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SUMMARY

Aqueous waste streams from nuclear fuel processing operations may contain trace quantities of heavy metals such as uranium. Conventional chemical and physical treatment may be ineffective or very expensive when uranium concentrations in the range of 10-100 g/m^3 must be reduced to 1 g/m^3 or less. The ability of some microorganisms to adsorb or complex dissolved heavy metals offers an alternative treatment method. Uranium uptake by Saccharomyces cerevisiae NRRL Y-2574 and a strain of Pseudomonas aeruginosa was examined to identify factors which might affect a process for the removal of uranium from wastewater streams. At uranium concentrations in the range of 10-500 g/m^3, where the binding capacity of the biomass was not exceeded, temperature, pH, and initial uranium concentration were found to influence the rate of uranium uptake, but not the soluble uranium concentration at equilibrium.

INTRODUCTION

Some aqueous effluent streams emanating from energy production processes contain dissolved heavy metals which, due to their chemical or radiological properties, can pose a hazard to the environment. The concentrations of

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such metals must be reduced to acceptable levels before the streams can be discharged to the environment. Processing steps in the nuclear fuel cycle generate wastewater streams containing a variety of dissolved heavy metals including uranium. Conventional methods for removing heavy metals from aqueous streams include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, and evaporative recovery. Such processes may be ineffective or extremely expensive when initial heavy-metal concentrations are in the range of 10-100 g/m^3 and discharge concentrations are required to be less than 1 g/m^3.

Another processing method which may be considered is the sorption and/or complexation of dissolved metal species by microorganisms. One of the earliest references to such a wastewater treatment concept for the disposal of radioactive metals was made by Ruchhoft,[1] who cited the removal of plutonium-239 from water by activated sludge. He observed that about 96% removal could be accomplished in a single stage of treatment and pointed out that a two-stage process could be used if a greater degree of decontamination was required. The decontamination process was described as the propagation of a microbial population "having gelatinous matrices with tremendous surface areas that are capable of adsorbing radioactive materials."[1] Other investigators[2] emphasized that the essential role of the biological population would be to function as an adsorbent. The concern expressed that "wastes can only be treated biologically if they are free from acids, alkalis, and toxic substances"[2] indicates that little thought was given to decoupling the two steps: propagation of a microbial sorbent, and contact of a metal-contaminated stream with the sorbent. In

recent years, however, some attention has been given to the use of dried, irreversibly degraded mycelium as an effective sorbent for the removal of metal radionuclides from aqueous streams.[3]

Our approach has been to characterize a number of microbial species with respect to effectiveness in removing uranium from aqueous solution by resting (washed, resuspended) cells.[4] On the basis of this characterization, Saccharomyces cerevisiae and Pseudomonas aeruginosa were chosen for additional experiments to study the effects of initial metal concentration, temperature, and pH on the rate of uranium uptake in a well-mixed, single-stage contacting vessel. Our intent is to decouple the steps of biosorbent propagation and contact of an aqueous stream with the biosorbent for removal of dissolved heavy metals such as uranium. In this way, these steps can be optimized independently to yield a more effective overall process. The presence of growth-inhibiting substances in the wastewater stream would not have any detrimental effect on the biosorbent propagation step.

MATERIALS AND METHODS

Culture and Preparation of Cells

The yeast used in this study was Saccharomyces cerevisiae NRRL Y-2574 obtained from the Agricultural Research Service Culture Collection (Peoria, Ill.). The bacterium was a culture of Pseudomonas aeruginosa obtained from H. R. Meyers and J. Johnson, Colorado State University, which had been utilized in a study of plutonium uptake.

The general-purpose YM medium of Wickerham[5] was utilized for both culture maintenance and cell production. This medium contains glucose (1%), yeast extract (0.3%), malt extract (0.3%), and peptone (0.5%). All cultures were incubated at 28°C.

Sufficient cells for uranium uptake experiments were obtained by culturing the organisms for 24 hr in Fernbach flasks that contained 750 ml of YM medium and were shaken at 100 rpm (2-in. stroke). These flasks had been inoculated with 10 ml of a 24-72 hr shake culture started from an agar slant. Cells were recovered from the Fernbach cultures by centrifugation at 9000 x g for 20 min and washed three times with deionized distilled water (Milli-Q Reagent-Grade Water System, Millipore Corp., Bedford, Ma.). The washed cell paste (refrigerated overnight) was resuspended in deionized distilled water to yield approximately 1 g of wet cells per 10 ml of water. An aliquot of this cell suspension was dried at 110°C for determination of dry cell weight.

