BIOSORPTION OF THE URANYL ION BY *RHIZOPUS*

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Abstract

Fermentor-product biomass of eleven strains of Rhizopus was screened for uptake of the uranyl ion. All nine species examined grew in a distinctive manner and exhibited high uptake -- 150-250 mg U/g cells at 300 ppm U equilibrium concentration in solution and .100-160 mg U/g cells with 100 ppm U in solution. Components of the growth medium such as antifoam and nitrogen source affected the ability to sequester the uranyl ion.

Uptake of the uranyl ion by R. oligosporus decreased rapidly between pH 3 and pH 2. In shake-flasks uranium was recovered by reducing pH or adding salts of E.D.T.A., acetate or sulfate. A simple equilibrium model is presented to explain the constant reduction of uptake in the presence of ligands. Oxalate and thiocyanate form bridges in multi-nuclear complexes, enhancing uptake, while nitrate and chloride sodium salts had little effect on uptake.

R. arrhizus grew as small, strong spherical pellets which were packed in a column. During eight successive sorption/desorption cycles 60 % of the original capacity was freely reversible with acid elution -- the remaining 40% was not observed following the initial contacting. Studies of fluctuations of the effluent pH indicated ion-exchange during biosorption.

Résumé

La détermination de l'adsorption des ions uranyl a été effectuée par onze souches de Rhizopus. Les neuf espèces examinées ont presenté des morphologies différentes et de hautes capacités d'adsorption: 150-250 mg U/g cellules à une concentration de 300 ppm U en solution et 100-160 mg U/g cellules à une concentration de 100 ppm U en solution. Les composants du milieu de culture, tels que l'antimousse et la source d'azote peuvent modifier la capacité d'adsorption de l'ion uranyl.

L'adsorption par R. oligosporus a diminué rapidement avec le pH, de pH 3 à pH 2. En erlenmeyer agité l'uranium est récupéré en imposant une reduction de pH ou en ajoutant des sels d'E.D.T.A., d'acétate ou de sulfate. Un modèle simple d'équilibre est proposé pour expliquer la diminution constante d'adsorption par rapport au témoin. Des ponts s'établissent entre l'oxalate ou thiosulfate et l'uranyl ce qui augmente l'adsorption tandis que les sels de nitrate et de chlorure affectent peu l'adsorption de l'ion uranyl.

R. arrhizus se présente sous forme de petites billes qui ont été mises dans une colonne. Huit éxperiences d'adsorption/desorption ont été effectuées: 60% de la capacite initiale d'adsorption d'uranyl est recuperée en ajoutant de l'acide; les 40% restant sont perdues dès le premier contact. Des études de pH de l'effluent ont indiqué qu'un échange d'ions est survenu.

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INTRODUCTION

1.1 The Phenomena of Biosorption

It has long been known that aquatic organisms concentrate metal ions from surrounding waters. In fact, many metals were detected in seaweeds long before they could be detected in the oceans. [1] This uptake is often considerable and frequently selective. In recent years a term has been coined to describe this phenomena. 'Biosorption' is the sequestering of metal ions by solid materials of natural origin. This generic term is not specific with respect to the mechanism of uptake, which may be via: [2,3]

1. particulate ingestion or entrapment by flagellae or extracellular filaments
2. active transport of ions
3. ion exchange
4. complexation
5. adsorption
6. inorganic precipitation (e.g. via hydrolysis of sorbed species.)

While the first two mechanisms are associated with living cells, the latter mechanisms have been reported for living and dead microbes, as well as cellular debris. [3,4,5] There are as many locations of immobilization of metals within microbes as there are mechanisms. They may be anywhere from extracellular polysaccharides to cytoplasmic granules.

Most microbes in their native environment surround themselves with a polysaccharide sheath, the chemical composition of which varies considerably among species. Many of these polysaccharides are anionic, containing free carboxyl or phosphate groups which participate in cation-exchange, while other groups such as amine, ketone, hydroxyl and ester linkages may provide sites for complexation. [7,8] Extracellular polysaccharide gels may also adsorb species or entrap microscopic

particles such as hydroxide precipitates.[9,10]

Microorganisms exhibiting remarkably high uptake of metals frequently sequester them principally within the cell wall via two mechanisms. The first is a stoichiometric interaction, either ion-exchange or complexation, between the metal ions and active groups such as phosphodiester (teichoic acid), phosphate, carboxyl (glycosides) and amine (amino- and peptidoglycosides and bound protein) on the polymers making up the cell wall. Further uptake is the result of inorganic deposition via adsorption or inorganic precipitation such as hydrolysis. Deposition within the cell wall is often readily discerned by electron microscopy, and has been described by Tsezos[11] (uranium and thorium deposition in cell walls of Rhizopus arrhizus) and Beveridge[5] (Na, K, Mg, Ca, Mn, FeIII, Ni, Cu, AuIII in native and modified cell walls of Bacillus subtilis.)

Significant uptake of heavy metals associated with lipid membranes has been reported[12] but this phenomenon is not as common as sequestering by cell walls. Toxicity of heavy metals upon association with cellular membranes is due to changes in membrane permeability, while protective mechanisms evoked by bacteria result in increased deposition of metals within cell walls.[13] Genetically-determined exterior structures result in enormous differences in the response of microbes to metals in their environment.

Cellular cytoplasm is largely made up of water and is seldom an important site for sequestering of metals because the cellular membrane controls metal transport. Vacuole formation upon particle ingestion or inorganic precipitation may occur.

1.1.1 Roles of Microbes in Metal Immobilization.

The generic term 'biosorption' describes a wide variety of phenomena, and these have repercussions in just as many areas of practical science. Such areas include waste-water treatment in waterways, assessment of water contamination using biological indicator organisms, and the use of industrial biosorbents in process applications.

Decontamination of mining and smelting waste-waters has been reported as the water passed through meandering streams and ponds and the metals were entrained in algal blooms.[14] A more complicated limnological situation was described in Flin-Flon, Canada, where anaerobic digestion of sulfate produced hydrogen sulfide, and metal sulfide precipitation enhanced the cleansing of the waste-waters.[15] Water flowing through beds of plants or swamps has also been found to be effectively cleansed of heavy metals.[16] The efficiency of water purification exhibited by populations of macro-species which do not themselves appear to significantly concentrate heavy metals may be attributed to often-overlooked microbial populations which preferentially bind to the roots and stems.[17]

The assessment of heavy-metal contamination of waterways using biological indicator organisms such as fixed algae is attractive because time-averaging has been automatically carried out and the metals are concentrated with respect to the surrounding waters. Many studies of pollution from mining and metal-processing operations[18,19,20] as well as of radionuclides such as strontium-90[22-29] have been carried out. Difficulties are encountered as growth and metal concentration in plant tissues are affected by metal toxicity, turbidity associated with high pollution level, and changes in temperature and pH. For these reasons most correlations are qualitative.

Application of biosorption of metals by living microbes in industrial processes, apart from waste-water treatment in lagoons etc., is difficult because the metal toxicity often inhibits microbial growth.[10] In spite of this, metal concentration by denitrifying bacteria has been studied.[9] Separation of the biomass propogation and metal uptake steps is one logical solution to this problem and has led to the development of non-living biosorbent materials. Metal concentration by waste biomass as well as by immobilized cells and stiffened cell products has been described.[30-33] This approach allows one to control the conditions of growth and processing to increase metal uptake and reversibility and to improve material handling characteristics.

One approach to the problem of the production of such biosorbents is to use waste biomass from fermentation industries to take up metals from solution. This was done by Tsezos[34] who examined the uptake of uranium and thorium by a number of wastes including activated sludge, denitrifying bacteria and the moulds Penicillium chrysogenum and Rhizopus arrhizus. Of all these materials R. arrhizus was found to exhibit much greater uptake of both metals, even from very dilute solution. Further study revealed that the uptake of both metals was principally associated with the cell wall; uranium was deposited in layers while thorium was found on the exterior. Kinetic studies with fine particles in a vigorously-stirred reactor indicated that when diffusional film resistance was negligible, 70% of the equilibrium uptake (that he ascribes to complex formation with chitin nitrogen and adsorption) occurred within the first minute. Hydrolysis of the uranyl ion resulted in the rest of the sequestering; that took place after a half-hour lag.[35] The work presented here in this thesis is a continuation of that done by Tsezos, a study of the uptake of the uranyl ion by species of Rhizopus.

1.2 Chemistry of the Uranyl Ion

No rational study of sequestering of metal ions may be made without considering the chemistry of the material.

In the presence of air the hexavalent oxidation state of uranium is the most stable. Uranium VI does not exist as the U^{6+} ion; rather it forms the uranyl species $O{=}U{=}O^{2+}$. This linear oxo-ion is of exceptional chemical stability[36], and is the actual species of uptake.

The uranyl ion will complex with nitrogen and sulfur-containing ligands, but it is most noted for its affinity for oxygen-containing ligands. Ligands complex equatorially, and the typical coordination number is six. Water is often included among the bound ligands. Formation of cyclic complexes is highly favoured and strongly-bound anionic complexes may occur.[37] Appendix I contains a list of some complex stability constants for a number of ligands commonly encountered in uranium recovery operations.

1.3 Conventional Uranium Recovery

Any development of a novel method of recovering uranium from either waste or process streams must not be carried out in ignorance of the conventional technology.

There are few deposits of high-grade uranium-containing ore in the world; most is extracted from deposits containing less than 0.1% uranium by weight.[38] Secondary recovery from phosphoric acid production as well as from mine tailings and slime from gold and copper mines and even older uranium recovery operations is also feasible. Uranium is now being recovered from in-situ or tailings-heap leachates, process streams and mine and tailings drainage waters.[39]

After mining, physical beneficiation and grinding the solid, uranium VI is leached from the solid, either by acid or alkali, as the sulfate or carbonate complexes respectively. Simultaneous oxidation of the less soluble uranium IV is carried out using one of a variety of oxidants such as air, peroxide, chlorate or manganese dioxide.

Alkaline leaching is a simple process because extraction is relatively specific, the solutions are non-corrosive and the uranium may be recovered by direct precipitation upon increasing pH.[40]

Acid leaching is the more commonly-used approach because it is more effective with low-grade ores as it partially leaches the matrix. Uranium is recovered from acid leachates as the anionic sulfate complex by solvent extraction or ion exchange.[40]

Solvent extraction of uranyl complexes is generally performed with a kerosene-diluted organic phase. Weak-base alkyl amines with aromatic substituents are used to concentrate alkali leachates while alkyl phosphates are used for sulfate leachates. To avoid extraction of ferric ion it is reduced to the ferrous state.[41]

Ion exchange has many advantages in efficiency of extraction and handling of pulps.[39] In contrast to the phosphate species which extract by complexation in solvent extraction, cationic exchange results in binding of the uranyl sulfate complex. It is displaced by strong acids. Poisoning by thiocyantes, polythionates, cyanides, silica, and some heavy metals may occur.[42]

2 OBJECTIVES

The objectives of this work were:

1. To determine whether biosorption of the uranyl ion is a property common to the genus Rhizopus or is exhibited only by R. arrhizus.

2. To discover the fungus with the 'best' biosorption isotherm for the uranyl ion at pH 4 among eleven strains of Rhizopus, biomass samples of which were obtained by batch fermentation on a bench scale.

3. To determine the effect of the timing of the harvesting of biomass during the fermentation on the biosorptive uptake of the uranyl ion.

4. To study the desorption of the uranyl ion in the presence of anionic ligands, by decreasing pH or by increasing ionic strength.

