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MICROBIAL UPTAKE OF URANIUM, CESIUM, AND RADIUM*

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SUMMARY

The ability of diverse microbial species to concentrate uranium, cesium, and radium was examined. *Saccharomyces cerevisiae, Pseudomonas aeruginosa,* and a mixed culture of denitrifying bacteria accumulated uranium to 10 to 15% of the dry cell weight. Only a fraction of the cells in a given population had visible uranium deposits in electron micrographs. While metabolism was not required for uranium uptake, mechanistic differences in the metal uptake process were indicated. Uranium accumulated slowly (hours) on the surface of *S. cerevisiae* and was subject to environmental factors (i.e., temperature, pH, interfering cations and anions). In contrast, *P. aeruginosa* and the mixed culture of denitrifying bacteria accumulated uranium rapidly (minutes) as dense, apparently random, intracellular deposits. This very rapid accumulation has prevented us from determining whether the uptake rate during the transient between the initial and equilibrium distribution of uranium is affected by environmental conditions. However, the final equilibrium distributions are not affected by those conditions which affect uptake by *S. cerevisiae*. Cesium and radium were concentrated to a considerably lesser extent than uranium by the several microbial species tested. The potential utility of microorganisms for the removal and concentration of these metals from nuclear processing wastes and several bioreactor designs for contacting microorganisms with contaminated waste streams will be discussed.

INTRODUCTION

The use of microbial cells as biosorbents for heavy metals offers a potential alternative to existing methods for the decontamination and/or recovery of heavy metals from a variety of industrial aqueous process streams (4,16,23,24,29). Biosorptive extraction of uranium from natural waters has been proposed as well (10,18). Metal accumulation by biosorption is generally considered to be a rapid physical/chemical phenomenon using preexisting biomass. Biosorption mechanisms have not been as extensively studied as growth-associated metal accumulation by microorganisms and other life forms.

Our investigations have been directed at the use of microbial biosorption of metals for the removal of radionuclides (e.g., uranium) located in waste streams from the nuclear fuel processing industry (23,24). While the emphasis has been on process development, we also desired an understanding of the mechanism of uranium uptake to determine if it could be enhanced through environmental or genetic manipulation of the microbial cells. Two organisms, *Saccharomyces cerevisiae* and *Pseudomonas aeruginosa*, were selected from an earlier survey (23) for a detailed characterization of uranium uptake. Both had been used in previous metal uptake studies (H. R. Meyer, Ph.D. thesis, Colorado State University, Fort Collins, 1977; 11,14,17), and many aspects of their physiology, structure, etc., were documented in the literature. The selection was fortuitous in that the evidence we report suggests two separate biosorption mechanisms.

To gain a better understanding of the uranium uptake phenomenon, we determined the physical location of cell-bound uranium and examined the effect of environmental conditions, chemical agents (including metabolic inhibitors), and potentially inferfering metal ions (i.e., calcium) on

uranium uptake. In addition, the uranium complexing capability of cell-surface components (phosphorylated polysaccharides, amino acids) which might be participating in metal binding was measured.

The ability of a variety of microorganisms to accumulate cesium and radium was considered in regard to potential treatment processes for two radioactively contaminated waste solutions.

MATERIALS AND METHODS

Radionuclide uptake experiments. The organisms used in this study and their sources are listed in Table 1. Radionuclide uptake by resting cells was determined as follows: Suspensions of washed cells were added to solutions containing a known concentration of the radionuclide and previously equilibrated to the desired temperature. Additions or adjustments to the solutions were made prior to cell addition. In those instances where the cells were chemically pretreated, the washed cells were exposed to the chemical agent at room temperature for a given time, then rewashed (3X, de-ionized, distilled water) before resuspension and addition to the radionuclide solution. The radionuclide-cell mixture was shaken at 100 rpm (2.54-cm stroke, rotary shaker). At the desired time interval, cells were removed from aliquots of the primary suspension by centrifugation. The remaining soluble uranium was assayed spectrophotometrically using Arsenazo III reagent (17,22,24). ^{137}Cesium and ^{227}Ra were assayed by γ radiation counting. Cell-free controls were run concurrently in all experiments.

Growth-associated ^{137}Cs and ^{226}Ra accumulation was determined as above after growth of the organisms in the appropriate medium (see text) containing these radionuclides.

Electron microscopy. Transmission electron photomicrographs and energy dispersive x-ray data were obtained by L. K. West (Department of

Table 1. Microorganisms and their sources

Organism	Source	Culture medium
Saccharomyces cerevisiae NRRL Y-2574	ARS Culture Collection Peoria, Ill.	YM (Ref. 7) Synthetic (Ref. 28)
Pseudomonas aeruginosa CSU	H. R. Meyers and S. Johnson Colorado State University Fort Collins, Colorado	YM (Ref. 7)
Ashbya gossypii NRRL Y-1056	ARS Culture Collection Peoria, Ill.	YM (Ref. 7)
Penicillium chrysogenum NRRL 807	ARS Culture Collection Peoria, Ill.	YM (Ref. 7)
Chlorella pyrenoidosa ATCC 7517	American Type Culture Collection Rockville, Maryland	WS pond water
WS "algae"	Natural blook of blue-green and green algae from pond water	WS pond water
Mixed culture of denitrifying bacteria	C. W. Hancher Chem. Tech. Division ORNL, Oak Ridge, Tenn.	Denitrifying medium (Ref. 10) SRSSW

Biochemistry, University of Tennessee, Knoxville, Tenn.). The general procedure involved fixation or uranium-exposed cells with 1% osmium tetroxide in 0.1 M cacodylate buffer (pH 7.2, 3.5 h), dehydration in a graded series of acetone solutions and embedment in plastic. Thick (250-nm) sections were used to obtain the energy dispersion x-ray data.

Calcium effect. The uranyl nitrate solution (100 g/m^3 uranium) was supplemented with 8×10^{-2} Ci/m^3 $^{45}CaCl_2$ (New England Nuclear, Boston, Mass.) and 100 g/m^3 unlabeled $CaCl_2$. After contact with the cells, soluble uranium was measured using the standard Arsenazo III assay, except that 8×10^{-4} M ethylenediaminetetraacetic acid (EDTA) was included in the assay mixture to complex uranium (19). Soluble and cell-bound (sulfuric acid digest) calcium were determined by standard liquid scintillation counting techniques.

Phosphomannan. Phosphomannans from *Hansenula holstii* NRRL Y-2449 and *H. capsulata* NRRL Y-1842 (kindly supplied by M. E. Slodki, Northern Regional Research Center, Peoria, Ill.) were dissolved in de-ionized distilled water and mixed individually with uranyl nitrate hexahydrate solutions to give final uranium concentrations of either 478 to 1044 g/m^3 and a final phosphomannan concentration of 0.2% (w/v). After stirring for 1 h at room temperature, the phosphomannans were precipitated by the addition of 1.5 volumes of 95% ethanol and 0.1% KCl (w/v). The precipitates were removed by centrifugation at 7000 x g for 20 min, and soluble uranium was determined in the supernatant by the Arsenazo III assay. A uranyl nitrate hexahydrate solution was treated in the same manner as a control.

RESULTS

Uranium

The microbial cells used in these experiments were cultured in the absence of uranium. Additionally, they were washed free of extraneous

nutrients before exposure to uranium. Therefore, the measured uranium uptake is considered to be an inherent property of the cells and one not associated with cell growth. This is further emphasized by the rapid rates of metal uptake by *S. cerevisiae* and *P. aeruginosa* as illustrated in Fig. 1. The rate of uptake by *P. aeruginosa* is 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. Centrifugation to separate the cells from the metal solution significantly extends the time of exposure. Filtration has proved impractical since the cells rapidly plug membrane and glass filters, and the filter materials themselves adsorb some uranium. Preliminary results obtained by separating the cells from the uranium solution by rapid passage through a bed of ion exchange resin (AG® 50W-X12, 200-400 mesh, hydrogen form, Bio-Rad Laboratories, Richmond, Calif.) indicate that the uranium is associated with the cells within 5-10 seconds after exposure. Thus in the experiments presented below, the apparent lack of an effect of imposed conditions on uranium uptake by *P. aeruginosa* may be due to our inability to monitor the time course of reaction within a time frame of several seconds. However, uranium accumulation by *S. cerevisiae* is sufficiently slow so that the time required for centrifugation (3 to 5 min) is a less significant fraction of the total time before an equilibrium is reached between soluble and cell-bound uranium.