Uranium Uptake Experiments

Uranyl nitrate hexahydrate (J. T. Baker Chem. Co., Phillipsburg, N.J.) solutions were prepared with deionized distilled water so that the addition of a given volume of cell suspension provided the proper dilution to give the desired initial uranium concentration. Generally, 10 ml of the cell suspension was added to 40 ml of the uranium solution (pre-equilibrated at the required temperature) contained in a 250-ml Erlenmeyer flask. The mixture was shaken at 100 rpm at either 25°C or 40°C in a water bath shaker (Aquatherm, Model G-86, New Brunswick Scientific Co., Inc., New Brunswick, N.J.). At desired time intervals, samples were withdrawn, the cells were removed by centrifugation, and the uranium content of each supernatant fraction was determined. Distribution coefficients were calculated on the basis that the uranium removed from solution was sorbed onto biomass. Cell-free controls were run to ensure that the uranium removed from solution was associated with biomass.

Uranium Assay

A spectrophotometric assay based on the reaction of uranium with Arsenazo III was used.[6,7] A 0.1% solution of Arsenazo III (Aldrich Chemical Co., Milwaukee, Wis.) was prepared by dissolving the dry powder in 0.1 N NaOH. This solution was acidified to pH 1.5 with concentrated H_2SO_4 and finally diluted 1:10 with 0.1 N H_2SO_4. The reagent was added at a ratio of 5:1 (v/v) to sample solutions that had been appropriately diluted to contain 1-20 ppm uranium. The solutions were mixed and allowed to stand approximately 10 min; then the absorbance of each was measured at 650 nm. Concentration was calculated from a standard curve.

RESULTS AND DISCUSSION

A number of investigators[8-12] have indicated that microbially synthesized polymers extending from the outer membrane of a cell are responsible for the binding of metal ions from solution. Metal cations may be complexed by negatively charged sugar units at the end of a polysaccharide chain. Rothstein and co-workers[8,13,14] have cited evidence that exocellular polyphosphate groups, associated with sugar metabolism, are responsible for the binding of uranium (uranyl ion) from aqueous solution. Polyphosphates complex metal ions by chelation through negatively charged oxygen atoms.[15] The chelating power or capacity of a chain polyphosphate increases with increasing chain length. The nature of these suggested binding units and other potential uranium binding groups on the cell surface indicates that the binding might be dependent on the cell environment (i.e., the solution in intimate contact with the cell). The complex aqueous chemistry of uranium must be considered as well. However, from a practical standpoint,

our initial concern has been focused on the identification of factors which would affect the association of uranium with biomass in a wastewater treatment process. Such factors might include soluble uranium concentration, pH, and temperature.

Figure 1 shows the rate and degree of uranium removal from solution by S. cerevisiae cells for a range of initial uranium concentrations from 10 to 500 g/m^3. Even at an initial uranium concentration of 500 g/m^3, the equilibrium solution concentration was just slightly higher than that which was reached with an initial uranium concentration of 10 g/m^3. Thus, the binding capacity far exceeded the uranium loadings used in these experiments.

The rate of uranium removal from solution was significantly affected by temperature at the higher initial uranium concentration as shown in Figure 2; the rate was considerably greater at 40°C than at 25°C. This pronounced effect is seen again in Figure 3, which summarizes data from experiments wherein cells were sequentially contacted with solutions with initial uranium concentrations of 86 g/m^3. The equilibrium uranium concentration in solution after the second exposure is just slightly higher than that reached after a single exposure to an initial uranium concentration of 100 g/m^3 (Figure 1).

Figures 4a and 4b illustrate the effect of pH on the rate of uranium removal from solution. The rate was greatest when the solution pH was maintained at 5.5; when no effort was made to control the solution pH, it gradually increased to a value between 5.5 and 6.0 as uranium was being sorbed or complexed by the yeast cells. The rate of uranium removal in this case was greater than when the pH was controlled at 3.9.