5. To mathematically formulate the equilibrium uptake behaviour.

6. To study biosorption of the uranyl ion in packed columns of R. arrhizus beads.

3 THEORETICAL ASPECTS OF EQUILIBRIA

When the biosorption mechanism involves ion exchange or complexation both the kinetics and quantity of uranium sequestered at equilibrium are of importance. The equilibrium behaviour of the system biosorbent plus solution is considered below as a series of examples.

3.1 General Case of Uptake by Many Sites

Given uncomplexed uranyl ion in solution in equilibrium with one of a variety of complexation sites on the biomass, one may write the reactions and equilibrium constants

$$\begin{aligned} U + W_1 &\rightleftharpoons UW_1 & k_1 &= \frac{uw_1}{u' \cdot w_1} \\ U + W_2 &\rightleftharpoons UW_2 & k_2 &= \frac{uw_2}{u' \cdot w_2} \\ U + W_n &\rightleftharpoons UW_n & k_n &= \frac{uw_n}{u' \cdot w_n} \end{aligned} \qquad (1)$$

where the lower case designates the average concentration of the appropriate species in the limited volume being considered. If the upper case designates the total concentration of either wall sites or uranium, the fraction of a particular type of site occupied by uranium is

$$f_i = \frac{uw_i}{w_i} \qquad (2)$$

so that

$$k_i = f_i / u' (1 - f_i) \qquad (3)$$

From the mass balance on uranium in solution and sequestered it follows that

$$U = u' \left(1 + \sum_{i=1}^{n} W_i \cdot k_i - \sum_{i=1}^{n} f_i \cdot W_i \cdot k_i\right) \qquad (4)$$

The first sum is a constant characteristic of the biomass, which will be designated as C. Note that as the biomass approaches saturation or f_i approaches unity, U approaches u'. The quantity of uranium taken up by the biomass is

$$U - u' = U\,(C - \sum_{i=1}^{n} f_i \cdot W_i \cdot k_i)/\,(1 + C - \sum_{i=1}^{n} f_i \cdot W_i \cdot k_i) \qquad (5)$$
$$= \sum_{i=1}^{n} f_i \cdot W_i$$

3.2 General Case of Uptake, with Complexation in Solution

If the possibility of complexation within the solution also exists, and the complexed uranyl-ligand species are not taken up by the biosorbent, then a series of equilibria analogous to that between the biosorbent and solution exists between the complexed and uncomplexed uranyl ions in solution.

$$U + L_i \rightleftharpoons UL_i \qquad k_i' = \frac{ul_i}{u' \cdot l_i}$$

If the ligands are present in large excess so that $(1-f_i')$ approaches unity, then of the total quantity of uranium in solution represented as u, only the uncomplexed quantity u' is available for uptake

$$u' = \frac{u}{(1 + C')} \qquad (6)$$

so that the new mass balance is

$$U - C' \cdot u' = u'\,(1 + C - \sum_{i=1}^{n} f_i \cdot W_i \cdot k_i) \qquad (7)$$

The uptake of uranium is

$$U - u = \sum_{i=1}^{n} f_i \cdot W_i = U\,(C - \sum_{i=1}^{n} f_i \cdot W_i \cdot k_i)/(1+C-\sum_{i=1}^{n} f_i W_i k_i + C') \qquad (8)$$

Consider two systems, one with and one without ligands present, but with identical concentrations of 'biosorbed' uranyl ion. No ligands are promoting the solubility of uranium so the system without ligands will have a lower total concentration of uranium. Representing the total uranium concentration as U_{wi} and U_{wo} respectively from mass balances and equations (5) and (8)

$$\frac{U_{wi}}{U_{wo}} = \frac{\sum_{i=1}^{n} W_i \cdot f_i + u' + C' \cdot u'}{\sum_{i=1}^{n} W_i \cdot f_i + u'} = \frac{1 + C - \sum_{i=1}^{n} f_i \cdot W_i \cdot k_i + C'}{1 + C - \sum_{i=1}^{n} f_i \cdot W_i \cdot k_i} \quad (9)$$

In such an instance the concentration of uranium in solution is greater by the constant factor (1+C') when ligands are present. This formulation allows calculation of both C and C'.

3.3 Multi-Component Equilibria in Solution and in the Biosorbent

There are other sorts of equilibria which affect the quantity of uranium taken from solution, as well as the situations described above. For clarity the following formulation has been simplified to consider only one type of site and ligand (this is not serious because expansion is straightforward and in many instances one type of site or ligand dominates.) Four possible reactions are considered: complexation of free uranium (U) by the biomass (W) or by a ligand (L) in solution, complexation of a uranium-ligand species (UL) by the biomass, and a competing reaction of another species (H) such as hydrogen ion or another metal ion with the site of biosorption.

$$\begin{aligned} U + W &\rightleftharpoons UW & k_W &= \frac{uw}{u \cdot w} \\ U + L &\rightleftharpoons UL & k_L &= \frac{ul}{u \cdot l} \\ UL + W &\rightleftharpoons ULW & k_{LW} &= \frac{ulw}{k_L \cdot u \cdot l \cdot w} \\ W + H &\rightleftharpoons WH & k &= \frac{wh}{w \cdot h} \end{aligned} \quad (10)$$

The mass balances on all four components are:

$$\begin{aligned} U &= u' \left(1 + k_L\, l + k_W w' + k_{WL}\, k_L\, w'\, l\right) \\ W &= w' \left(1 + k\, h + k_W\, u' + k_{WL}\, k_L\, u'\, l\right) \\ L &= l \left(1 + k_L\, u' + k_{WL}\, k_L\, w'\, u'\right) \\ H &= h \left(1 + k\, w'\right) \end{aligned} \quad (11)$$

The quantities of uranium in solution and on the biomass are

$$u' + u\mathfrak{l} = u = u' (1 + k_L \mathfrak{l}) \qquad (12)$$

and

$$uw + u\mathfrak{l}w = u' (k_w + k_{WL} k_L) (H/h - 1)/k / (1 + k_L \mathfrak{l}) \qquad (13)$$

From equation (13) the effects of the existence of reactions 2 - 4 are evident. Competitive complexation of the uranyl ion by high concentrations of ligands reduces uptake by a constant fraction, as does competition for sites if $H/h >> 1$. If uranyl complexes react with the biomass then the shape of the isotherm is changed.

4. MATERIALS AND METHODS

4.1 Maintenance of the Culture Collection

Ten strains of Rhizopus were obtained from the American Type Culture Collection, and a strain of Rhizopus arrhizus was obtained from Canada Packers (Toronto). The cultures were rejuvenated from lyophilized form and under visual inspection were maintained in morphologically stable form on agar slants with routine transferring every four months. Table 4-1 is a list of the cultures, while Figure 4-1 contains a description of the medium.

Table 4-1 Rhizopus Strains

	A.T.C.C. culture no.
R. arrhizus	--
R. chinensis saito	22958
R. delemar var. multiplici-sporus	24864
R. formosaensis	26612
R. japonicus	24836
R. javanicus	22580
R. oligosporus	22959
R. oryzea	12883
R. stolonifer	14037
R. stolonifer	14038
R. stolonifer	62276

Figure 4-1 Medium for the Growth of Rhizopus

Nutrients
0.5% (w/v) peptone
0.5% (w/v) neopeptone
2.0% (w/v) sucrose
Inorganic salts (buffer)
0.1% (w/v) KH_2PO_4
0.1% (w/v) $NaNO_3$
0.05% (w/v) $MgSO_4 \cdot 7H_2O$
- 2% agar added for preparation of slants.
- Dow Corning Antifoam AF added to fermentor as needed to prevent excessive foaming. (~ 20 drops). (Contains stearates and silicates.)

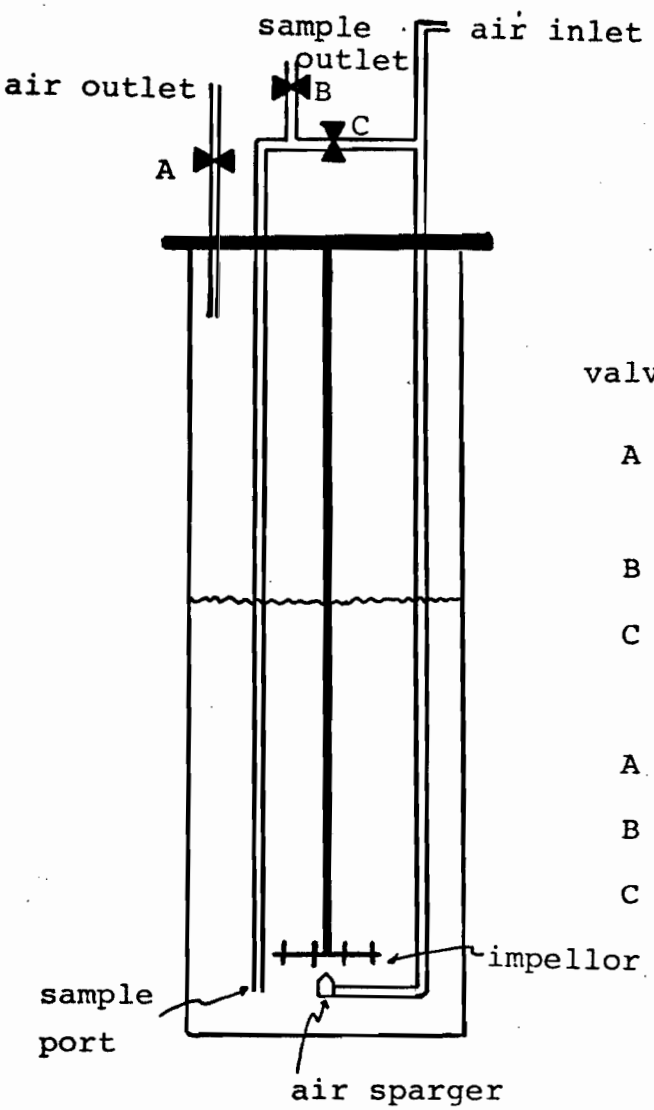
4.2 Propagation of Biomass

Five 100 mL quantities of broth, sterilized in 500 mL erlenmeyer flasks, were inoculated with 1 mL of spores suspended in sterilized distilled water. The cultures were grown 24-36 hours at 25°C, agitated at 200 rpm on a New Brunswick rotary shaker with 2.5 cm displacement. This disperse inoculum was added to 3L of broth in a 7 L fermentor (Microferm, New Brunswick Scientific, New Jersey) which had been steam-sterilized for 30 minutes at 105 kPa(g) and 120°C. Air was supplied at a flowrate of 3L/min through a single fine sparger beneath the impellor and the temperature was maintained at 25°C with cooling water in baffles. One four-blade turbine impellor rotating at 600 rpm provided agitation.

Representative sampling of broth and biomass was carried out as illustrated in Figure 4-2. Growth was followed as determination of dry biomass in the broth. Between 50 and 100 mL of broth were filtered and

Figure 4-2

Schematic Diagram of Fermentor Broth Sampling



Normal Operation

valve

A cracked open to keep sample port clear

B closed

C open

During Sampling

A closed

B open

C closed

washed on a pre-dried, preweighed filter (Whatman no. 4) and the solids were dried to constant weight at 85°C. Scorching occurred at temperatures above 90°C.

The disperse biomass was harvested by filtration within three hours of the beginning of stationary phase. The solids were soaked in distilled water and refiltered five times, then broken up and dried at 85°C. The final filtrate was clear and colourless.