Despite differences in the rates of uranium uptake, the total capacity for metal accumulation, 10 to 15% of the dry cell weight, was the same for both species.

Electron microscopic examination and energy dispersive x-ray analysis showed that uranium accumulated as needle-like fibrils in a layer 4 to 5 nm thick on the surface of *S. cerevisiae* (Fig. 2). Little or no uranium was

found within the cells. In contrast, uranium formed dense intracellular deposits in *P. aeruginosa* (Fig. 3). This is surprising in view of the rapid rate of metal uptake.

It is evident in Figs. 2 and 3 that not all of the cells possessed visible uranium deposits. Those cells which had uranium deposits (32% and 44% of the total cells of *S. cerevisiae* and *P. aeruginosa* respectively) showed no apparent structural differences from those which did not and for *S. cerevisiae* included both budding and nonbudding cells. M. McKracken (a summer student trainee in our laboratory from the University of Vermont) developed a technique whereby uranium-exposed cells of both species could be separated into two bands by light centrifugation (2000 to 3000 x g, 5 to 10 min) after layering on 40% (w/v) $CsCl_2$. As indicated by reaction with Arsenazo III reagent, the heavier cell band contained the bulk of the sorbed uranium. We were able to culture a few viable cells on streak plates from the separated bands, but there was no difference in uranium uptake by isolates of either species as compared to the parent cultures.

The conditions used to culture the cells can affect uranium uptake. *S. cerevisiae* grown on a synthetic medium (28) had a faster rate of uptake, by a factor of 2.5, than when grown on a rich organic medium (18). Although not quantitated, the growth rate and cell yield were reduced in a 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 organisms were separately exposed to uranium in the presence of the metabolic inhibitors 2,4 dinitrophenol (5×10^{-3} M)

or sodium azide (1×10^{-4} M) or pretreated 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 in that no organisms could be cultured from the treated cell preparations. Uranium uptake by both organisms was not affected by either metabolic inhibitor (Fig. 4). Although formaldehyde and $HgCl_2$ treatment had no apparent effect on *P. aeruginosa*, the rate of uranium uptake by *S. cerevisiae* (Fig. 5) was increased.

Temperature and the initial solution pH had a dramatic effect on metal uptake by *S. cerevisiae*. As shown in Fig. 6, the rate of uptake increased with temperature between 20 and 50°C. Although the initial uptake rate increased as the pH was raised from pH 2.5 to pH 5.5, maximal equilibrium distributions were obtained between pH 3.0 to 4.0 (Fig. 7). There was no discernible response of *P. aeruginosa* to temperature or pH although it must be remembered that we were unable to observe the transient 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. Nitric acid (0.1 M), disodium EDTA (0.1 M), and ammonium carbonate (0.1 M) removed only 59.3%, 72.3%, and 83.5% respectively of the bound uranium. To determine if surface binding sites were altered by these treatments, the treated cells were washed and re-exposed to uranium. As shown in Fig. 8, all three treatments enhanced the initial rate of uranium uptake, but nitric acid and sodium EDTA greatly reduced the concentration of uranium on the cells at equilibrium. Two other agents (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 that of ammonium carbonate. Whereas ammonium carbonate treatment before or after the initial exposure to uranium increased the metal uptake rate, sodium citrate and potassium oxalate were only effective after prior exposure of the cells to uranium. Sodium EDTA and nitric acid were not tested in this regard.

It is known (6) 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 analysis of a casamino acid solution before and after exposure to uranium tentatively implicated cysteine and glutamic acid as the responsible amino acids. To determine if these and other amino acids were strong enough complexing agents to compete with cells for uranium, they were added individually (0.1% w/v) to the cell-uranyl nitrate mixture. The presence of several amino acids had no effect on uranium uptake by *P. aeruginosa*. While glutamic acid and aspartic acid (dicarboxylic amino acids) strongly inhibited uranium uptake by *S. cerevisiae* Fig. 9), none of the monocarboxylic amino acids, including the sulfur containing 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 with the corresponding organic acids (e.g., acetic acid) did interfere with metal uptake.

Potassium (100 g/m^3 K^+ as KCl) had no effect on uranium uptake by either organism, nor did Ca^{2+} (100 g/m^3 as $CaCl_2$) interfere with *P. aeruginosa*. ^{45}Calcium was taken up concomitantly with uranium in this organism. Both the initial rate of uranium uptake and the ultimate equilibrium distribution with *S. cerevisiae* are altered by Ca^{2+} (Fig. 10).

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.

Phosphorylated polysaccarides (phosphomannans) comprise a portion of the cell wall of yeasts (8). Phosphate groups are potential binding sites for uranium. As shown in Table 2, two preparations of yeast phosphomannan were effective in complexing uranium.

^{137}Cesium

The ability of microorganisms to remove ^{137}Cs from solution was examined with both resting cells (biosorption) and growing cultures. The results are shown in Table 3. SRSSW is a solution of reagent grade chemicals designed to simulate a high level radioactive waste solution. It contains several other metals and high concentrations of nitrate. Unlabeled $CsCl_2$ was added to the concentration shown along with $\sim$50 μCi $^{137}CsCl_2$ for a tracer. As shown in Table 3, the distribution coefficients were relatively low and in all cases the bulk of the cesium (as evidenced by soluble radioactivity measurements) remained in solution. Little, if any, growth of the denitrifying bacteria occurred in the presence of cesium (ethanol as a carbon source, incubated anaerobically under a nitrogen atmosphere).

^{226}Radium

^{226}Radium incorporation by microbial cells was considered with regard to another radioactive waste problem, namely contaminated waste storage ponds. In addition to ^{226}Ra, the water contains a variety of other metal species and 1.3% nitrate. Samples of the pond water were treated as indicated in Tables 4 and 5. ^{226}Ra incorporation was determined for resting cells and under growth conditions. While some organisms do accumulate ^{226}Ra, the distribution coefficients are relatively low.

Table 2. Complexation of uranium by yeast phosphomannans

Phosphomannan[a]	Mannose/Phosphate ratio[b]	Solution uranium concentration, g/m^3 Initial	Final	Phosphomannan uranium concentration %[c]
Hansenula holstii	∿5	478	224	12.7
NRRL Y-2448		1044	754	14.5
Hansenula capsulata	∿2.5	478	48	21.5
NRRL Y-1842		1044	432	30 6

[a] 0.2% w/v final concentration.
[b] Mannose/phosphate ratios supplied by M. E. Slodki, Northern Regional Research Center, Peoria, Ill.
[c] $\frac{\text{g U}}{\text{g phosphomannan}} \times 100$.

Table 3. ^{137}Cesium uptake by microorganisms

Organism	Conditions		Cesium concentration (g/m^3)	Cell concentration (g/100 ml)	Distribution coefficient[a]
Pseudomonas aeruginosa	Resting cells (in SRSSW)	pH 4	0.12	0.4	11
		pH 7	0.12	0.4	12
		pH 10.2	0.12	0.4	9
	Growth[b]		0.017	0.2	16
Saccharomyces cerevisiae	Resting cells (in water)	pH ∿6	0.006	0.6	26
	Growth[b]		0.017	0.4	37
Mixed culture of denitrifying bacteria	Growth - denitrifying[c] medium		0.013	0.002, 0.003	228, 469
			0.013	0.04	9
			0.013	0.04	15
	Growth - SRSSW[c]		0.013	0.006, 01010	95, 137
			0.013	0.03	21
			0.013	0.05	49

[a]Distribution coefficient: $\frac{\text{cpm } ^{137}\text{Cs per g cells}}{\text{cpm } ^{137}\text{Cs per g solution}}$.