The rate and degree of uranium removal by P. aeruginosa cells for a range of initial uranium concentrations from 10 to 100 g/m^3 are shown in Figure 5. As is evident, the removal rate was much greater than that obtained using the yeast cells (Figure 1). The data also indicate that the uranium concentration in solution had approached the equilibrium value after a contact time of only 10 min. This rapid uptake is similar to the effect observed by Zajic and Chiu[12] when using a growing Penicillium culture.

Soluble metal concentration, pH, and temperature are among the more readily determined and controlled parameters to be considered in the design of a process for the removal of a heavy metal from wastewaters by association with biomass. Additional factors that are being considered include the type, number, and reactivity of metal binding sites as affected by cell growth history and species differences, resistances to mass transfer, and other constitutents in the waste stream (e.g., competing ions).

CONCLUSIONS

Resting (washed, resuspended) microbial cells have been shown to effect rapid removal of uranium from aqueous solution. The rate and degree of uranium isolation from solution make this approach to heavy-metal removal quite promising as a means for the decontamination of process wastewaters from the nuclear fuel cycle. The complexed, concentrated uranium could conceivably be removed from the microbial cells and recycled. A bench-scale process is being developed to test this concept for the removal of radioactive heavy metals from aqueous process streams.

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FIGURE CAPTIONS

Fig. 1. Effect of initial uranium concentration on uranium sorption by *Saccharomyces cerevisiae*.

Fig. 2. Effect of temperature on uranium sorption by *Saccharomyces cerevisiae*.

Fig. 3. Uranium sorption by *Saccharomyces cerevisiae* during sequential exposure.

Fig. 4a. Effect of pH on uranium sorption by *Saccharomyces cerevisiae*: pH controlled at 3.9.

Fig. 4b. Effect of pH on uranium sorption by *Saccharomyces cerevisiae*: pH controlled at 5.5.

Fig. 5. Effect of initial uranium concentration on uranium sorption by *Pseudomonas aeruginosa*.

