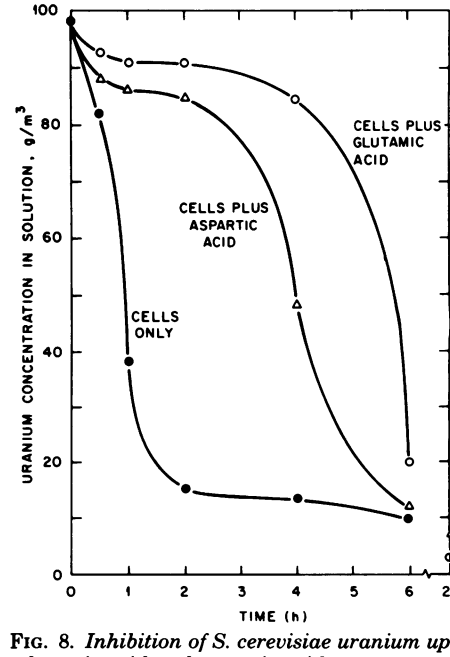

FIG. 8. *Inhibition of S. cerevisiae uranium uptake by glutamic acid and aspartic acid.*

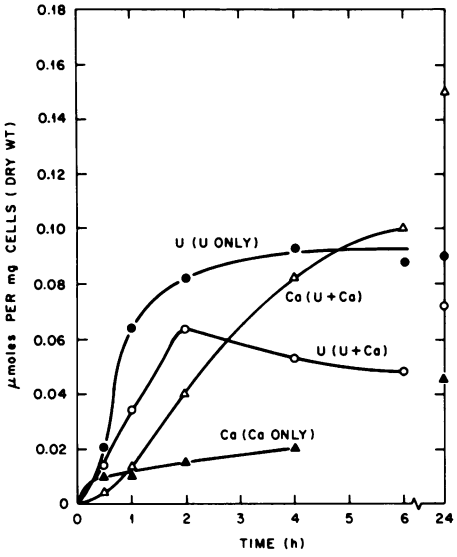

FIG. 9. *Effect of Ca^{2+} on uranium uptake by S. cerevisiae.*

TABLE 1. *Complexation of uranium by yeast phosphomannans*

Phosphomannan from:[a]	Mannose/phosphate ratio[b]	Solution uranium concn (g/m³)		Phosphomannan uranium concn (%)[c]
		Initial	Final	
H. holstii NRRL Y-2448	~5	478	224	12.7
		1,044	754	14.5
H. capsulata NRRL Y-1842	~2.5	478	48	21.5
		1,044	432	30.6

[a] Final concentration, 0.2% (wt/vol).

[b] Mannose/phosphate ratios were supplied by M. E. Slodki, Northern Regional Research Center, Peoria, Ill.

[c] Percentages were calculated as follows: [(grams of uranium)/(grams of phosphomannan)] × 100.

phosphomannans was related to their phosphate contents.

DISCUSSION

Uranium uptake by *S. cerevisiae* and uranium uptake by *P. aeruginosa* differ in many respects. The surface-associated accumulation of uranium exhibited by *S. cerevisiae* is consistent with the view that metal biosorption occurs by the complexation of positively charged metal ions with negatively charged reactive sites (e.g., $R\text{-}COO^-$, PO_4^{2-}) on the cell surface (2–4, 17) or in extracellular polymers (8). As expected, metal uptake was affected by environmental parameters, such as pH, temperature, and competing cations (i.e., Ca^{2+}). Not only can environmental changes affect reactive metal-binding sites, but also the solution chemistry of uranium is quite complex. In the pH range of the optimal uranium uptake (pH 3.0 to 4.5), soluble uranium exists as UO_2^{2+} (1) and other hydrolysis products [$(UO_2)_2(OH)_2^{2+}$, $UO_2(OH)^+$, $(UO_2)_3(OH)_5^+$]. Since carbon dioxide was not excluded in our experiments, carbonate complexation reactions with the uranyl ion could also take place. Attempts to determine the chemical state of uranium as it exists on cell surfaces have been unsuccessful. We have observed that as uranium is taken up by cells, the pH increases from ~4.0 to 5.5 to 6.0, indicating a release of free hydroxyl ions. This suggests that UO_2^{2+} could be the form of the bound metal, and in fact, UO_2^{2+} complexes readily with a variety of anions (7).