[b]Complex organic medium (see ref. 7).

[c]Ethanol (0.5% v/v) as carbon source (see ref. 10).

Table 4. Uptake of ^{226}Radium from contaminated pond water[a]

Organism[b]	Conditions	Distribution coefficient[c]
Pseudomonas aeruginosa	pH 4	<<1[d]
	pH 6.9	<<1[d]
Saccharomyces cerevisiae	pH 4	<<1[d]
	pH 6.9	<<1[d]
Ashbya gossypii	pH 7.7	344
Penicillium chrysogenum	pH 7.7	624
Mixed culture of denitrifying bacteria	pH 3.9	7
	pH 5.7	95
	pH 7.7	38

[a] ^{226}Ra concentration 345 pCi/ℓ.

[b] Resting cells (washed, resuspended in H_2O) were added to the waste solution and the mixture shaken at 25–27°C.

[c] Distribution coefficient: $\frac{\text{pCi } ^{226}\text{Ra/g cells}}{\text{pCi } ^{226}\text{Ra/g solution}}$.

[d] pCi ^{226}Ra/g cells essentially equivalent to background.

Table 5. ^{226}Ra content of microorganisms cultured in contaminated pond water

	Total pCi ^{226}Ra			Distribution coefficient[d]
	Initial	Solution	Cells	
Mixed culture denitrifying bacteria				
Glucose grown[a]	48	41, 35	3.5, 2.6	104, 119
Ethanol grown[a]	48	32, 34	1.9, 3.1	124, 126
Chlorella pyrenoidosa				
Shake culture[b]	48	40	0.45	51
Static culture[b]	48	56	0.63	41
WS algae[c]				
Shake culture[b]	48	42	0.37	46
Static culture	48	60	0.60	32

[a] 20 days incubation. ∿10 fold increase in cell mass.

[b] 17 days incubation.

[c] Mixed algal, blue-green algal population isolated from pond water.

[d] $\frac{\text{pCi } ^{226}\text{Ra/g cells}}{\text{pCi } ^{226}\text{Ra/g solution}}$.

DISCUSSION

Uranium

Uranium uptake by *S. cerevisiae* and *P. aeruginosa* differ from each other 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., $R\text{-}COO^-$, $PO_4^{=}$) on the cell surface (2,3,20) or in extracellular polymers (7). 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 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 the cell surface have been unsuccessful. We have observed that as uranium is taken up by the cells, the pH rises from ~4.0 to a pH of 5.5 to 6.0, indicating a release of free hydroxyl ions from the hydrolysis products of uranium which are present in an aqueous solution under these conditions. This suggests that UO_2^{+2} could be the form of the bound metal and, in fact, UO_2^{+2} readily complexes with a variety of anions (6).

The relatively large amounts of uranium accumulated (10 to 15% of the dry cell weight) was of concern. This value is similar to that obtained for a mixed culture of denitrifying bacteria (S. E. Shumate II et al., Biotech. Bioeng., in press) and *Rhizopus arrhizus* (B. Volesky, personal

comm.). 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 uranium, the metal concentration on that fraction of the cells approached 50% on a dry weight basis. Beveridge (2) also observed a nonstoichiometric accumulation of metals on isolated cell walls of *Bacillus subtilis*. He suggested that metal complexes with existing reactive sites and that additional metal "crystallizes" on the bound molecules. It would help to know the chemical nature of the bound metal. While no physiological or genetic basis for the distribution of metal uptake in the cell population has been found to date, the phenomenon is not only of basic scientific interest but also of great practical importance. If the entire population could be induced to accumulate metal through environmental or genetic manipulation, process potential would be enhanced.

Rothstein and Meier (20) gave evidence that polyphosphate groups of *S. cerevisiae* complex uranium. Our finding that uranium complexed with yeast phosphomannans and that the uranium complexation capacity of the polymers appeared to be related to the phosphate content supports the role of phosphate groups in metal binding. Carboxyl groups are also active in metal complexation (6; T. J. Beveridge and R. G. E. Murray, Abstr. Annu. Meet. Am. Soc. Microbiol., 1978, J5, p. 77; T. H. Matthews and R. J. Doyle, Abstr. Annu. Meet. Am. Soc. Microbiol., 1979, K86, p. 159). 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 content of the cell wall, which is known to occur in yeast cultured at reduced growth rates (15). It would be worthwhile to examine the effects of growth conditions and nutrient limitation in both batch and continuous culture on cell wall composition of the metal binding and nonbinding yeast cells separable by $CsCl_2$ centrifugation. Unfortunately, we have yet to find a method

of removing bound uranium without affecting the cell surface (as evidenced by changes in subsequent metal uptake).

Dounce and Flagg (6) carried out extensive studies on the complexation of uranium with organic acids, proteins, and a few amino acids using a titration technique. Organic acids and proteins were very effective in complexing with uranium. However, little interaction occurred between uranium and serine or glycine, which the authors were unable to explain. The ability of certain amino acids to interfere with uranium complexation by whole cells of *S. cerevisiae* appears to be a means of addressing this question. As we observed, monocarboxylic and N-substituted amino acids were ineffective in this regard, but dicarboxylic amino acids and organic acids did interfere, presumably by competitive complexation of the metal. Molecular models indicated no basis for a steric hindrance to uranium complexation with monocarboxylic amino acids. We have tentatively 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. Unofrtunately, raising the pH to remove the charge on the amino group decreases the solubility of uranium. Although the above interpretation would imply that there are only a limited number of carboxyl groups available for metal binding in a protein, the situation must be more involved since proteins are effective complexing agents for uranium. Consideration should be given to the determination of the effects of the charge interactions within a protein molecule and the close physical proximity of carboxyl groups (multiple linkage of metal to more than one carboxyl group). It is necessary to remember, however, that in a whole cell, amino acids are not the only components possessing carboxyl groups.

There has been some indication that reducing the positive charge on cell wall components by chemical treatment can enhance metal uptake (4).

Since the active metal binding sites on the surface of *S. cerevisiae* have not not been established, we have no explanation for the increase or decrease in uranium uptake rates resulting from chemical pretreatment (e.g., ammonium carbonate, H_gCl_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 response of uranium uptake by *S. cerevisiae* to pH, temperature, interfering metal ions (e.g., Ca^{2+}) can be understood if likened to an ion-exchange-type mechanism. How do we explain, however, the rapid intracellular uptake of uranium by *P. aeruginosa*? Within the limits of our ability to measure uranium uptake, the process in this organism appears insensitive to environmental conditions, and we don't 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 a prokaryotic organism (17), is involved. Interestingly, in another bacterial system we have examined (S. E. Shumate II et al., Biotech. Bioeng., 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 apparent insensitivity to environmental parameters). This mixed culture is believed to be predominantly comprised of pseudomonad-like organisms (6). 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 have originally hoped to be able to extrapolate the results of our studies with the two organisms used in order to enhance metal biosorption in a variety of biomass sources. That there are at least two different biosorption systems, subject to different parameters, precludes any generalizations. Enhancement by whatever means will be dependent on the type of biosorption system involved. Future studies will be directed toward elucidating the two mechanisms observed. It is of particular interest why only a portion of the cells in a population take up uranium and how this proportion can be increased.

Cesium

Literature reports of microbial cesium accumulation indicate that both growth related and adsorptive phenomena occur (9,11,18,25,26,27). The stage of growth for maximum accumulation varies in these reports. Also it was found that cesium was not permanently associated with growing cells and that with time, firm binding was more significant in old cells and detritus.