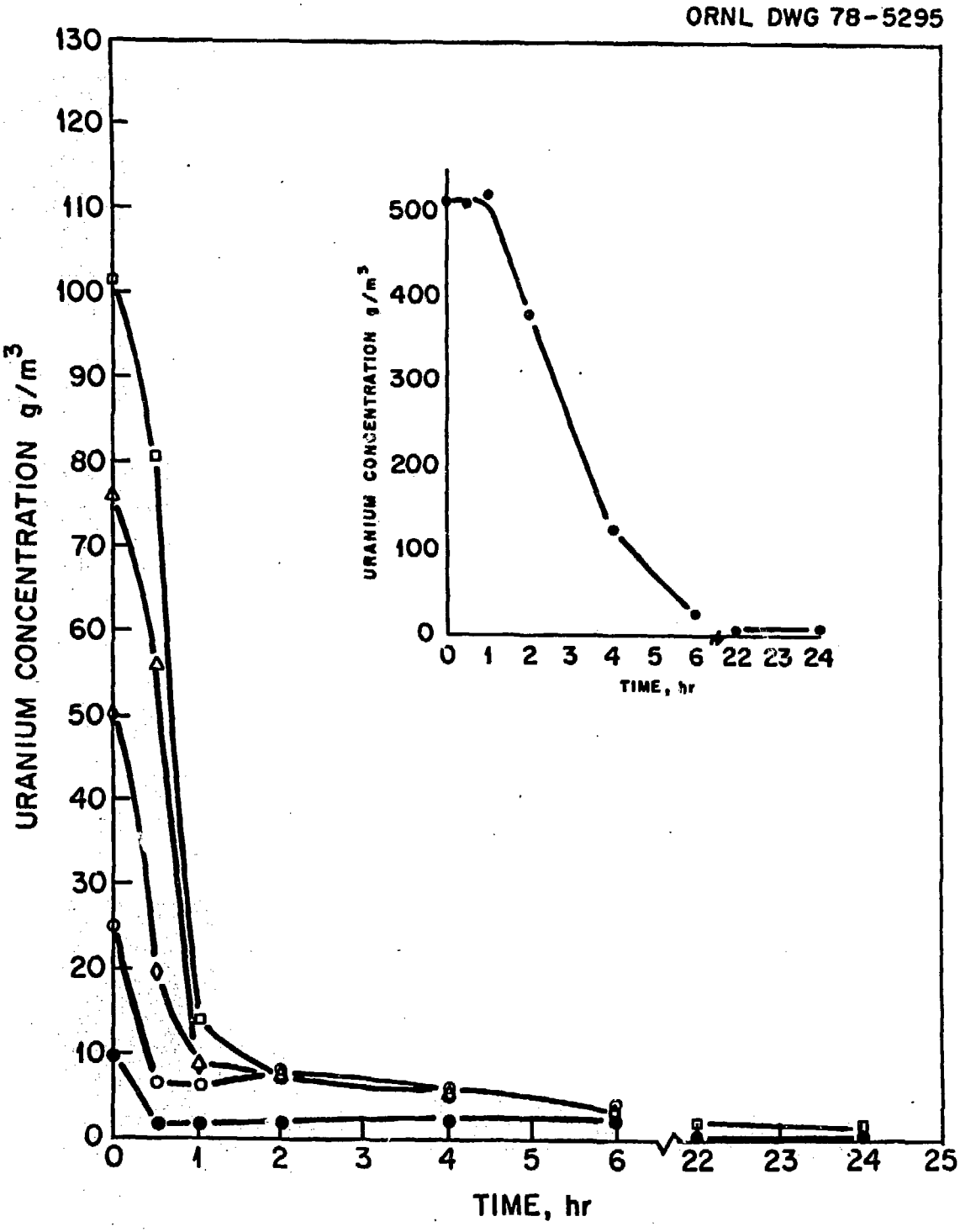

Fig. 1. Effect of initial uranium concentration on uranium sorption by Saccharomyces cerevisiae.