4.3 Analysis of Uranium

The concentration of uranium in solution was determined by measuring the absorbance of the uranyl-arsenazo III* complex using a 1 cm path-length quartz cell in a Bausch and Lomb 'Spectronic 70' spectrophotometer. This complex has a maximum absorbance between pH 1.5 and 3 so a sulfate buffer (pK = 1.92) was chosen for the determination. Five mL of 0.1% arsenazo III (F.W. 776.37) in 0.1M NaOH were combined with a sample containing 50 - 250 µg of uranium and diluted to 50 mL with sulfuric acid and distilled water so that the final concentration of sulfuric acid and sulfate was 0.0067M. Absorbance was measured at 665 nm. As illustrated in Figure 4-3 this wavelength is not at the maximum absorbance; rather it is on a slight shoulder of the complex absorption spectrum. It is, however, near the maximum in the ratio of sample/blank absorbance and for this reason the calibration curve was linear to unity absorbance. The slope was 2.3×10^{-3} absorbance units per µg U in 50 mL. Replicate samples displayed identical absorbance within the reading accuracy of the instrument; accuracy was instrument-limited.

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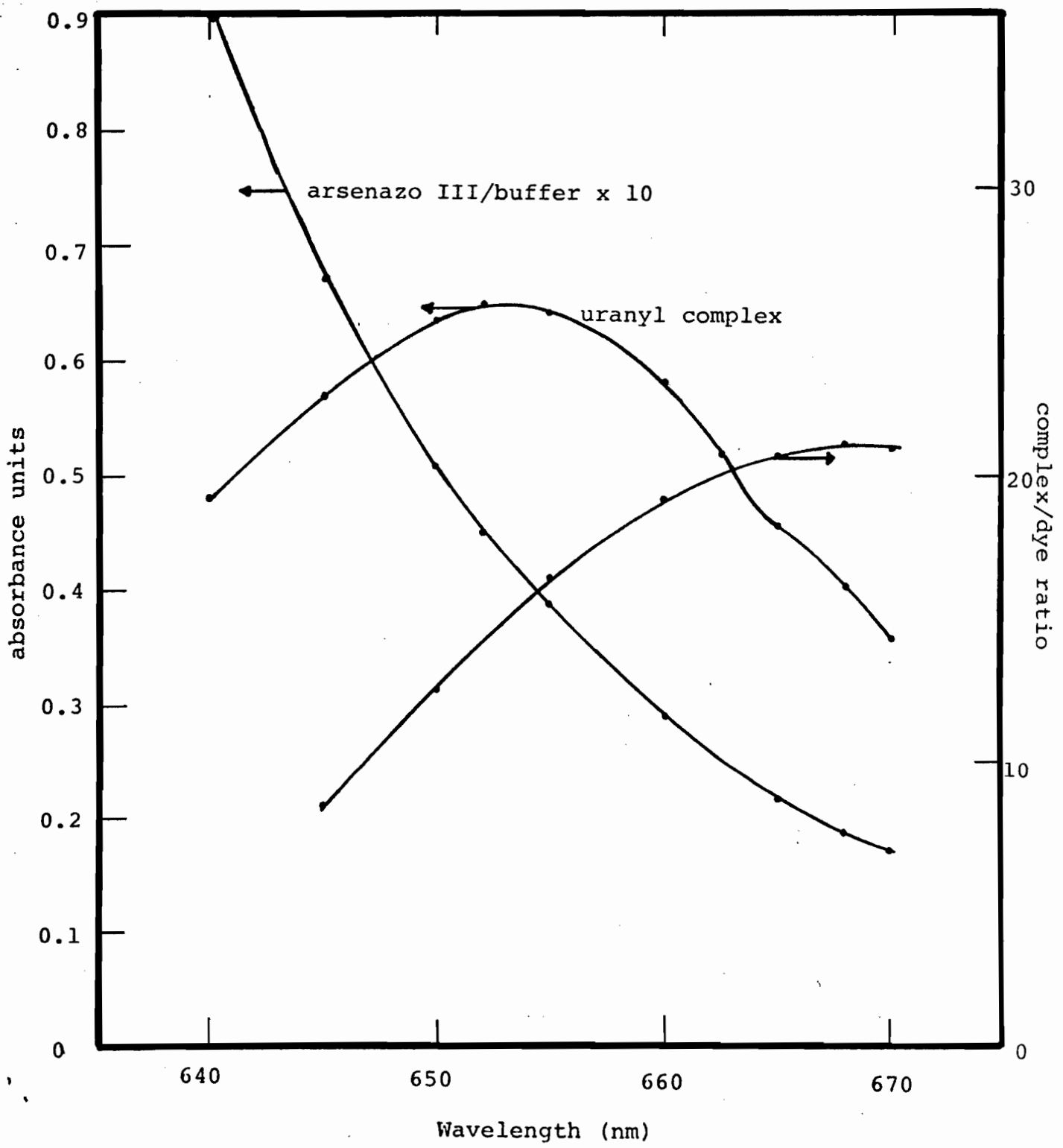
*. reagent Arsenozo III is 1,8 - dihydroxynaphthalene - 3,6 - disulfonic acid - 2,7 - bis (< - azo - 2) - phenylarsonic acid)

Figure 4-3

Absorption Spectra of Arsenazo III/Sulfate Buffer Mixture and Uranyl-Arsenazo III Complex



Interference in this method occurs in the presence of some other complexing anions as they compete with the dye. Calibration curves determined with the appropriate concentrations of competing anions were all linear. With 0.002 M anion in the final solution arising from the sample, reductions in absorbance are summarized in Table 4-2.

Table 4-2 Anion Interferences in Uranium Determinations

Anion (0.002M)	% reduction in absorbance
oxalate / oxalic acid	8.9
acetate	2.7
sulfate (in addition to buffer)	2.5

4.4 Determination of Uranium uptake in Shake-flask Experiments

An aliquot (0.1g) of ground biomass was transferred to a 500 mL erlenmeyer flask. Either 100 or 200 mL of the appropriate solution was added and the flask was fitted with a ground-glass stopper sealed with silicon grease. The flasks were shaken at 150 rpm on a shaker-table (New Brunswick Scientific) in a room in which the temperature was controlled at 25 ± 0.5°C. Cell-free blanks were run simultaneously to test for the possibilities of adsorption of uranyl ion on the surface of the glass and/or evaporation during long contact times (neither occurred during the reported experiments). Equilibrium was considered to have been reached after 10 hours.

After shaking, a sample of the solution was filtered through a 0.45 μm pore-size 25 mm diameter cellulose-acetate membrane filter (Millipore) in a holder fitted with a 10 mL 'plastipak' syringe. The first 5 mL were used to rinse the filter while the second half was collected for subsequent determination of uranium concentration. No difference in the measured

uranium concentration of filtered and unfiltered solutions was observed in the absence or presence of any of the anions studied.

All glassware was routinely washed with 'Alconox' detergent and rinsed with tap water followed by distilled, deionized water.

Standard uranium solutions were prepared with analytical grade uranyl nitrate ($UO_2(NO_2)_2 \cdot 6H_2O$) ('Analar', BDH Chemicals). For the screening experiments the solution was buffered with 0.005 M potassium biphthalate. Analytical-grade salts or acids were added to make up the indicated concentrations of anions in the corresponding studies. Distilled deionized water was used in all solutions. In the studies of desorption the biomass was allowed to equilibrate for 13 hours with 200 mL of a solution of uranyl nitrate (500 ppm). Only then was 50 mL of a concentrated solution containing the appropriate anion added to bring the resulting 250 mL to the desired concentration.

Uptake of uranyl ion (U, mg U/g cells) was calculated by difference knowing the initial and final concentrations of uranium (c_i and cf, ppm U), the solution volume (V, mL) and the mass of cells (m, g).

$$U = \frac{V \times (c_i - c_f)}{1000 \times m}$$

4.5 Titration of Rhizopus Biomass with Aqueous Base

A sodium hydroxide solution was prepared in distilled deionized water which had been boiled to remove CO_2, standardized with oxalic acid and diluted to 0.0196M. The Orion pH meter and combination electrode were calibrated at pH4 and pH8. An accurately weighed 0.5 - 1.0 g sample of Rhizopus biomass was soaked in 10-20 mL of distilled deionized water, then was titrated very slowly, allowing the pH to reach a constant value between additions of base.

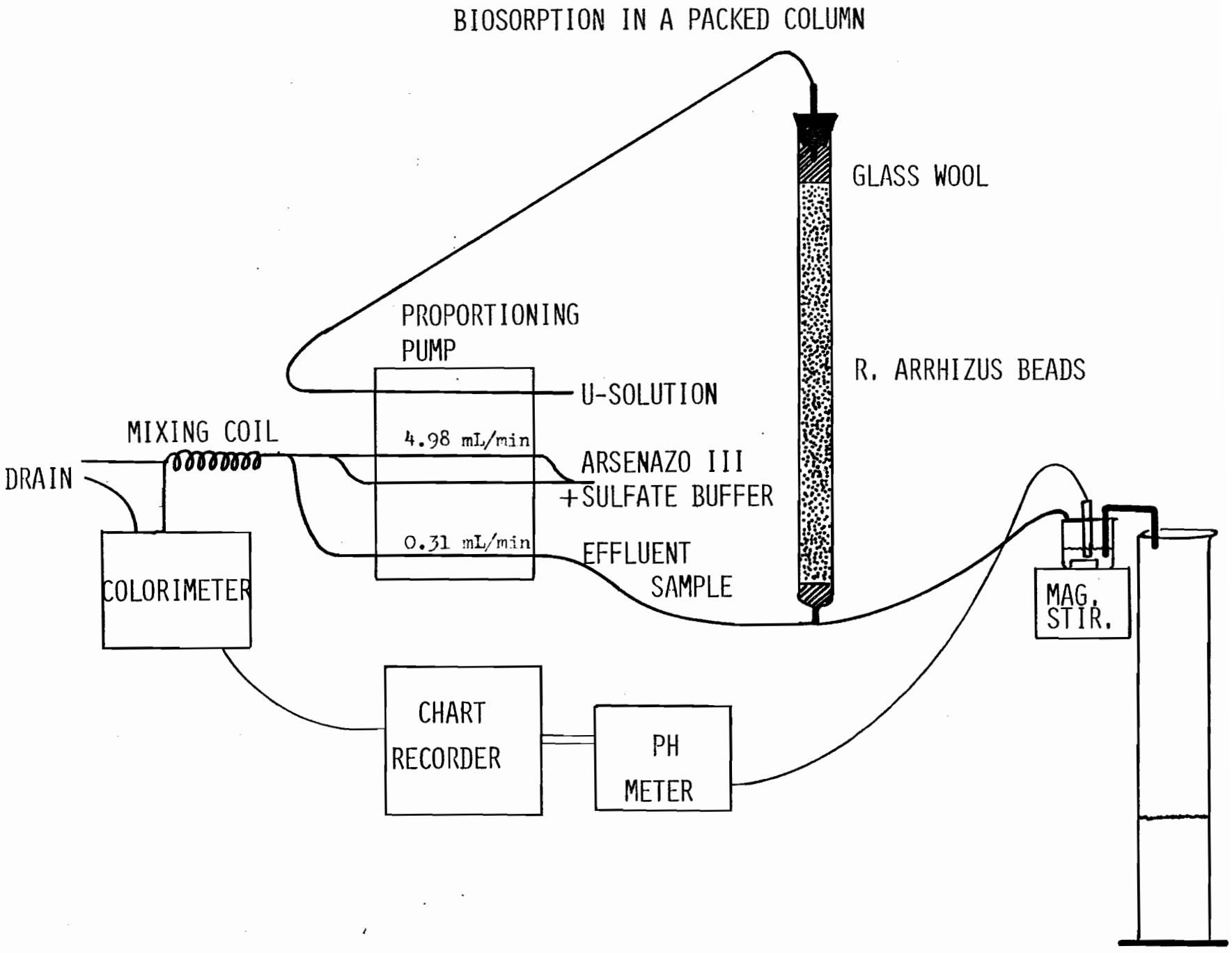
4.6 Study of the Uptake of the Uranyl Ion by a Packed Column of Beads of R. Arrhizus

Dried pellets of the biomass of R. arrhizus were screened to separate them into fractions 20-30 mesh and 30-50 mesh. One fraction was packed into a glass column of 0.80 cm I.D. and a solution of 90 ppm U was passed through. As illustrated in Figure 4-4, both the outlet uranium concentration and the pH were monitored continuously. The uranium was eluted from the pellets with acid (usually 0.1M H_2SO_4) and the biomass was washed to constant pH with distilled deionized water before repeating the contacting with uranium solution. During desorption the sample to be analysed for uranium was diluted by a factor of 18 in an additional delay-coil.