We attempted to compare our results with those in the literature. Notably, it has been shown that cesium accumulation factors or distribution coefficients decreased as the cesium concentration was increased. For example, Fisel et al. (9) observed maximum accumulation by *Paecilomyces marquandii* (34% of added ^{137}Cs) at the lowest cesium concentration tested ($\sim$0.008 mM Cs). With *Chlorella pyrenoidosa* (Williams, 25), increasing the cesium concentration from a tracer level (10 μCi $^{137}Cs/\ell$) to 0.5 mM decreased the concentration factor by three-fold. Williams (25) also found that a cesium concentration of 0.15 mM significantly reduced the concentration

factor in *Euglena* intermedia. Cesium incorporation studies done by other workers (11,18,26) involved very low ^{137}Cs levels (2 to 20 x 10^{-5} µCi/ml) and relatively high distribution coefficients were obtained.

The results in the literature as well as our own are presented as distribution coefficients or accumulation factors. These values are a function of both the maximum cellular loading capacity for cesium and the solution cesium concentration. When maximum loading of the cells has been achieved, increasing levels of residual cesium depress the distribution coefficient. We have observed this with microbial uranium uptake (24). It would seem, based on the available data that high cesium levels may not be inhibitory, but rather, the cells have a low maximum capacity for cesium. Hence, when the cells have reached this maximum capacity, additional unbound cesium depresses the distribution coefficient or accumulation factor. We were unable to deduce the loading capacity from the data in the literature, and our own experiments were not designed to obtain this value. However, a very rough estimate from our data indicated a loading capacity of less than 10 µg Cs/g cells. In all cases, the greater portion of cesium remained in solution providing evidence that the cells were saturated with cesium under the conditions imposed. Additionally, Plato and Denovan (18) and Williams (25) reported that cesium accumulation factors were cell density dependent and our own results (Table 3) also show this.

^{226}Radium

Little is known about the microbial accumulation of radium. Jilek et al. (14) observed the removal of ^{226}radium from a waste stream by chemically derivitized mycelia of *Penicillium chrysogenum* but no distribution coefficients or cellular capacities were reported. P. A. Taylor of the Chemical Process Department, Y-12 Development Division, Nuclear Division, Union Carbide Corp., Oak Ridge, Tenn. (unpublished) found 95%

of the ^{226}Ra removed during long-term (months) growth of denitrifying microorganisms in the same pond water we examined. Methanol was used as a carbon source. In contrast, we found very little ^{226}Ra removal either by biosorption or during short-term (weeks) growth experiments. Further experimentation will hopefully resolve these differences.

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FIGURE CAPTIONS

Fig. 1. Removal of uranium from aqueous solution by *S. cerevisiae* and *P. aeruginosa*.

Fig. 2. Electron micrograph of *S. cerevisiae* showing surface accumulation of uranium. X35,000.

Fig. 3. Electron micrograph of *P. aeruginosa* showing intracellular accumulation of uranium. X27,000.

Fig. 4. Effect of 2,4 dichlorophenol and sodium azide on uranium uptake by *S. cerevisiae* and *P. aeruginosa*.

Fig. 5. Effect of H_gCl_2 and formaldehyde pretreatment on uranium uptake by *S. cerevisiae*.

Fig. 6. The influence of temperature on uranium uptake by *S. cerevisiae*.

Fig. 7. The effect of initial pH on uranium uptake by *S. cerevisiae*.

Fig. 8. Uranium uptake by *S. cerevisiae* (pre-exposed to uranium) after treatment with nitric acid, disodium ethylenediaminetetraacetic acid or ammonium carbonate.

Fig. 9. Inhibition of *S. cerevisiae* uranium uptake by glutamic acid and aspartic acid.

Fig. 10. Effect of Ca^{2+} on uranium uptake by *S. cerevisiae*.

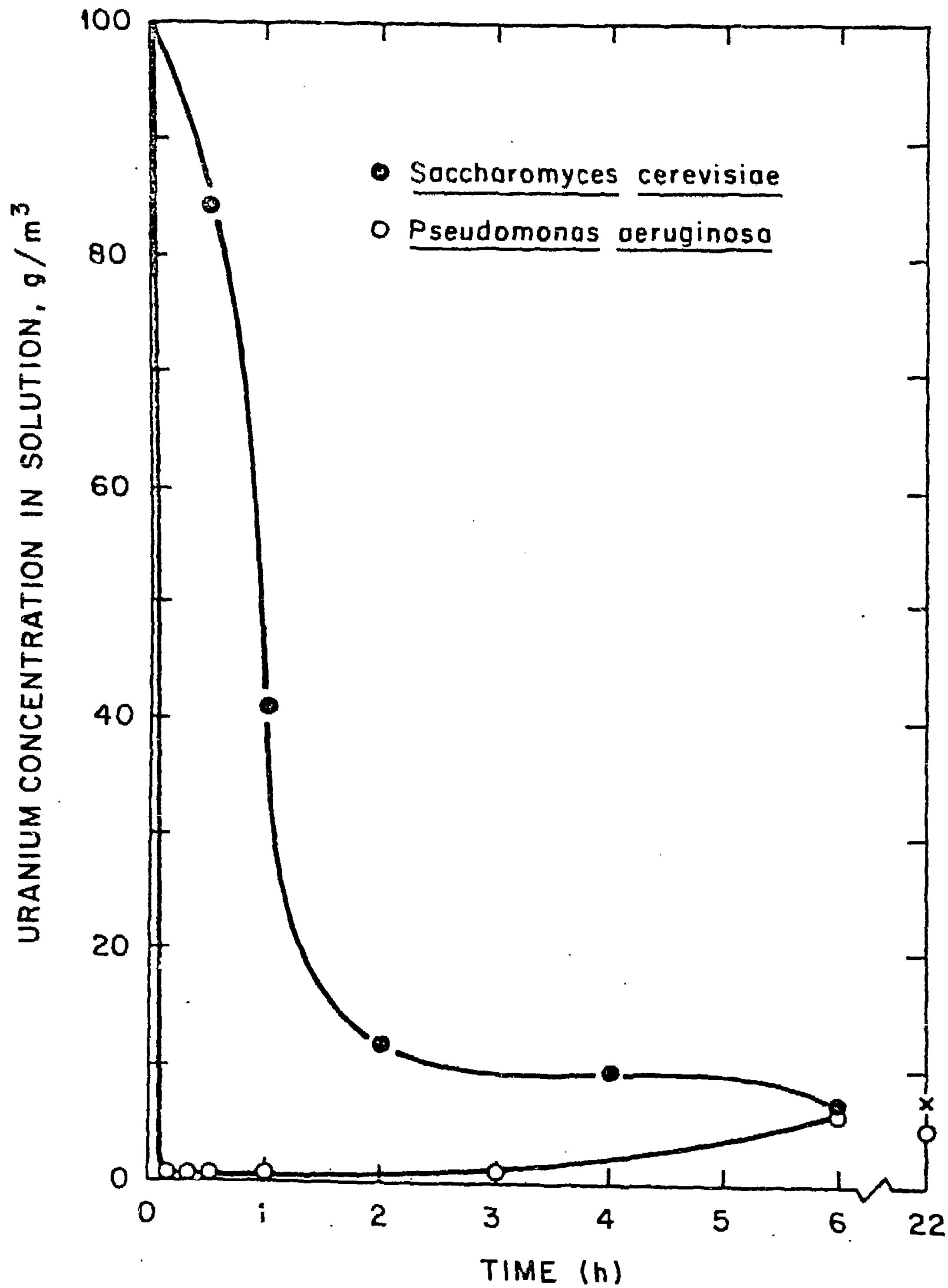
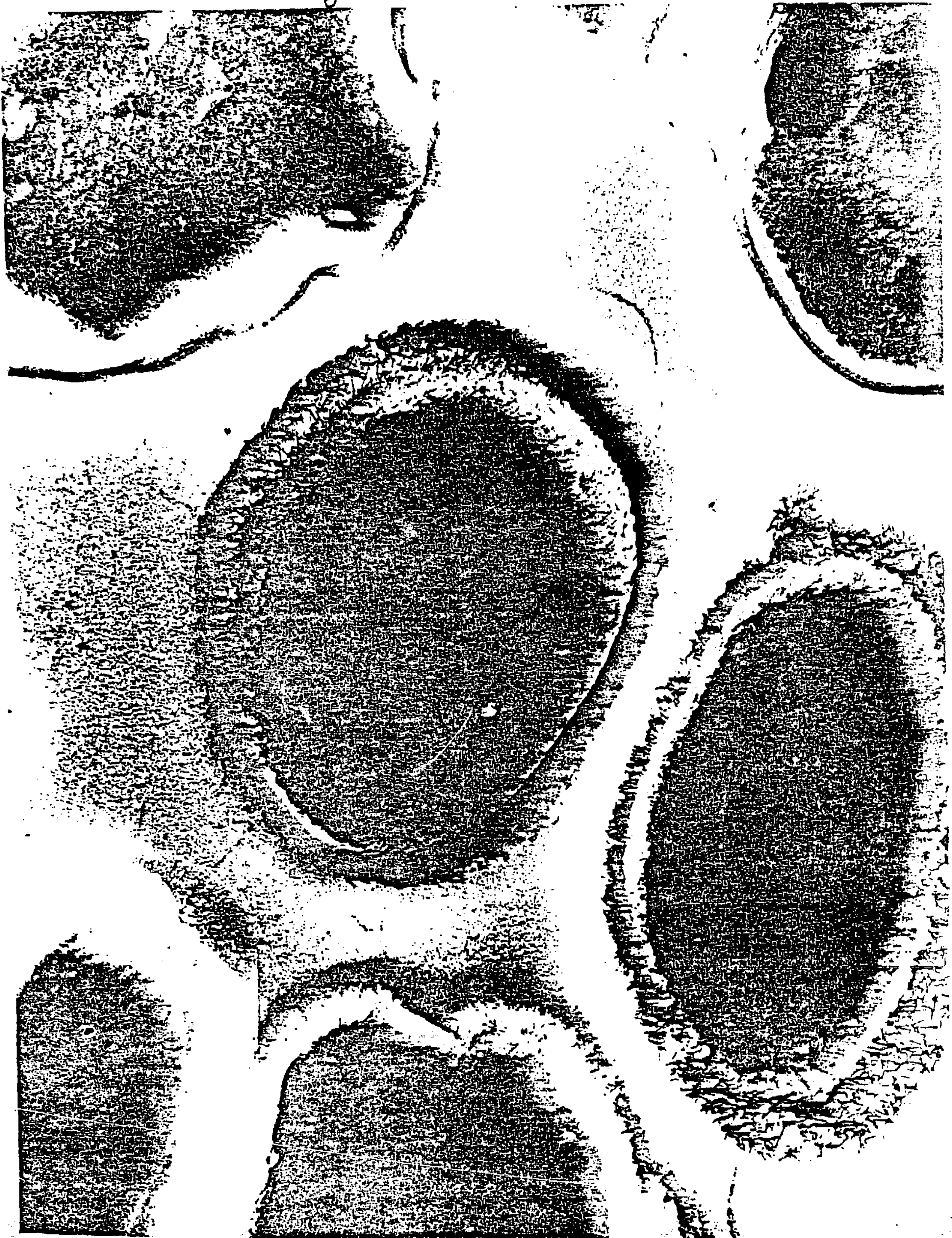