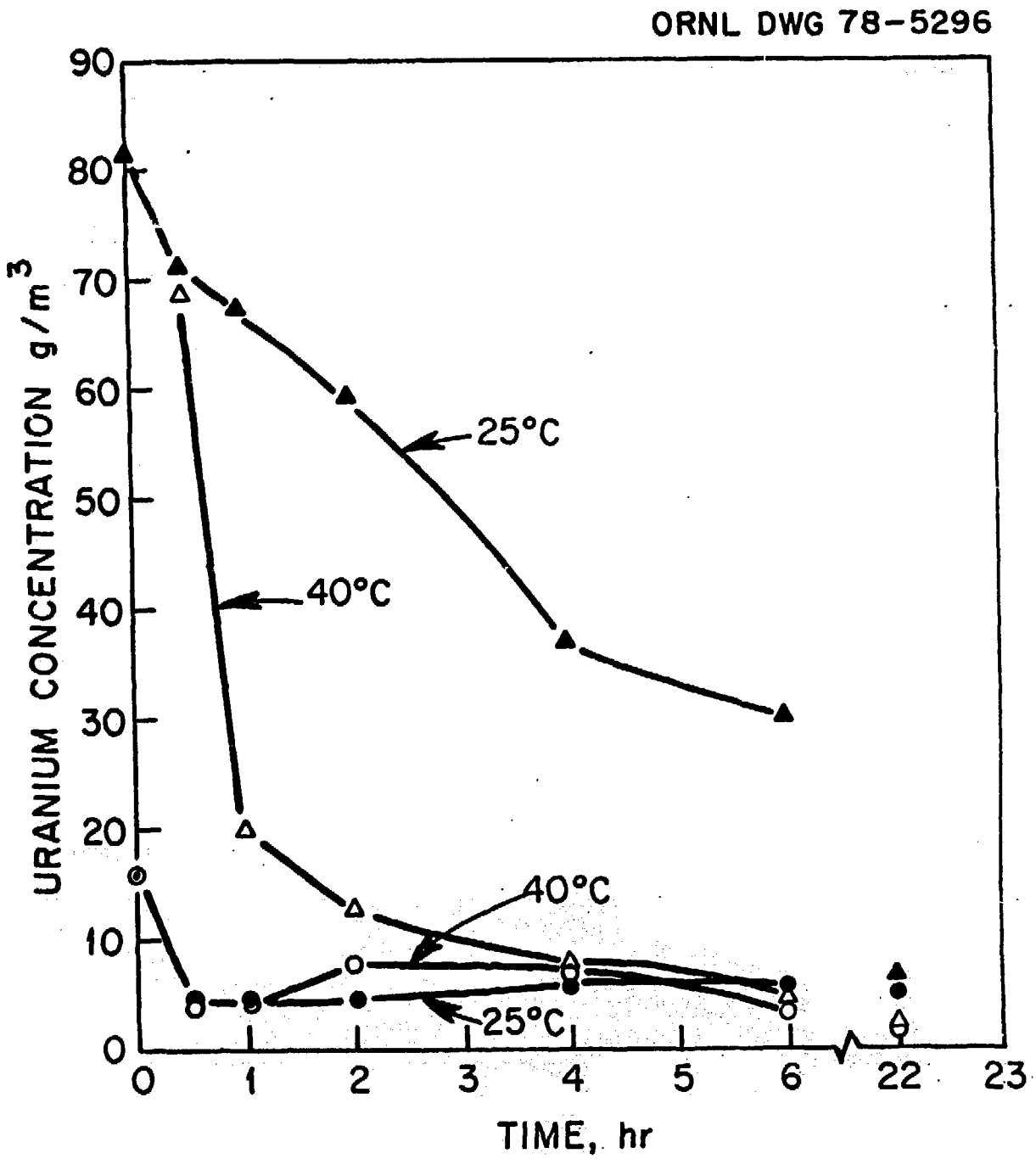

Fig. 2. Effect of temperature on uranium sorption by Saccharomyces cerevisiae.