Voltage output of the pH meter was linear with pH, while the Autoanalyser I colorimeter gave a logarithmic output with concentration, corresponding to the Beer-Lambert Law. The voltage was measured directly between the sample photocell and zero (ground). A 0.5 cm path-length cell, and thin-layer interference filters with a 10 mm band-width and maximum transmittance of 43% at 660 nm were used. The apparatus was in a room maintained at 25°C.

Daily calibration of the uranium analytical network was fitted with the first program in Appendix II. The shapes of the curves on the chart-recorder were fitted by least-squares to an equation 3rd to 6th order in time. Finally the uranium or hydrogen ion absorbed or desorbed was integrated by Gaussian quadrature using the third program in Appendix II.

Figure 4-4


BIOSORPTION IN A PACKED COLUMN
GLASS WOOL
R. ARRHIZUS BEADS
PROPORTIONING PUMP
U-SOLUTION
4.98 mL/min
ARSENAZO III +SULFATE BUFFER
0.31 mL/min
EFFLUENT SAMPLE
MIXING COIL
DRAIN
COLORIMETER
CHART RECORDER
PH METER
MAG. STIR.

5 RESULTS

5.1 Macroscopic Physiology and Growth of Rhizopus Cultures

5.1.1 R. arrhizus

On agar slants R.arrhizus grew as fine, fluffy mycelia which tended to spread over the surface of the agar before filling the tube. Fine grey spores matured into larger, darker clumps.

In suspension R.arrhizus was unique in the distinctive formation of<1-3 mm diameter spherical pellets which were slightly denser than the broth or water. This culture was grown twice to check repeatability of experimental methods and the biomass material was indistinguishable, both in appearance and uptake of uranyl ion. A specific growth constant of 0.11 h^{-1} was observed in the last twelve hours of fermentation. The final broth was bright yellow and the washed mycelia were a pale 'cream' colour. Little collection of biomass on the fermentor impellor occurred. On a sucrose basis >25% yield of biomass was attained.

5.1.2 R. chinensis saito (ATCC#22958)

After a few transfers on the standard-medium agar slants R.Chinensis saito lost vitality; growth was sparse and the small light-brown spores did not mature to the characteristic dark spore clumps. Smith[43] suggests that this may occur if 'life is too easy'. Substitution of starch for sucrose in the slants led to good growth of fine, fluffy, white mycelia with small grey spore clusters.

In suspension R. chinensis saito grew as light fluffy irregular pellets of sizes ranging from <1 mm to larger than 5 mm. It exhibited a pronounced tendency to foam; the Dow Corning Antifoam AF was only partially effective. This, along with a tendency to collect on the impellor led to a very low yield of usable product in the fermentor.

5.1.3 R. delemar var. multipli-sporus (ATCC#24864)

Rapid growth of frequently-branching mycelia was observed on the agar slants. Early appearance of many medium-sized brown-black spore clumps preceded the slant turning rather black. The tube was well filled with growth.

A specific growth rate of 0.09 h^{-1} was observed in the fermentor for porridge-like growth. Foaming was not a severe problem and little biomass adhered to the impellor or baffles. The biomass floated. On the basis of the mass of sucrose in the fermentor a yield greater than 30% was calculated.

5.1.4. R. formosaensis (ATCC#26612)

In agar slants, fine fluffy mycelia which displayed a phototropic tendency were formed. Brown spore clumps darkened upon maturation. Growth was not particularly luxuriant.

In suspension R. formosaensis grew as porridge-like disperse mycelia, with little adherance to the impellor.

5.1.5 R. japonicus (ATCC#24836)

A distinctive thick white mycelial mat formed on agar slants. After several days when the slant was drying out a few huge black spore clumps formed.

In shake-flasks, R. japonicus grew as a few large lumps. When broken up and inoculated into a fermentor it repeatedly foamed a great deal and adhered to the impellor so only a low yield of large, irregular pellets was obtained from the suspension.

5.1.6. R. javanicus (ATCC #22580)

On agar slants R. javanicus was similar to R. japonicus although it was more readily sporulating and the spore clumps were smaller. Growth in suspension was similar as it foamed and adhered to the impellor so that after 10 hours only a small quantity of material was harvested from the broth.

5.1.7. R. oligosporus (ATCC #22959)

R. oligosporus grew as a low mat of fine, fluffy mycelia with fine grey spore clumps which enlarged and darkened as they matured. After storage at 5°C it displayed yellow-orange exudations.

In suspension the lag-phase after a spore-transfer was dramatically reduced. In a standard neopeptone + peptone broth the biomass was disperse mycelia. The specific growth rate was 0.2 h^{-1}. In a broth with peptone substituted for the neopeptone the off-white growth was slightly pelletized; very small denser but fuzzy clumps formed. Growth was much slower as the specific growth rate was 0.07h^{-1}.

5.1.8. R. oryzea (ATCC #12883)

Luxuriant fluffy dense growth was evident on agar slants. The spore clumps were large and brown-black.

Very rapid growth was observed in the fermentor as on two occasions the specific growth rate was measured as 0.27 and 0.29 h^{-1}. The porridge-like growth tended to compress a great deal upon filtering so that although it could be easily suspended for washing it was not as easily broken up for drying. In consequence the product was always hard and dark.

5.1.9 R. stolonifer (ATCC#14037, 14038, 62276)

All three strains of R. stolonifer appeared identical to one another yet distinct from the other species of Rhizopus. On agar they grew as very long, sparsely-branched white mycelia with fairly large, dark spore clusters at the end. They were very noticeably phototropic.

In suspension this species grew as large pellets in shake-flasks and as huge lumps on the impellor in the fermentor. After a day the culture broth was always clear. Sharpening the bottom of the impellor blade and increasing the air flow rate did not deter R. stolonifer from adhering to it.

5.2 Screening of Rhizopus Species for Uptake of the Uranyl Ion

Equilibrium isotherms of the uptake of uranyl ion by species of Rhizopus at 25°C, pH 3.7 - 3.9 are plotted on a log-log scale of uptake versus equilibrium concentration in Appendix III as Figures III-1 to III-8. Table 5-1 summarizes the growth and uranium uptake ability of the species of Rhizopus studied.

To obtain an indication of the variation in ability to sequester the uranyl ion as a function of the stage of growth, samples of R. arrhizus drawn from the fermentor to follow the growth curve were assayed. This ability was found to decrease exponentially during the exponential growth phase. The growth curve and the variation in uptake are presented in Figure 5.1. The most remarkable observation is that uranium uptake by shake-flask (inoculum) biomass was 130% greater than the uptake by the fermentor product at the same equilibrium uranium concentration (Figure III-1).

To obtain biomass for the pH and anion experiments reported below, two fermentations of R. oligosporus were carried out. This

Table 5-1

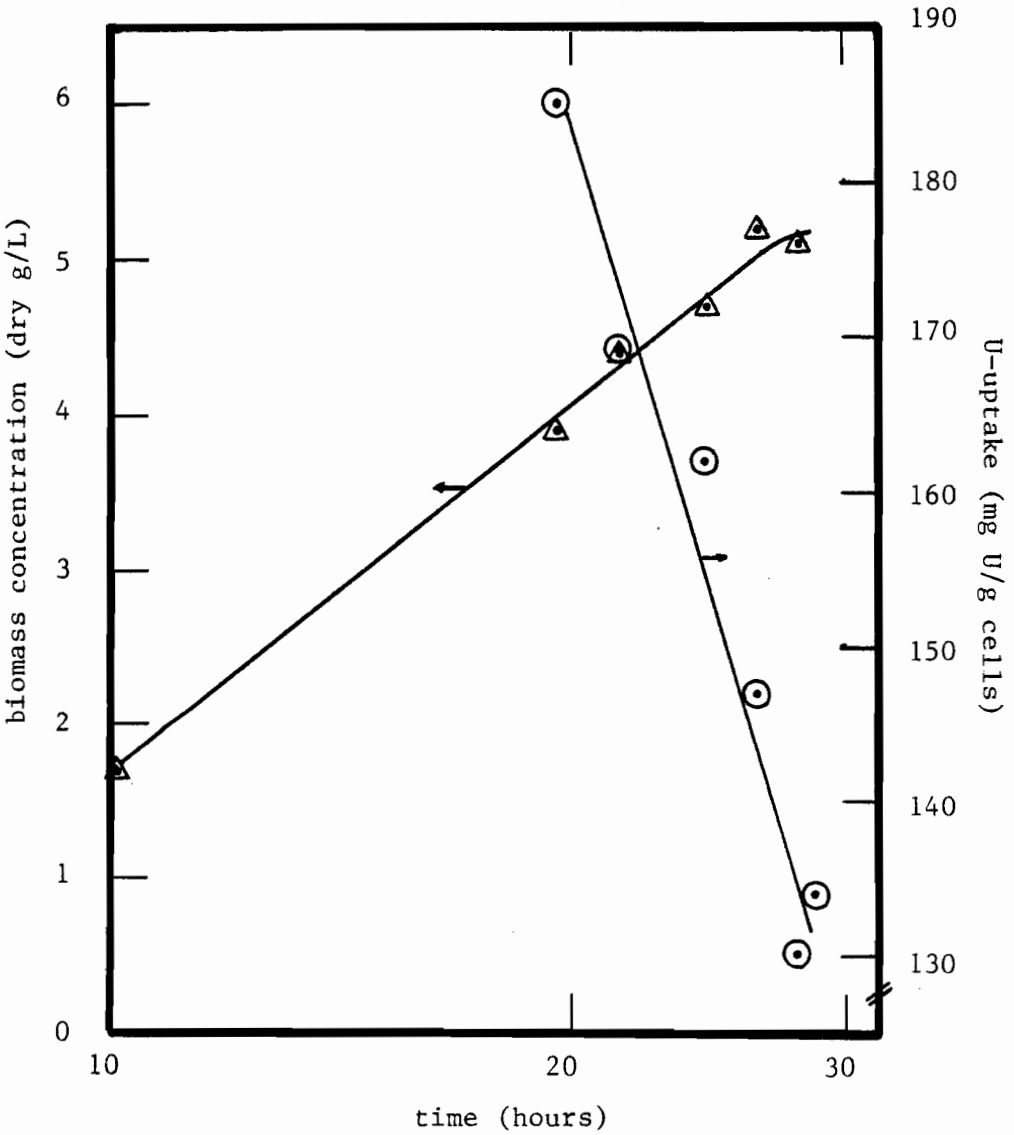
Screening of *Rhizopus* Species

	Growth	UO_2^{2+} Uptake (mg U/g cells) at equilibrium concentrations of 100 ppm U	300 ppm U
R. oligosporus	0.2 h^{-1} S.G.R.* light, cottony	165	250
R. oryzea	0.3 h^{-1} S.G.R. poor filtering	160	260
R. javanicus	stuck to impellor	160	250
R. formosaensis	0.1 h^{-1} S.G.R. light, cottony	160	200
R. chinensis saito	large lumps, 2-10 mm	160	190
R. japonicus	stuck to impellor	160	200
R. stolonifer	stuck to impellor	130	170
R. arrhizus	0.11 h^{-1} S.G.R. small, strong beads, 1-3 mm	110	130
R. delemar var. *multiplici-sporus*	0.09 h^{-1} S.G.R. light, cottony	95	120