Fig 1
ORNL DWG 80-586
Saccharomyces cerevisiae
Pseudomonas aeruginosa
URANIUM CONCENTRATION IN SOLUTION, g/m3
0
20
40
60
80
100
0
1
2
3
4
5
6
22
TIME (h)

Fig. 2



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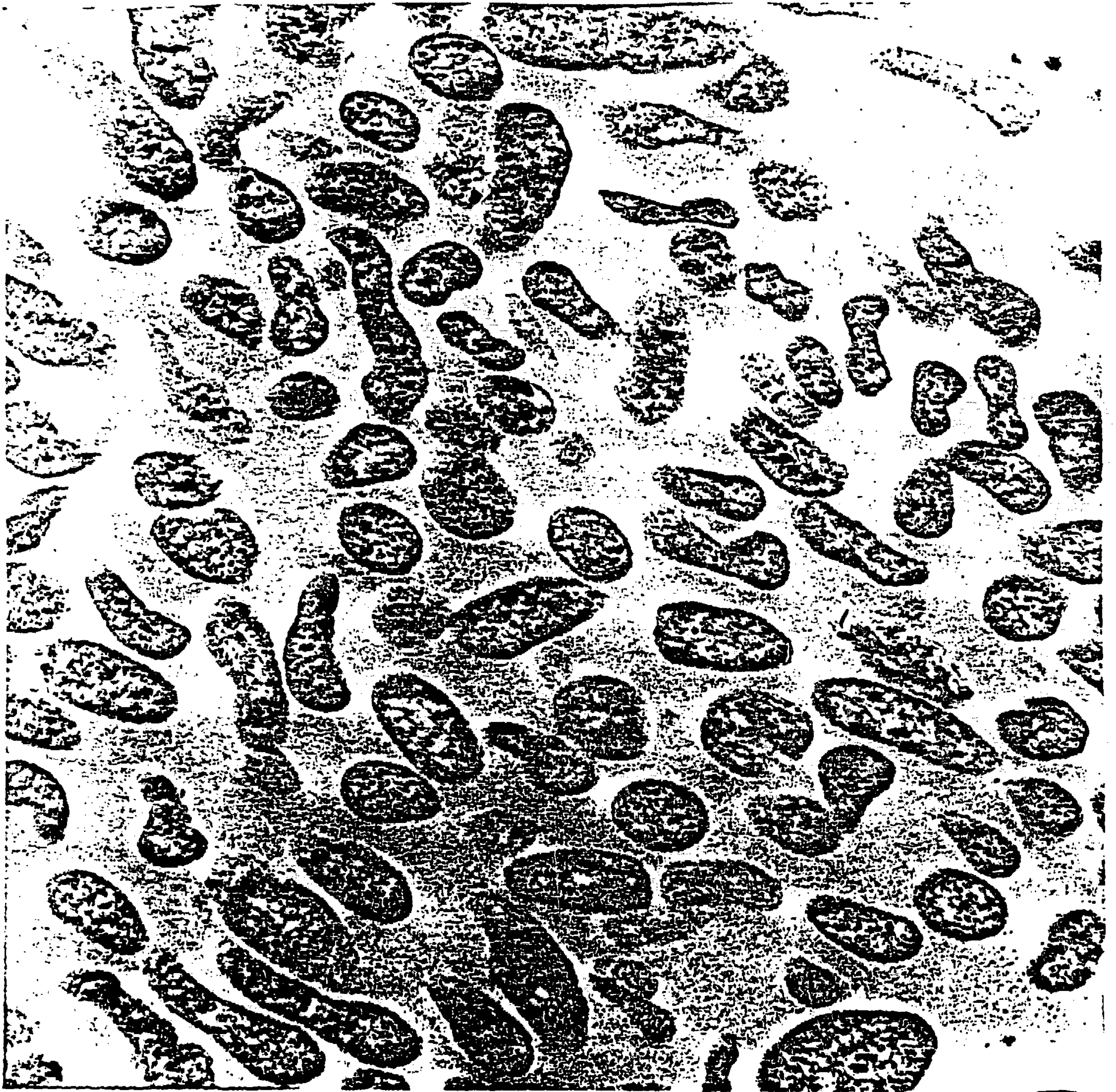