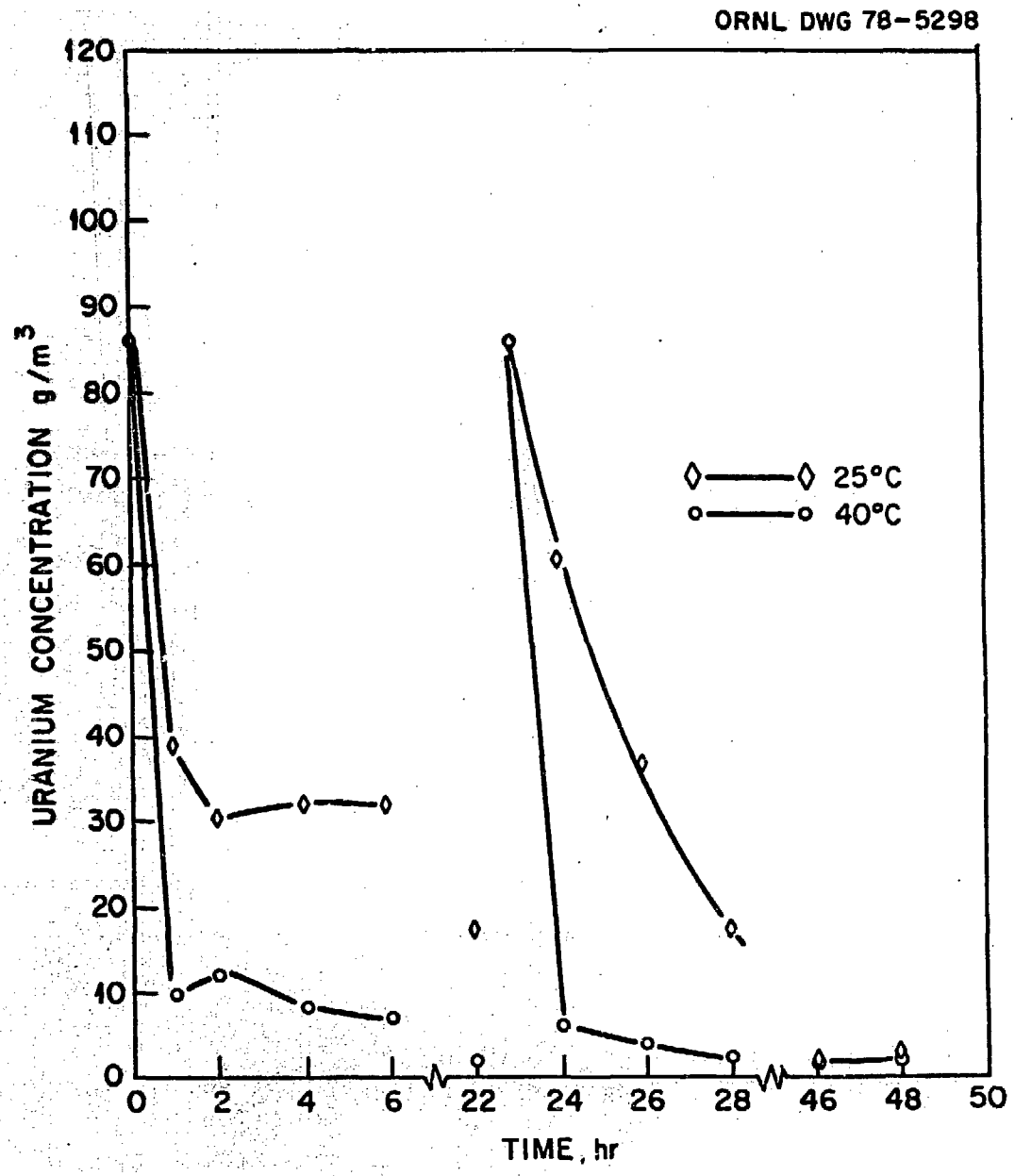

Fig. 3. Uranium sorption by Saccharomyces cerevisiae during sequential exposure.

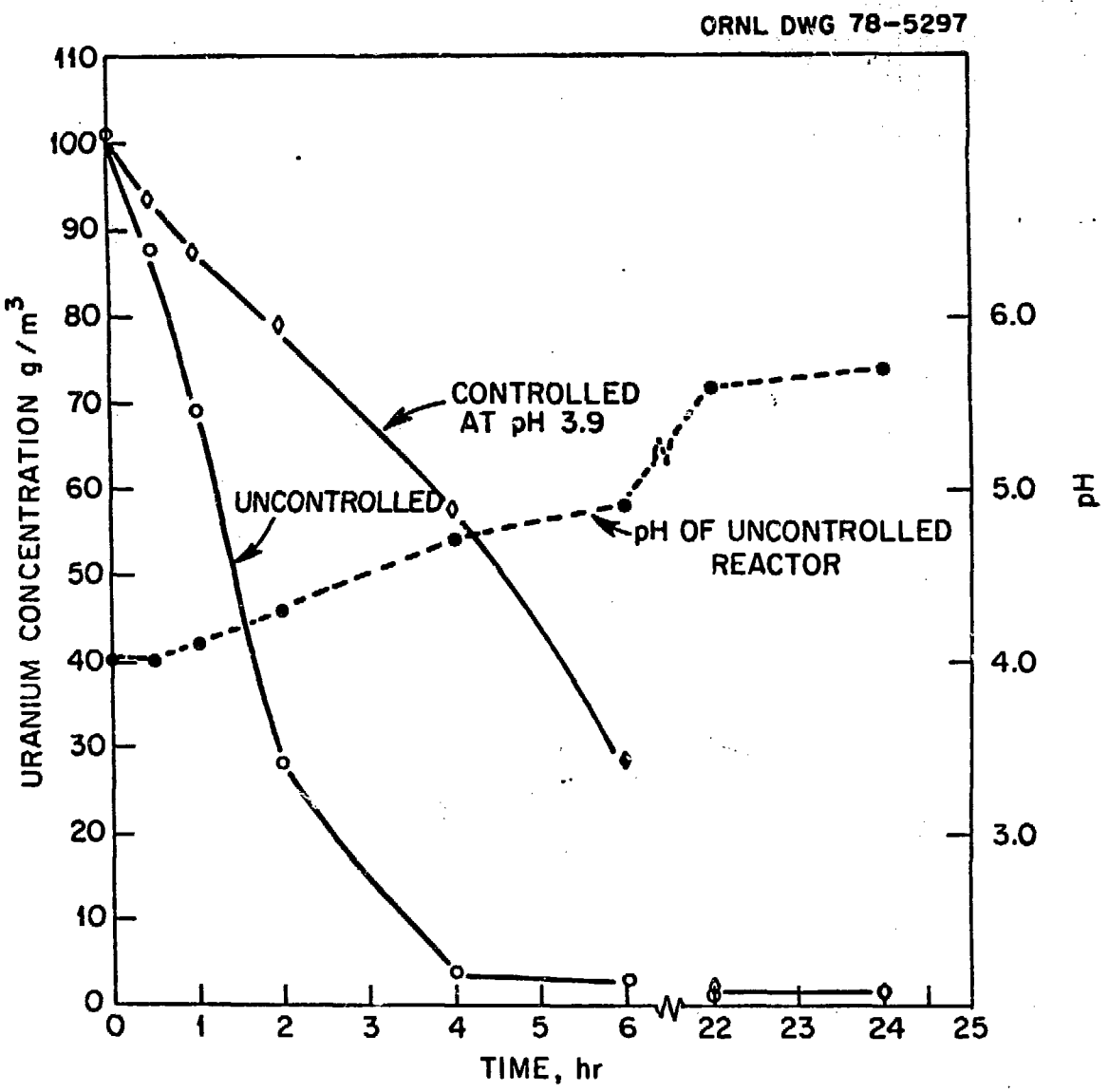

Fig. 4a. Effect of pH on uranium sorption by Saccharomyces cerevisiae: pH controlled at 3.9.