*S.G.R. - specific growth rate

Figure 5-1

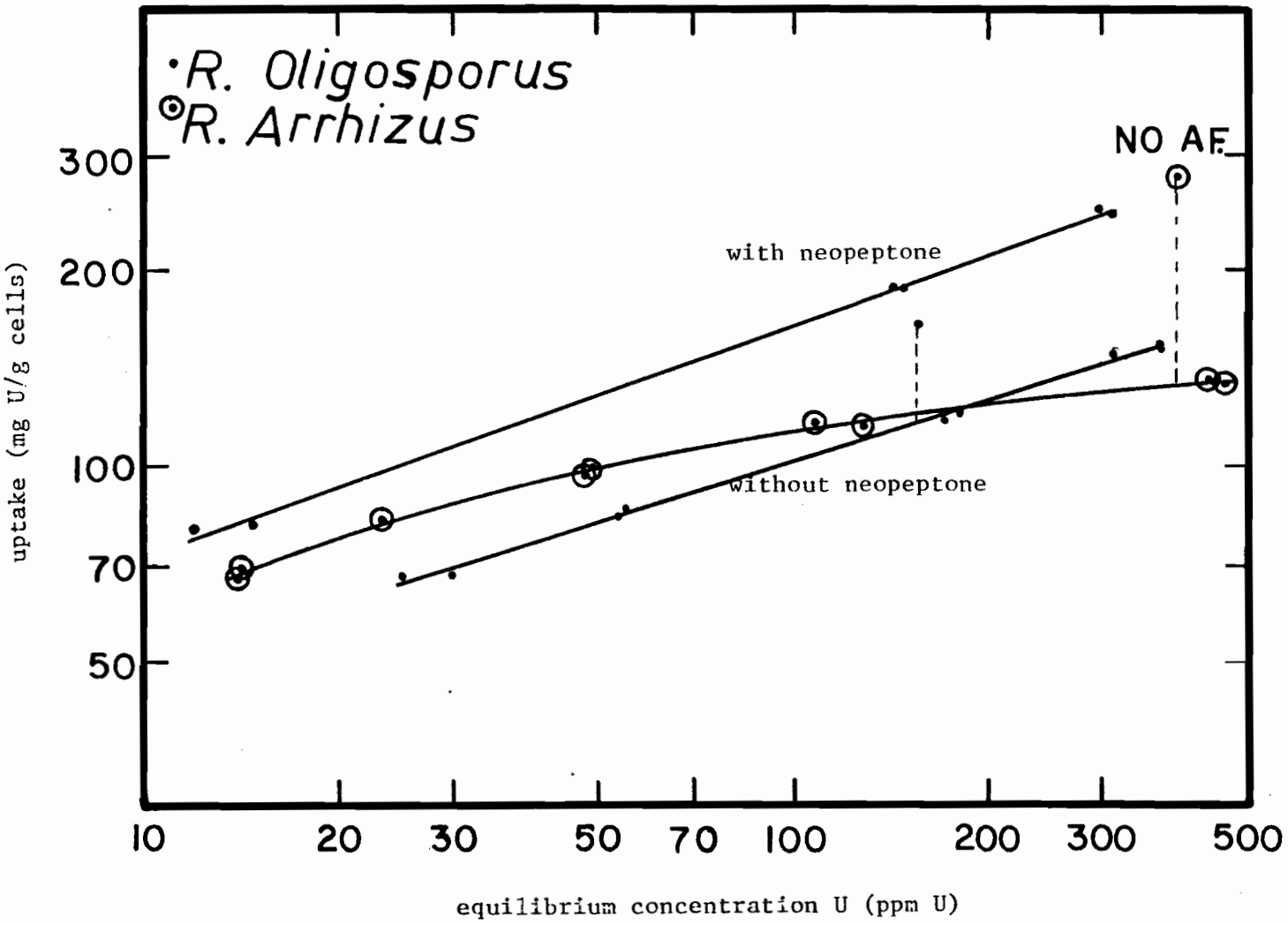
Effect of Stage of Growth on Uptake of UO_2^{2+} by Rhizopus arrhizus



Uptake conditions -- initial uranium concentration 480 ppm U
0.1 g cells / 200 mL solution

Inoculum uptake capacity -- 293 mg U / g cells

Figure 5-2
Effects of Media and Conditions of Growth
on UO_2^{2+} Uptake by R. oligosporus and R. arrhizus



species was chosen because it exhibited high uptake of the uranyl ion, it grew rapidly in the fermentor with little adherence to the impellor or baffles, and it was easily filtered and dried to form a light, porous mat. The log-log isotherm was linear. In the second fermentation peptone was substituted for the neopeptone in the medium. The resulting material exhibited a 32% lower uptake of uranyl ion, as indicated in Figure 5.1.

In light of the observed decrease in uptake of the uranyl ion during the fermentation of R. arrhizus R. oligosporus was grown in shake-flasks both with and without antifoam in a medium with peptone substituted for neopeptone. The uptake capacities of these samples of biomass are also indicated in Figure III-1. When grown in the presence of antifoam biomass samples from the fermentor and shake-flasks exhibited the same uptake of the uranyl ion, while the uptake by the biomass grown in the absence of the antifoam agent was 40% greater.

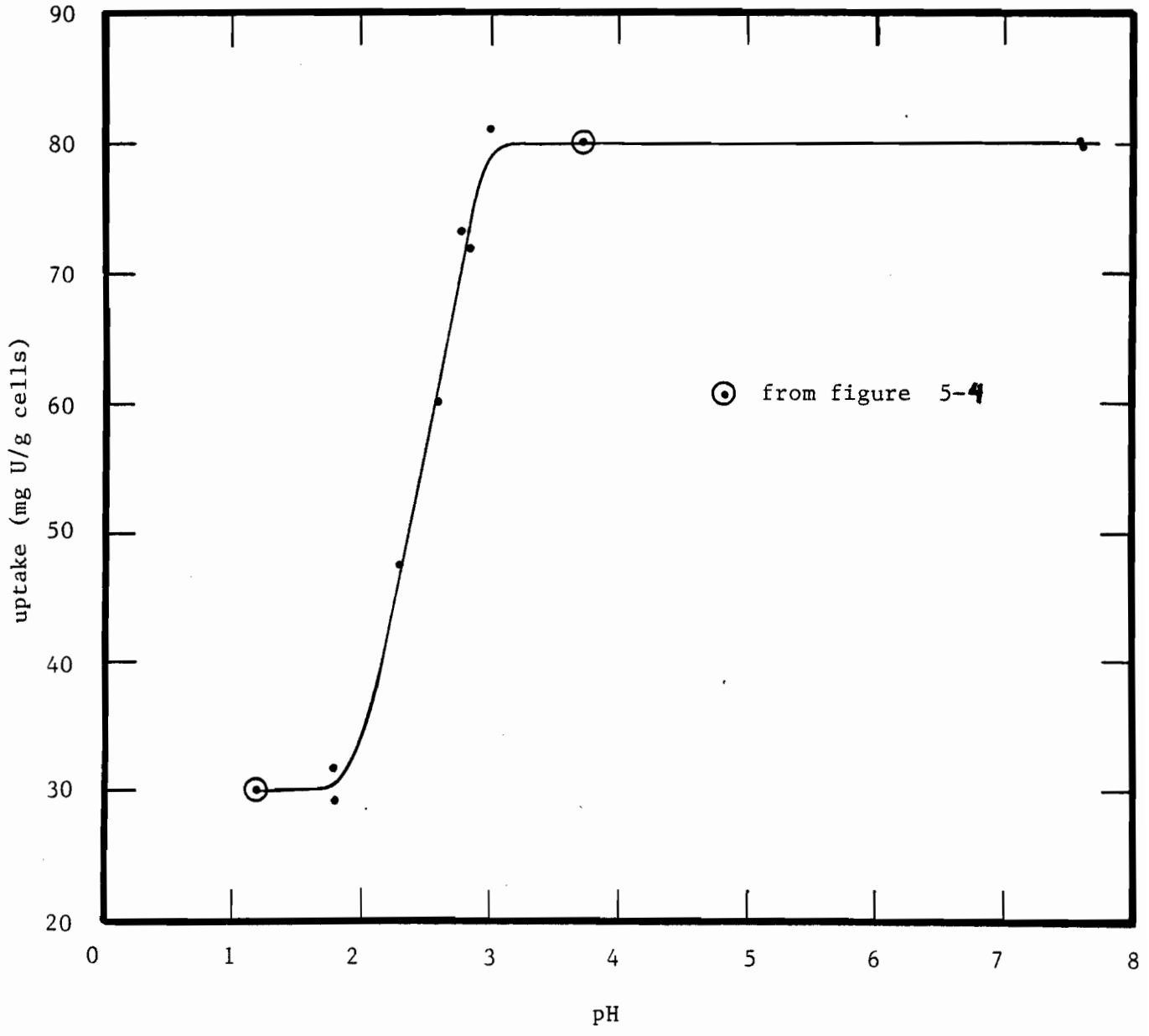
5.3 Effect of pH on the Uptake of the Uranyl Ion by R. oligosporus

Rhizopus oligosporus biomass, grown in a medium in which peptone was substituted for neopeptone, was shaken in contact with solutions of uranyl nitrate of various values of pH. The pH had been adjusted with sodium hydroxide or hydrochloric acid. As illustrated in Figure 5.3, a sharp drop in uptake occurred between pH 3 and 1.8, and an apparent pK of 2.5 was observed. Above pH 3.3 and below pH 1.6 the uptake of uranyl ion was relatively independent of pH.

Figure 5-3

Effect of pH on uptake of UO_2^{2+} by R. oligosporus

conditions -- pH adjusted with HCl or NaOH
initial uranium concentration 96 ppm U
0.1 g cells/200 mL solution



5.4 Effect of Anions and Ligands in Solution on the Uptake of the Uranyl Ion by R. oligosporus.

The biomass of R. oligosporus which was grown in the absence of neopeptone was also shaken in contact with uranium solutions containing one of a variety of anions including thiocyanate, oxalate, sulfate, acetate, chloride, nitrate and ethylenediamine-tetraacetate (E.D.T.A). Isotherms of uptake versus equilibrium uranium concentration are presented in Figures 5-4a, b and c for pH values > 3.3, 2.6 and < 1.4 respectively. In the presence of all anions except oxalate and thiocyanate the isotherms were very nearly parallel at all pH values, and uptake decreased with decreasing pH, and increasing complexation of the uranyl ion in solution.

The presence of thiocyanate enhanced uptake at high pH but had little effect on the uptake at low pH.

The presence of oxalate in solution led to the most peculiar behaviour. Uptake increased at low pH and decreased at high pH. Although the two isotherms in the presence of oxalate were parallel to one another, their slope was much greater than the others'.