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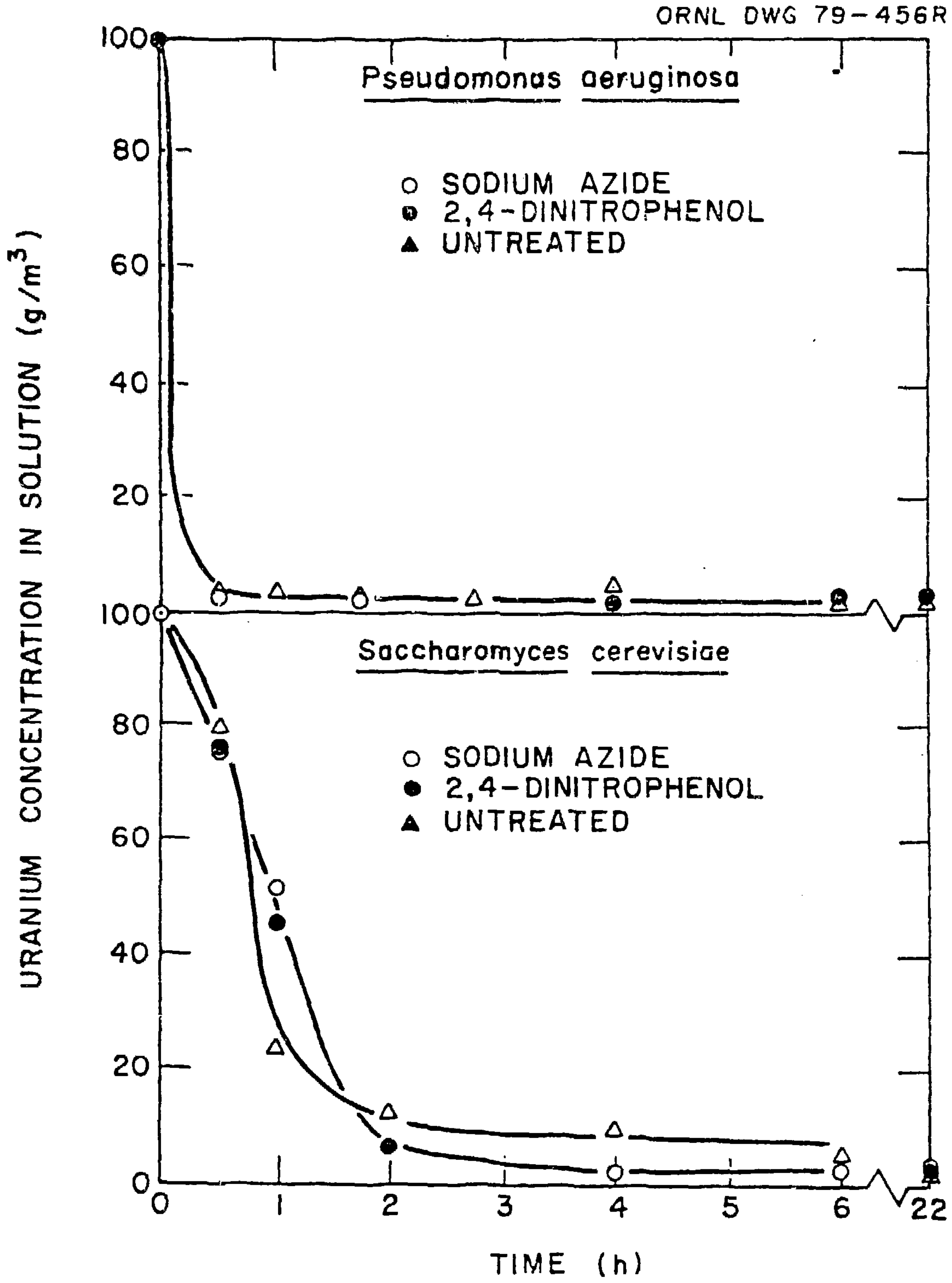

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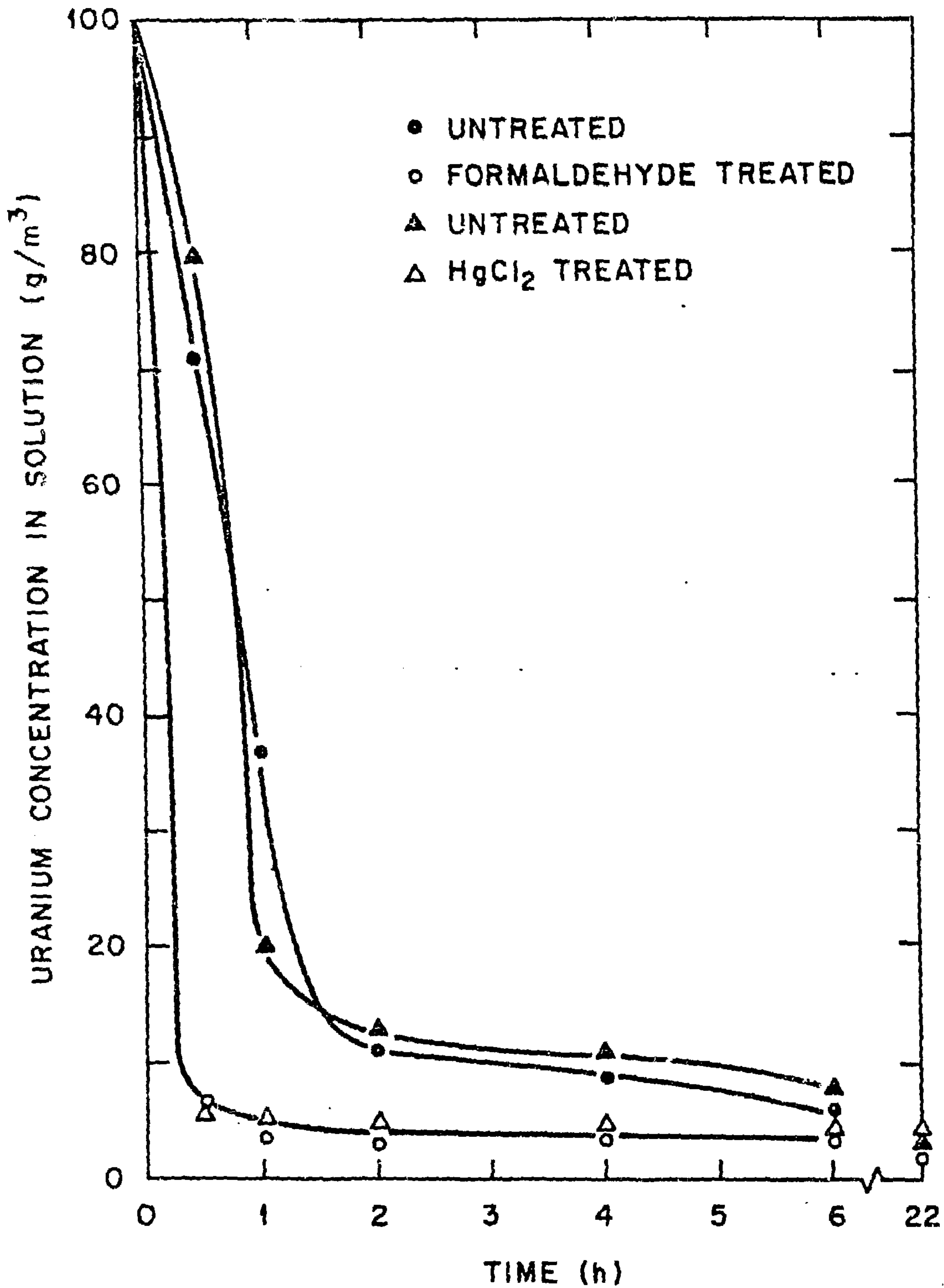