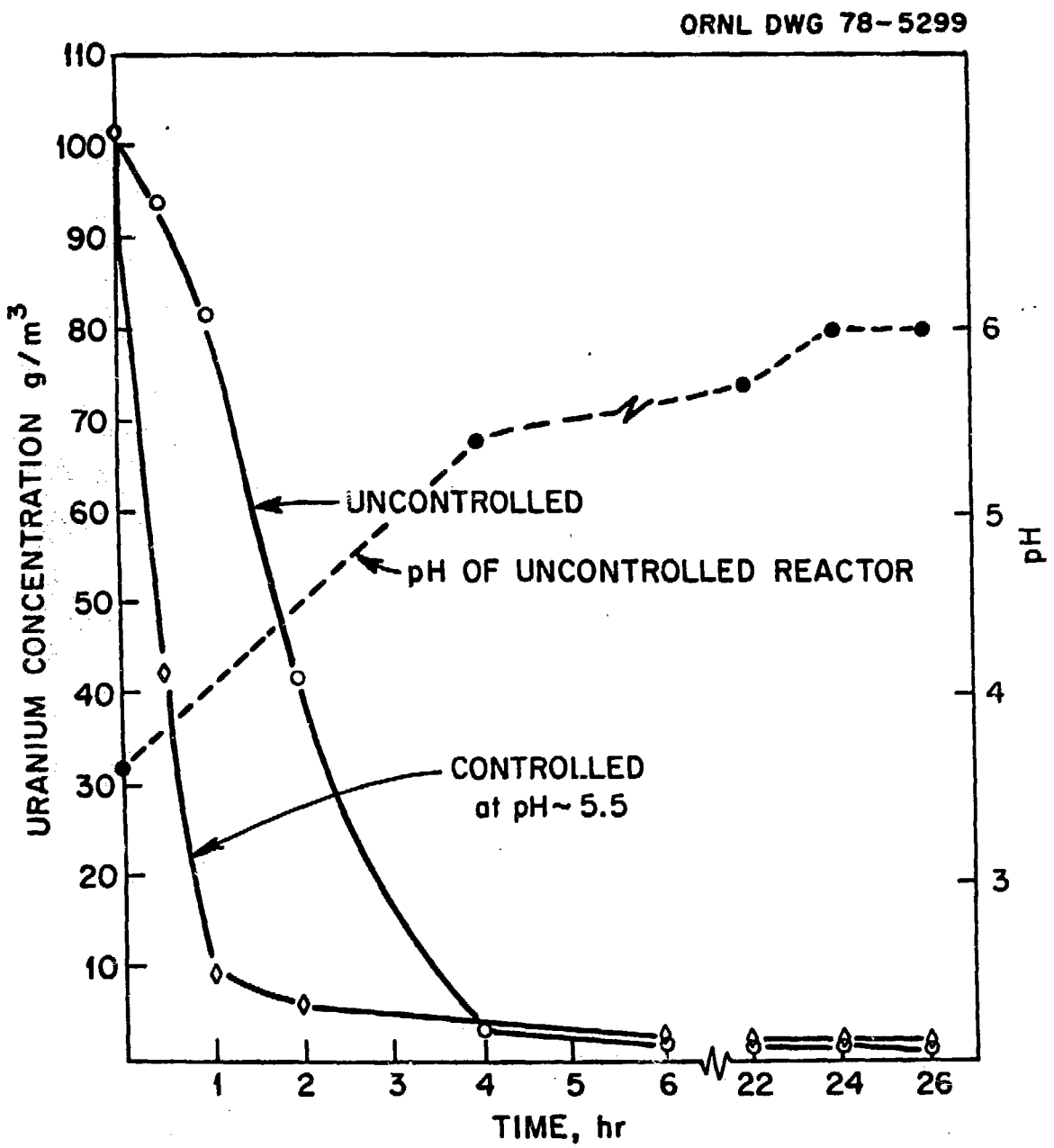

Fig. 4b. Effect of pH on uranium sorption by Saccharomyces cerevisiae: pH controlled at 5.5.