Studies of the uptake of uranyl ion as a function of time were carried out for most of the above cases. Two replicate flasks were analysed for each point; plots of uptake versus time are included in Appendix III. These were not kinetic studies; they merely provide evidence that equilibrium had indeed been attained. Qualitatively it appeared that equilibrium was reached more rapidly at low pH values.

Appendix III contains the results plotted in Figures 5-4a, b and c.

Figure 5-4

UPTAKE OF THE URANYL ION BY R. OLIGOSPORUS
IN THE PRESENCE OF ANIONS AND LIGANDS

all points are averages of two-three determinations which were within 3% of one another

Figure 5-4a Uptake at pH 3.3-4

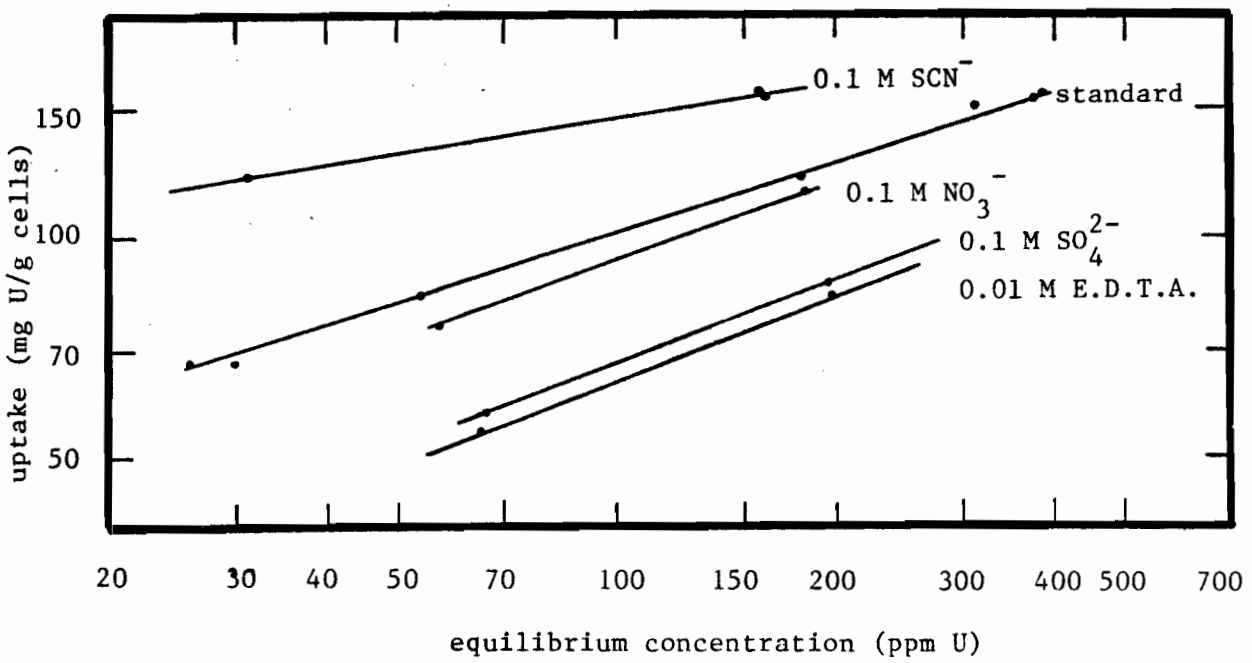


Figure 5- 4b Uptake at pH=2.6

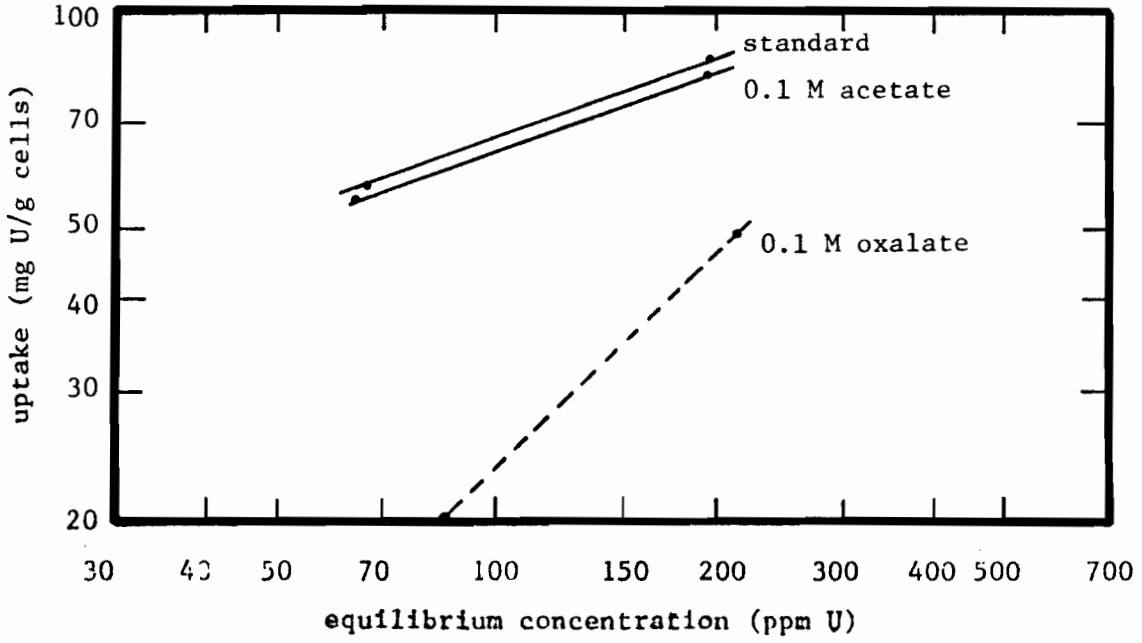


Figure 5-4c Uptake at pH < 1.4

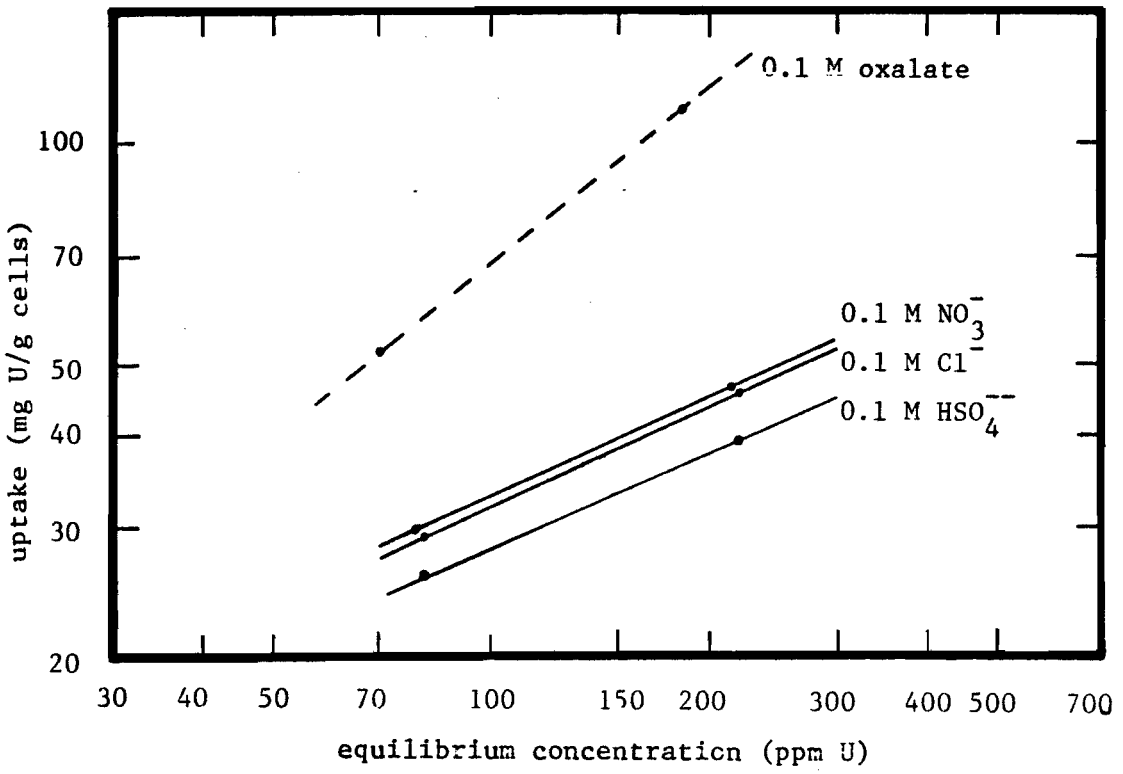


Table 5-2

Reversibility of Uptake of UO_2^{2+} by R. oligosporus

pH	Anion	final concentration (ppm U)	Uptake of UO_2^{2+} (mg U/g biomass) observed	expected according to Fig. 5-4
2.7	0.1 M acetate	341	91	95
4.2	0.1 M sulfate	327	129	110
2.6	0.1 M oxalate	354	63	62
1.2	0.1 M HCl	356	56	57
3.8	0.01 M E.D.T.A.	339	101	100
1.5	0.1 M SCN^- + HCl	82.9*	31	30

*Initial concentration = 100 ppm U

5.5 Recovery of Biosorbed Uranyl Ion by Addition of Ligands and Adjustment of pH

The same R. oligosporus which had been grown in medium containing peptone in lieu of neopeptone was shaken in contact with an unbuffered solution of 474 ppm U initial concentration for 13 hours. The final pH was 3.4. Fifty milliliters of solution were added to increase the ionic strength and adjust the anionic ligand concentration and pH. The contents of the flasks were analysed after equilibrium was attained. Table 5-2 contains the results of these analyses, and the values of uranyl ion uptake at the appropriate equilibrium concentration from Figures 5-4a to c are included for reference. It is evident that all the uranium that one could expect to recover was released by the biomass into the solution.

5.6 Titration of Rhizopus Biomass with Aqueous Base

Two titrations with aqueous sodium hydroxide of Rhizopus biomass are presented in Figures 5-5 and 5-6. In the former the pellets of R.arrhizus which had been contacted with and eluted of uranium eight times were used. A sample of R.oligosporus powder which had not been previously contacted with uranium was used in the second experiment. Two noteworthy observations are that both samples have marked buffering capacity between pH 6 and 9.5, and that the two curves are similar although not identical. This is partly because soluble species had not been washed from the R.oligosporus biomass. Table 5-3 summarizes the similarities between the two curves. Each plateau on a titration curve indicates the presence of significant concentrations of an ionizable group on the biomass, and similarities in their pK's suggest chemical similarities between the polymers.