The relatively large amounts of uranium accumulated (10 to 15% of the dry cell weight) were of concern. These values are similar to the value obtained for a mixed culture of denitrifying bacteria (Shumate et al., Biotechnol. Bioeng., in press) and *Rhizopus arrhizus* (B. Volesky, personal communication). However, it was difficult to imagine that there were sufficient binding sites to account for this much metal. Actually, since only 32% of the cells possessed measurable amounts of uranium, the metal concentration on that fraction of the cells approached 50% on a dry weight basis. Beveridge (2) also observed a non-stoichiometric accumulation of metals on isolated cell walls of *B. subtilis*. He suggested that metal molecules complex with existing reactive sites and that additional metal "crystallizes" on these bound molecules. It would help to know the chemical nature of the bound metal. Although no physiological or genetic basis for the distribution of metal uptake in cell populations has been found to date, this phenomenon is not only of basic scientific interest, but also of great practical importance. If an entire population could be induced to accumulate metal through environmental or genetic manipulation, the potential of the process would be enhanced.

Rothstein and Meier (17) provided evidence that polyphosphate groups of *S. cerevisiae* complex with uranium. Our findings that uranium complexed with yeast phosphomannans and that the uranium complexation capacity of the polymers appeared to be related to their phosphate contents support the hypothesis that phosphate groups play a role in metal binding. Carboxyl groups are also active in metal complexation (4, 7; Matthews and Doyle, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979). The fact that yeast cells grown on a synthetic medium showed enhanced uranium uptake rates might be due to an increase in the phosphate and protein contents of the cell walls, which is known

to occur in yeast cultured at reduced growth rates (13). It would be worthwhile to examine the effects of growth conditions and nutrient limitations in both batch and continuous cultures on the cell wall compositions of the metal-binding and -nonbinding yeast cells separable by CsCl centrifugation. Unfortunately, we have yet to find a method for removing bound uranium without an apparent effect on the cell surface (as evidenced by changes in subsequent metal uptake).

Dounce and Flagg (7) carried out extensive studies on the complexation of uranium with organic acids, proteins, and a few individual amino acids, using a titration technique. Organic acids and proteins were very effective in complexing with uranium. Although these authors concluded that the free carboxyl groups of proteins were the active sites of uranium complexation, they were unable to explain the very limited interaction between uranium and the monocarboxylic amino acids serine and glycine. Rothstein and Meier (17) considered these studies in interpreting the results of their own equilibrium dialysis experiments, which measured the affinities of various complexing agents, including proteins, for uranium. They observed that the maximum amount of uranium bound by a protein accounts for only 20% of the available "free" carboxyl groups. Their suggestion that "as in the case of copper, only those carboxyl groups which have adjacent accessory groups such as carboxyl and hydroxyl groups can form stable complexes with uranyl ion" was one of their reasons for discounting yeast cell surface proteins as important for uranium complexation.