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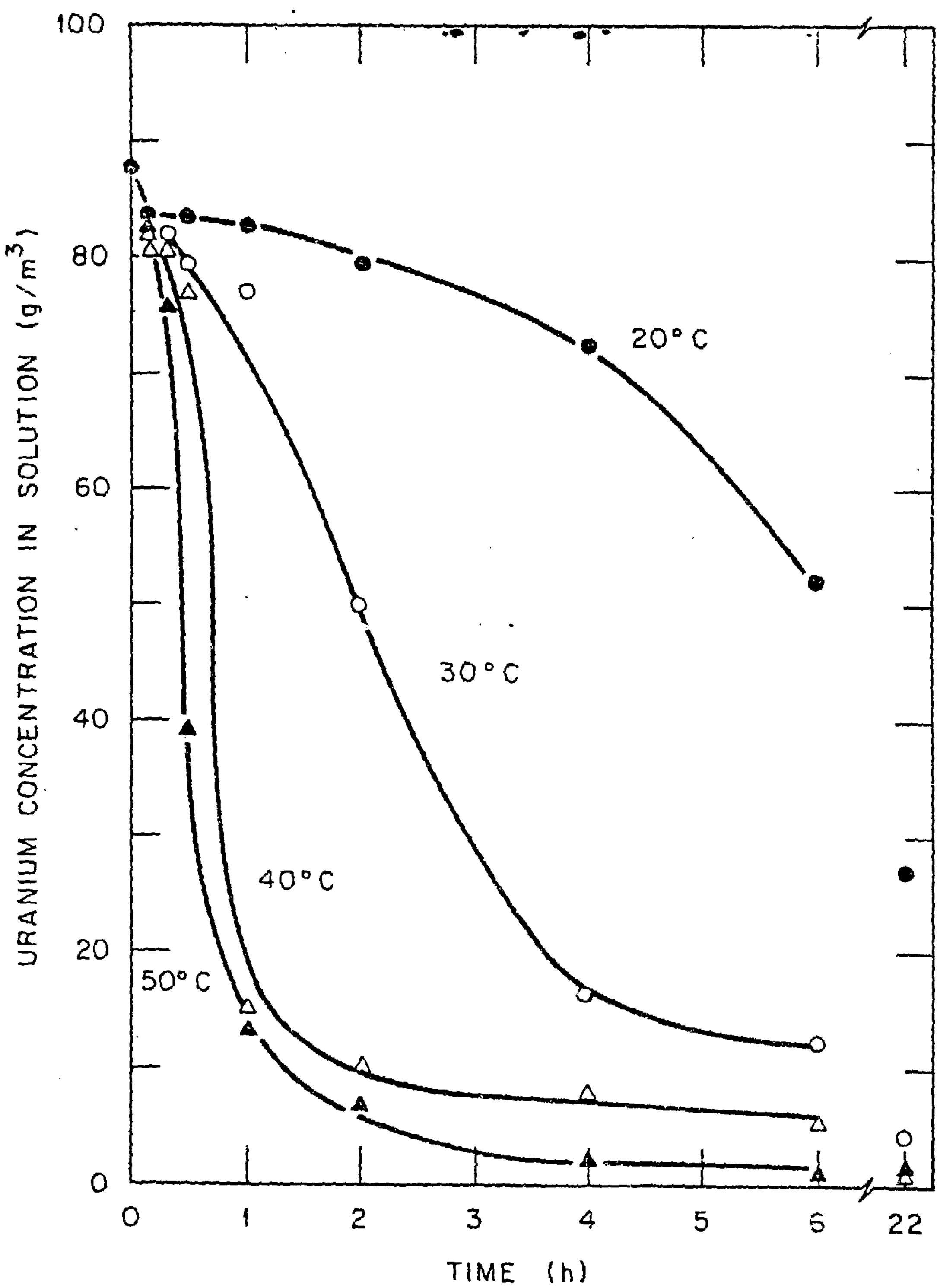

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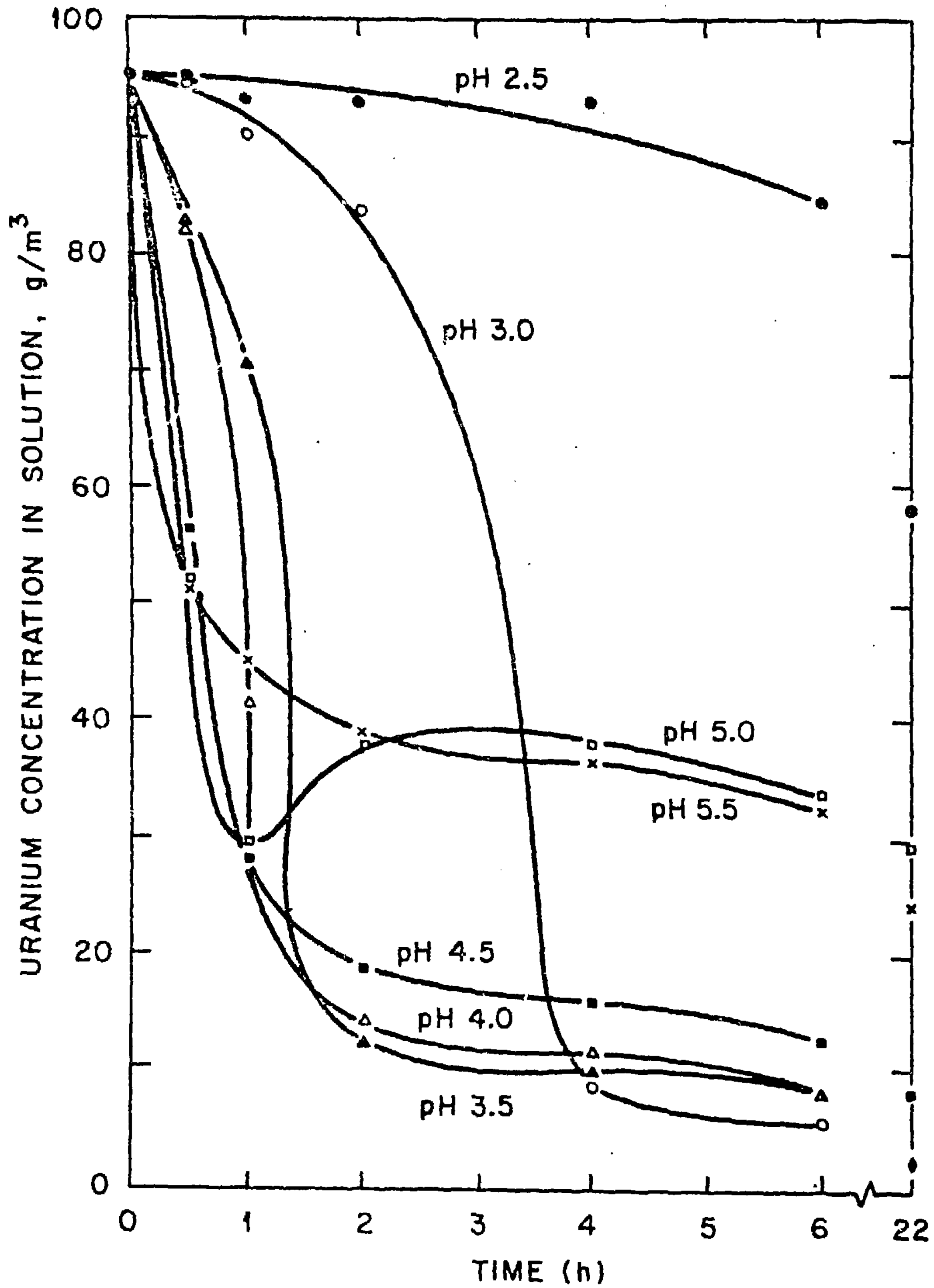