Figure 5-5

Titration of R. arrhizus biomass with Aqueous NaOH

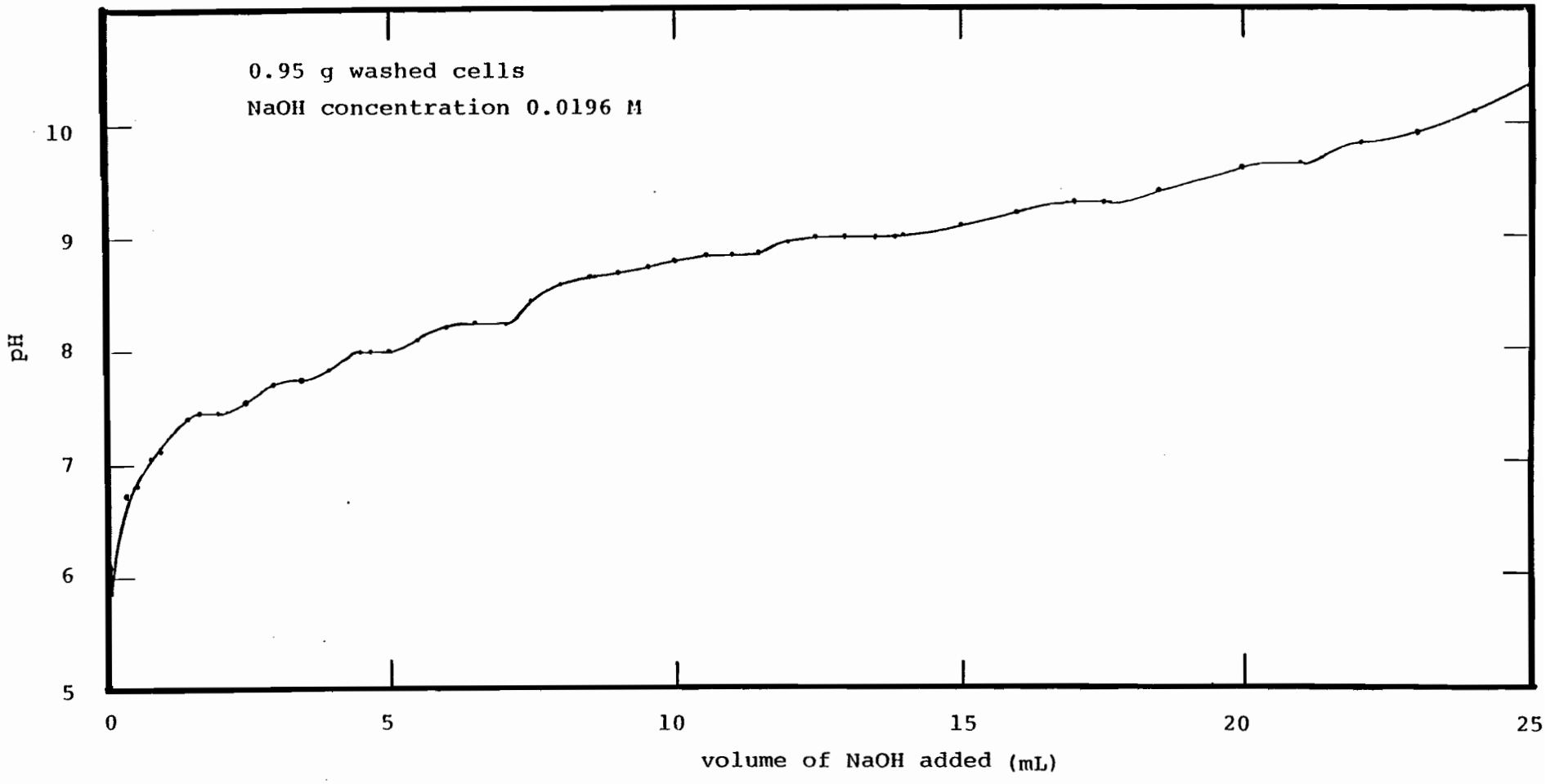


Figure 5-6

Titration of R. oligosporus biomass with Aqueous NaOH

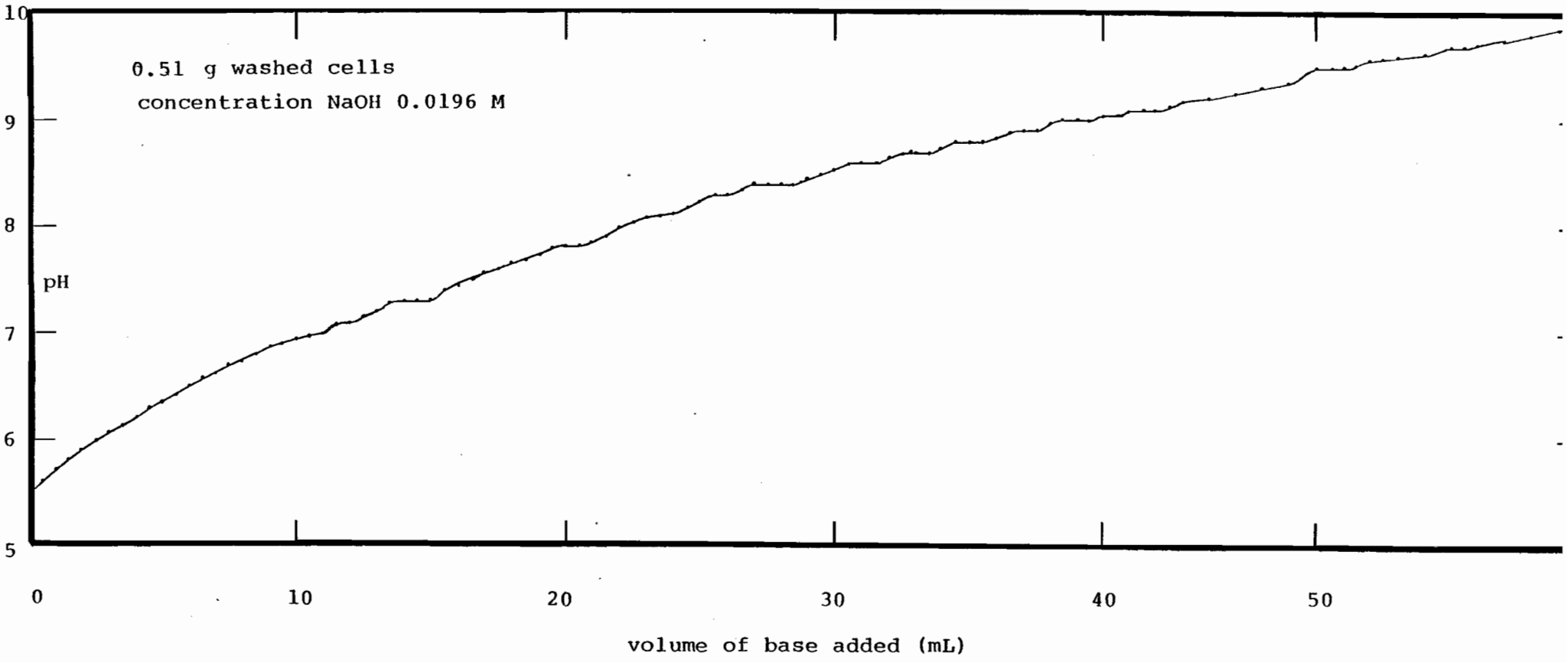


Table 5-3

Comparison of Hydroxide Titration of *R. arrhizus* and *R. oligosporus* Biomass Samples

pH of Plateaus		
R. oligosporus	*R. arrhizus*	groups commonly titrated at corresponding pH
7.0		free phosphate
7.1		
7.3 *	7.45	
7.6	7.75	
7.8 *		
8.1	8.03 *	
8.3	8.25 *	↑phosphate esters
8.4 *		
8.6	8.6	
8.7	8.75	
8.8		↓amino functions
8.9		
9.0 *	9.0 *	
9.1		
9.2		
9.5 *	9.6 *	
9.6		
9.7	9.8	

* relatively large quantities present

5.7 Studies of the Uptake of the Uranyl Ion by a Packed Column of Beads of R.Arrhizus

5.7.1 Uptake from an Unbuffered Uranium Solution

Uranium solution was pumped through columns packed with both 20-30 and 30-50 mesh pellets of R.arrhizus. Figures 5-7 and 5-8 are plots of the cumulative quantity of uranium taken up and hydrogen ion evolved into the solution. The molar ratios of H^+ : UO_2^{2+} are 1.95, 2.1 and 2.0 for the three experiments; very close to the value of 2 expected for pure ion exchange.

5.7.2 'Sorption / Desorption Cycling of R.arrhizus Beads

One column of 20-30 mesh R.arrhizus beads was cycled eight times. Table 5- 4 indicates the quantities of uranium taken up and eluted in the first 40 mL of acid each time. Although the column was washed until pH had stabilized at the pH of the acid, after the first cycle the capacity of the column was reduced. Thereafter it remained constant. The pellets remained intact and were still discrete when the column was unpacked. One difficulty was encountered in that at very low pH the pellets became less rigid or softer. Compaction of the column occurred during washing with distilled de-ionized water which was at a higher flowrate than the very slow elution with acid. As the column became compacted less surface area was directly available to the flow, channelling occurred next to the walls and the break-through curve was less sharp.

5.7.3 Elution of Uranium and 0.2N and 1.0N acid

Figure 5-9 illustrates the elution of similarly packed columns with 0.1M H_2SO_4 and 1.0 M HNO_3 (runs 4 and 5.) With higher acid strength the elution is much more rapid and the useful concentration factor is higher. There is much shorter tailing as the last sites are once again saturated with hydrogen ions.