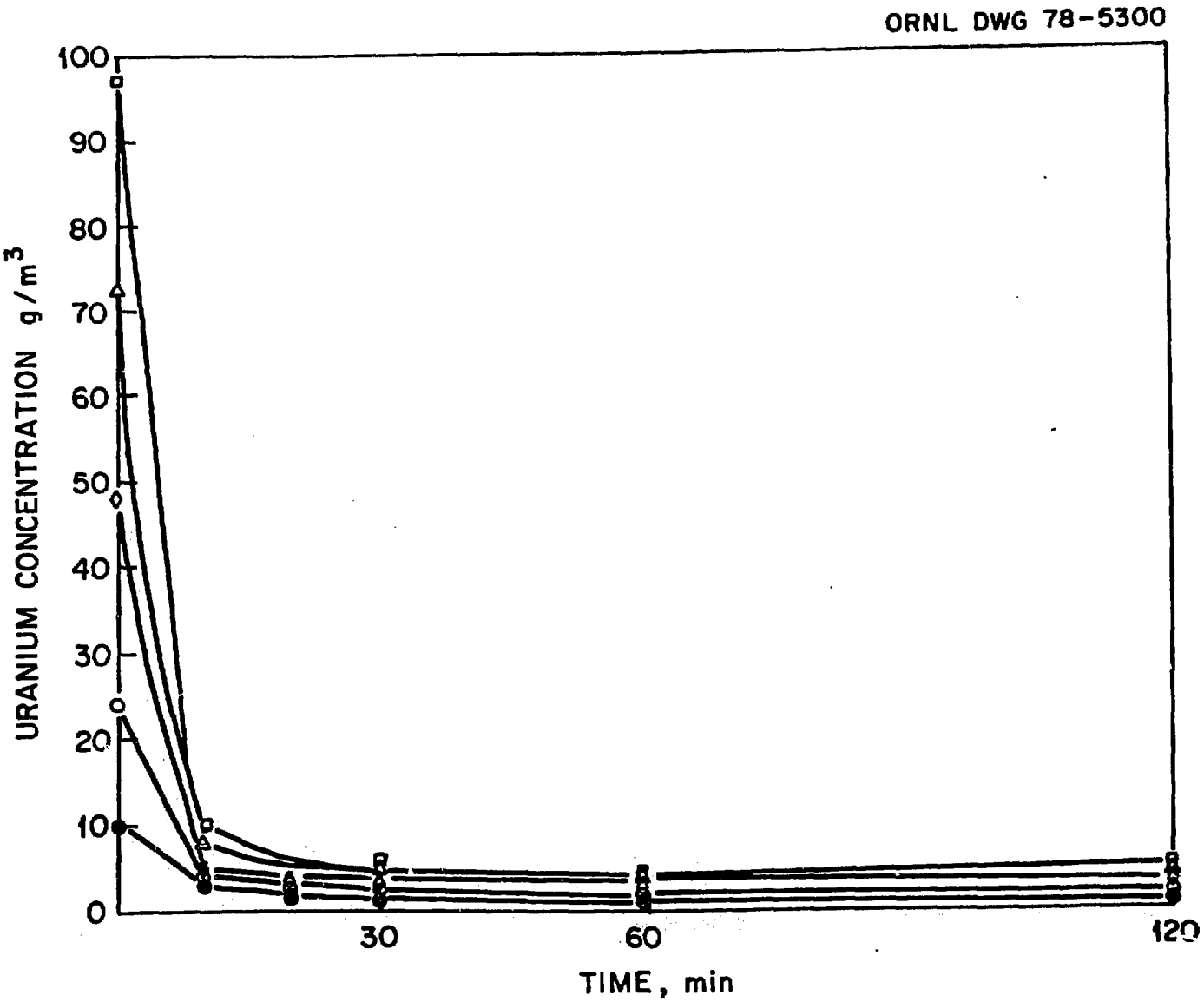

Fig. 5. Effect of initial uranium concentration on uranium sorption by Pseudomonas aeruginosa.