Fig 8

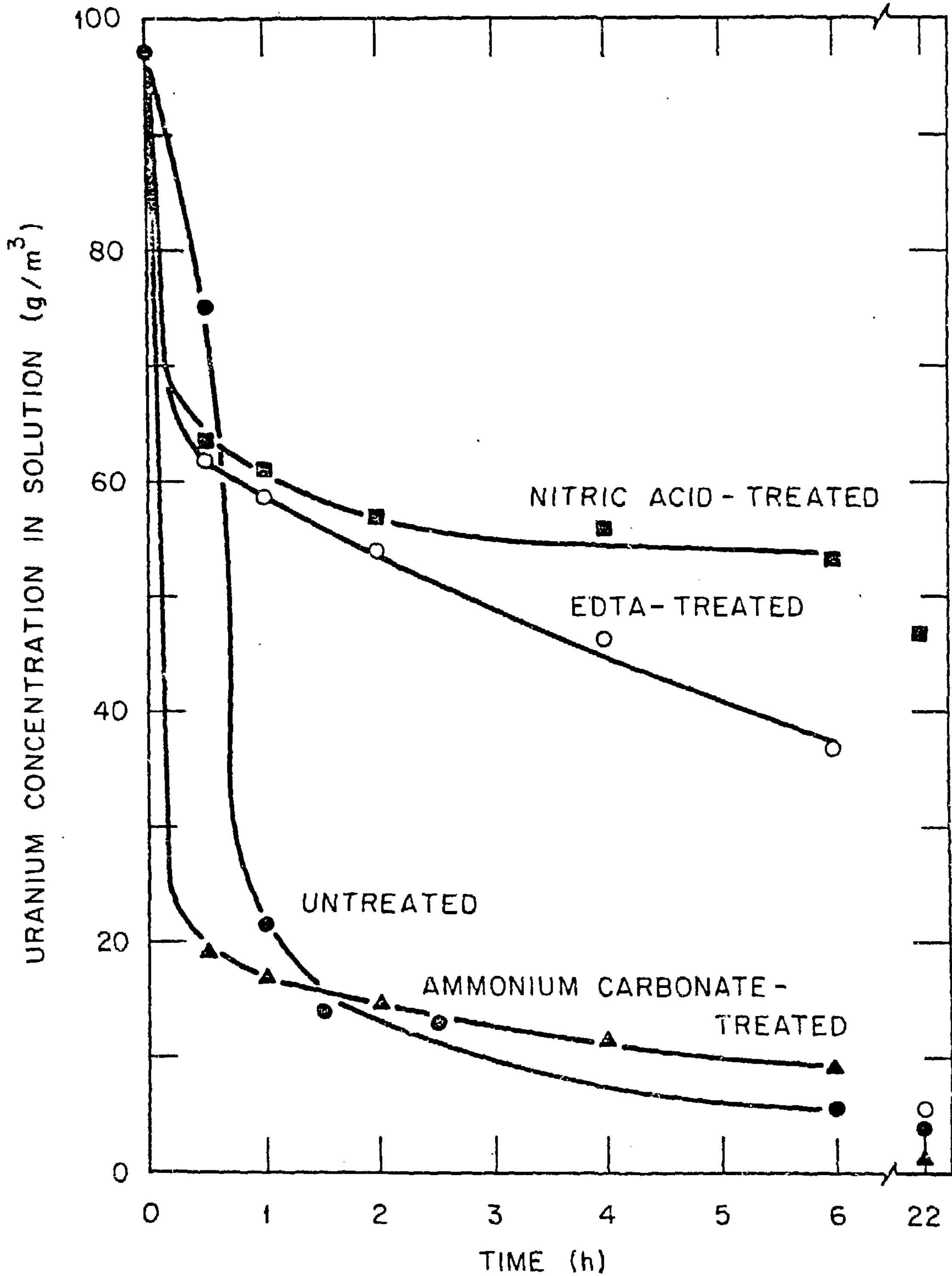

Fig 9

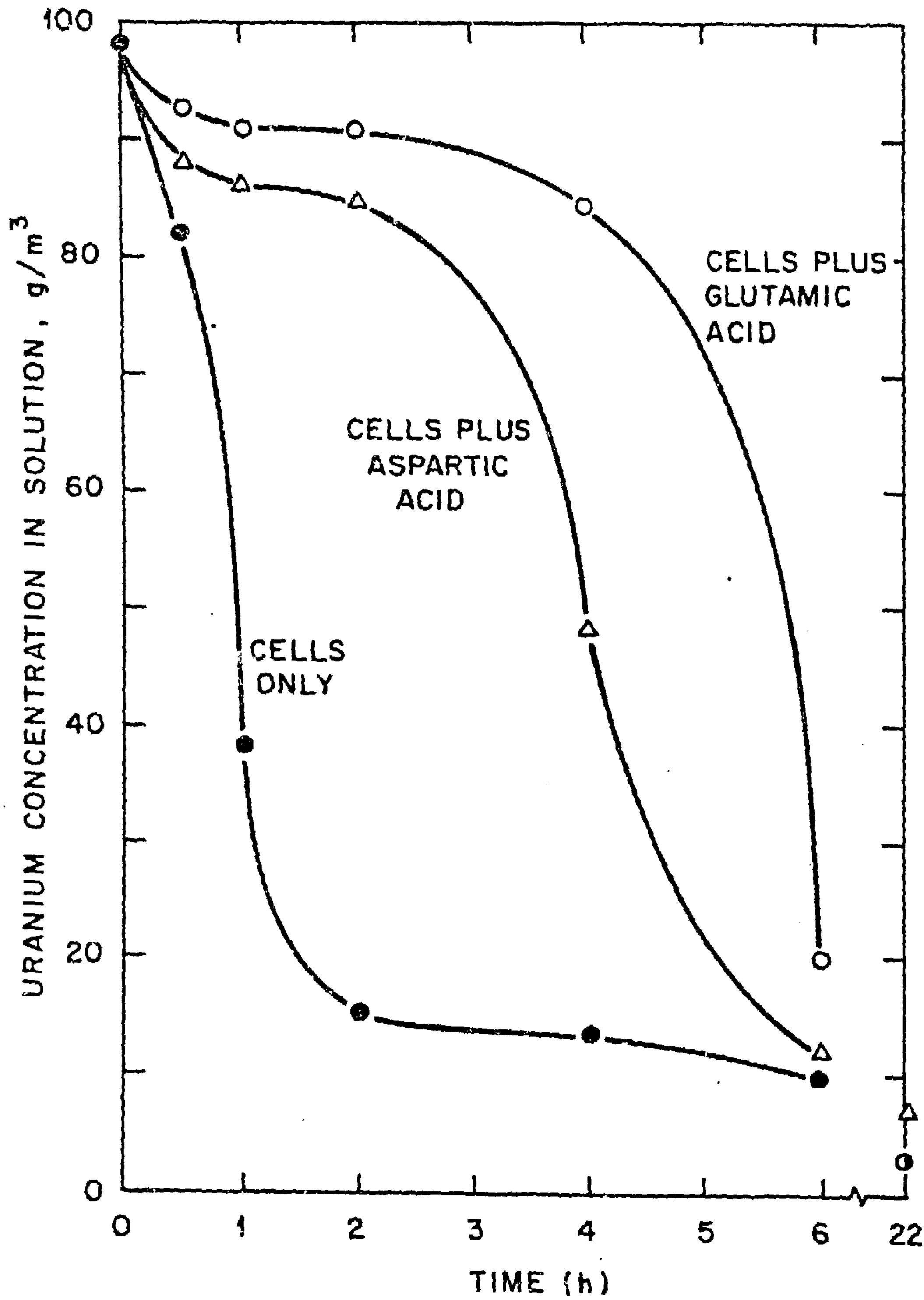

Fig 10