Our experiments on the competition between yeast cells and soluble amino acids for uranium showed that dicarboxylic amino acids are effective complexing agents for uranium. We observed that monocarboxylic and N-substituted amino acids were ineffective in the competition experiments. Molecular models indicated no basis for a steric hinderance of uranium complexation by these compounds. We have concluded that the positive charge on the amino group, which exists in the pH range of our experiments, is sufficient to prevent uranium from complexing with the proximal carboxyl group. The distant carboxyl group of the dicarboxylic amino acid is free to complex, as are the carboxyl groups of the corresponding organic acids. This interpretation would explain the lack of complexation by serine and glycine observed by Dounce and Flagg (7). It would also provide an alternative explanation for the less-than-stoichiometric complexation of uranium by the available free carboxyl groups in a protein, as reported by Rothstein and Meier (17); i.e., there are relatively few carboxyls (based on their proximity to an amino group) that are free to complex with uranium. Two other related points should be mentioned in regard to this general question. The first is that reducing the positive charge on cell wall components by chemical treatment (e.g., formaldehyde) can enhance metal uptake (5). The second is that there is recent evidence that the most effective site for metal complexation on the cell walls of *B. subtilis* is the carboxyl group of glutamic acid in the peptidoglycan (4).

With time, the competitive effect of glutamic and aspartic acids diminish, and uranium becomes associated with the cells (Fig. 8). We considered the possibility that the cells might metabolize these amino acids, thus relieving the competitive effect, but we did not attempt to measure changes in amino acid concentrations. However, initially there was a considerable amount of amino acid present (0.1%, wt/vol), and uranium is to some extent toxic to *S. cerevisiae* (17; unpublished data). Also, as mentioned above, uranium uptake is normally accompanied by an increase in the pH of the solution. No substantial change in pH occurred in the competition experiments. These factors indicate that relief of the competitive effect is not due to amino acid depletion. The fact that uranium becomes preferentially associated with the cells cannot be fully explained at this time. The chemistry of uranium is quite complex, and we do not know the chemical nature of the bound uranium. The theory of Beveridge concerning nucleization and crystallization of uranium on the cell surface deserves further attention. Rothstein and Meier (17) stated that the yeast-uranium complex was the most stable complex which they observed.

Since the presence of active metal-binding sites on the surfaces of *S. cerevisiae* cells has not been established, we have no explanation for the increase or decrease in uranium uptake rates resulting from chemical pretreatment (e.g., ammonium carbonate, $HgCl_2$, formaldehyde). As noted above, formaldehyde could enhance metal uptake by reducing the overall positive charge of cationic sites on cell walls. Again, both the theoretical considerations and the practical benefit of increasing microbial metal uptake are of importance.

The responses of uranium uptake by *S. cerevisiae* to pH, temperature, and interfering metal ions (e.g., Ca^{2+}) can be understood if the uptake process is similar to an ion-exchange type of mechanism. However, how do we explain the rapid intracellular uptake of uranium by *P. aeruginosa*? Within the limits of our ability to measure uranium uptake, the process in this

species appears to be insensitive to environmental conditions, and we do not know how uranium enters the cell so rapidly (metabolism has been discounted). Once inside the cell, uranium appears to be localized. Perhaps the heavy metal-binding protein metallothionein, whose presence has now been verified in the procaryote *Synechococcus* sp. (15), is involved. Interestingly, in another bacterial system which we have examined (Shumate et al., in press), a mixed culture of denitrifying bacteria behaves in a manner similar to *P. aeruginosa* (i.e., there is a very rapid uranium uptake and an apparent insensitivity to environmental parameters). This mixed culture is believed to be composed predominantly of pseudomonad-like organisms (10). The rates of uranium uptake in this culture were rapid enough to prevent an assessment of mass transfer phenomena.

Since only 44% of the cells in the electron micrographs of *P. aeruginosa* contained visible deposits of uranium, there is again the theoretical and practical question of whether genetic or environmental factors govern metal uptake.

We originally hoped to be able to extrapolate the results of our studies with the two species used to enhance our knowledge of metal biosorption in a variety of biomass sources. The fact that there are at least two different biosorption systems, which are subject to different parameters, precludes any generalizations. Enhancement by any means is dependent on the type of biosorption system involved. Future studies will be directed toward elucidating the two mechanisms observed. We are particularly interested in why only a portion of the cells in a population take up uranium and how this proportion can be increased. We also intend to include other radionuclides and heavy metals in our metal uptake studies, using the same general approach described in this paper.

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