Figure 5-7 Uptake of the Uranyl Ion by 30-50 mesh R. arrhizus Biomass

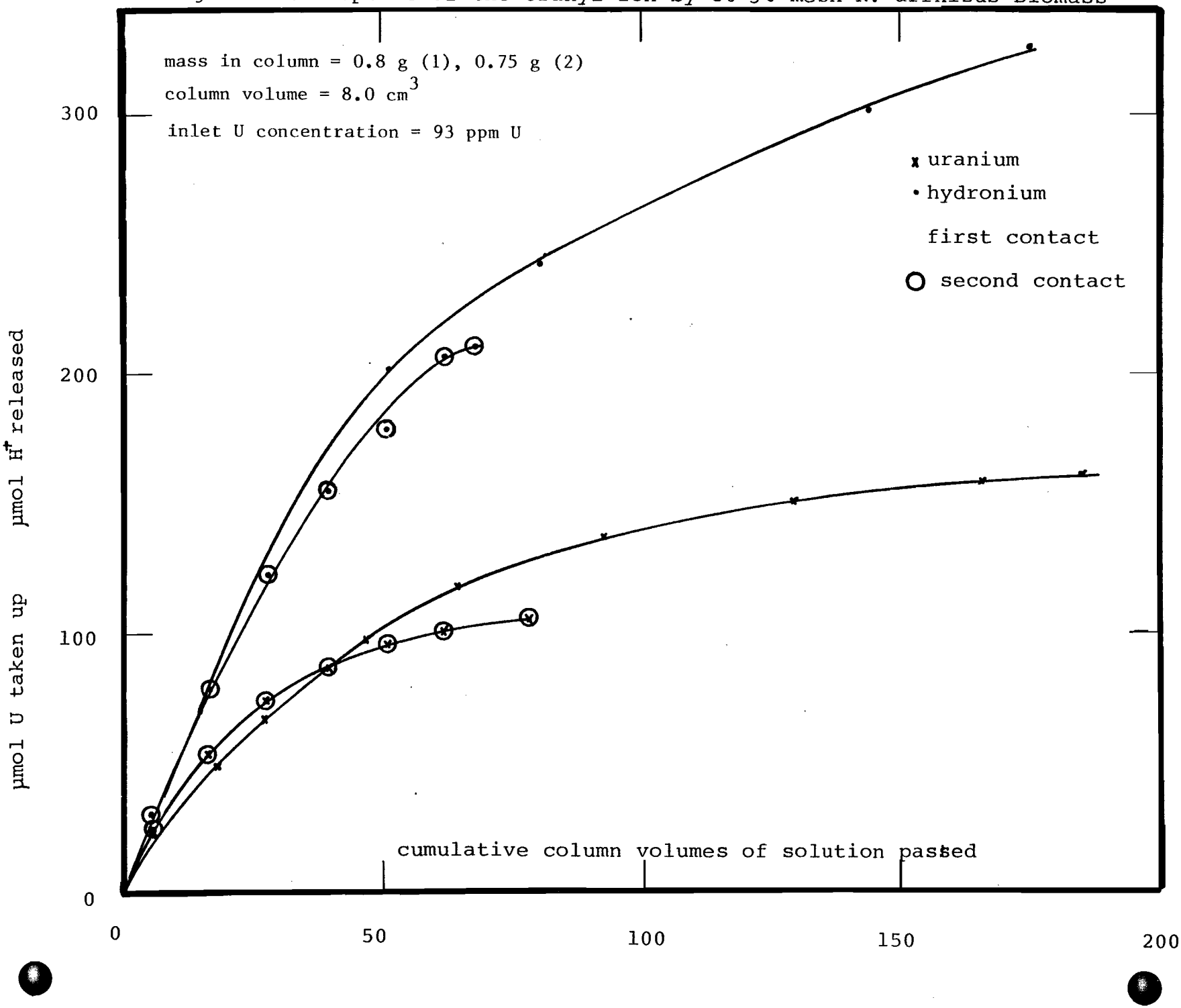


Figure 5-8

Uptake of the Uranyl Ion by 20-30 mesh R. arrhizus Pellets

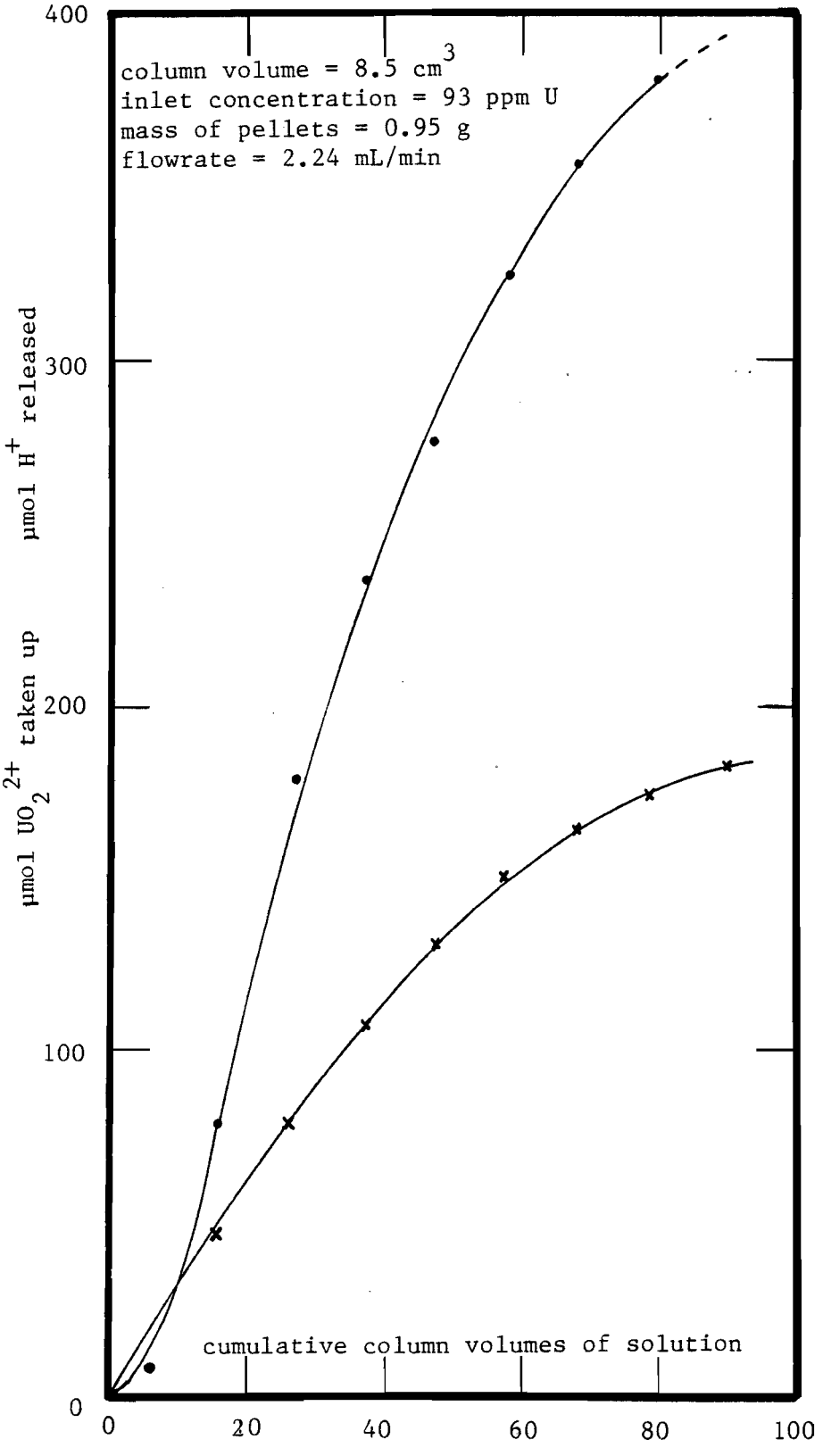


TABLE 5- 4

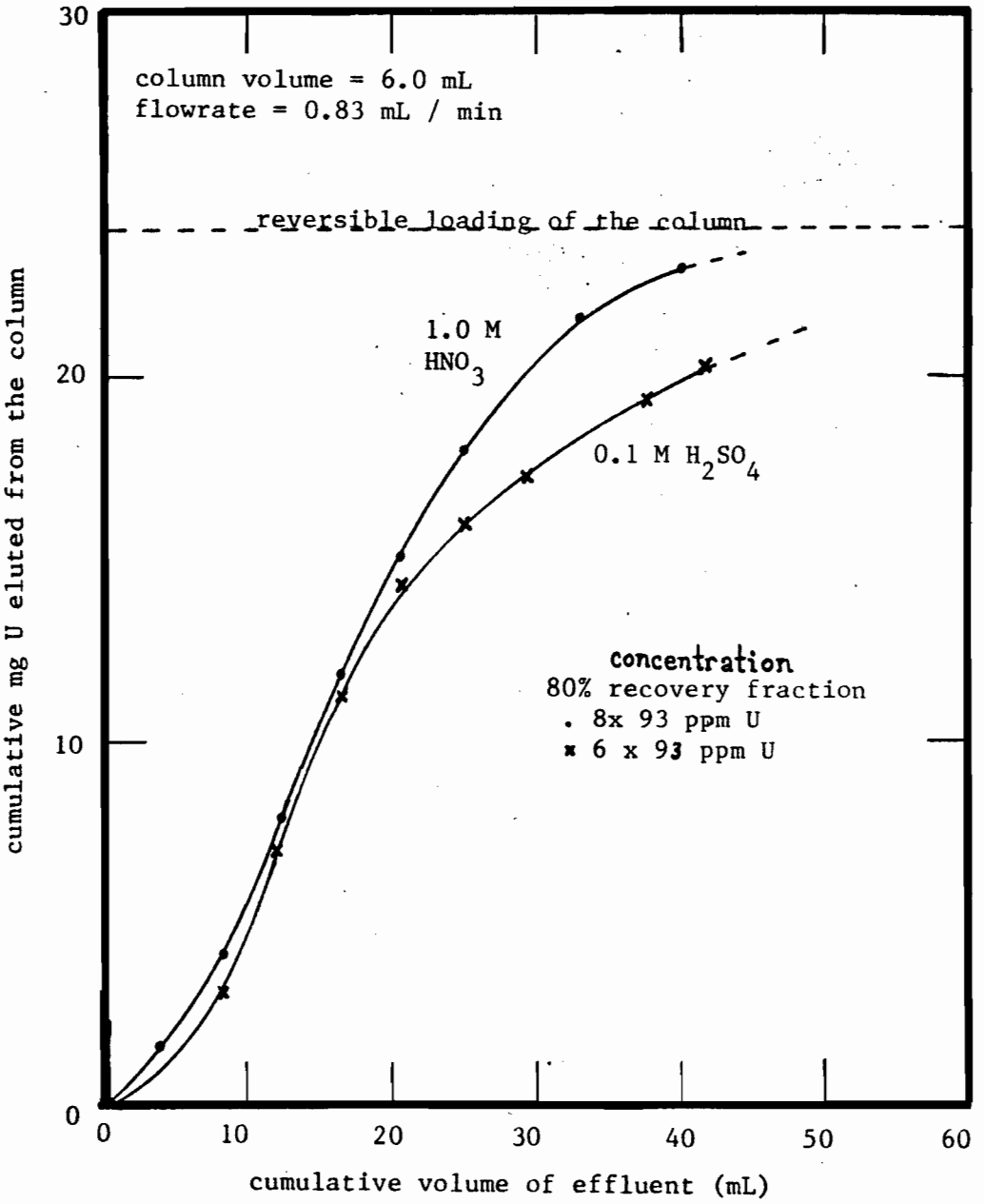
Uptake of the Uranyl Ion by Beads of R. Arrhizus during 'Sorption / Desorption Cycling

Run #	Uptake from 90 ppm U solution by .95 g biomass (mg. U)	mg U in first 40 mL of acid eluted
1	44	20
2	24	19
3	24	19
4	24	20
5	24	22 *
6	24	20
7	24	20
8	24	20

* 1.0 M HNO_3 used instead of 0.1 M H_2SO_4

Figure 5-9

Elution of Uranyl-Loaded Column with 0.2 N and 1.0 N Acid



6 DISCUSSION

6.1 Screening of Fermentor-Product *Rhizopus* Biomass for Uptake of the Uranyl Ion

The individual isotherms of the uptake of the uranyl ion by species of *Rhizopus* are presented as log-log plots of the uptake (mg U/g dry cells) versus equilibrium uranium concentration (ppm U). Most of the species exhibited a straight line, complying with the empirical Freundlich model. The model presented in Chapter 3 is an extension of the Langmuir model which involves assumptions as to the number and relative quantities of sites of adsorption. When the system is not approaching saturation, Langmuir behaviour also leads to a straight line on a log-log plot.

As is evident in Table 5.1, all species of *Rhizopus* examined exhibited high uptake of the uranyl ion. The uptake of the biomass propagated and assayed in this study is similar to that of the industrial-waste *R.arrhizus* biomass examined by Tsezos[4]. At 300 ppm U equilibrium concentration and pH 4 he observed 200 mgU/g cells uptake, which is the mid-point of the 150-250 mg U/g cells range measured here for a variety of *Rhizopus* species. Strandberg *et al.* reported 100-150 mgU/g cells uptake by the bacterium *Pseudomonas aeruginosa* and the yeast *Saccaromyces cerevisiae* in 100 ppmU uranyl nitrate solutions, which is also in line with these results.[47]

Uptake of 250 mg U/g cells at 300 ppmU equilibrium concentration were determined for *R.javanicus*, *R.oligosporus* and *R.oryzea*. This corresponds to slightly more than 1 mmol U/g cells 'biosorbed' from a 1.3 mM solution of uranyl nitrate. At 100 ppmU *R. chinensis saito*, *R. formosaensis* and *R. japonicus*, as well as the above, took up at least

160 mg U/g cells; that is, one mole of uranium was sequestered per 1500 g in contact with 0.4 mM uranyl nitrate at pH 4 and 25°C.

Although significant differences were observed in the uptake of the uranyl ion by the various species of Rhizopus studied, no definitive statement may be made as to which is the best or worst biosorbent. This is chiefly because the growth medium was constant for all species. It was not optimum most obviously because it would be preferable to have the mycelia grow in a disperse fashion rather than as large, glossy streamlined lumps on the impellor blades. Those of R. stolonifer grew only on the impellor while some biomass of R. chinensis saito R. japonicus and R. javanicus remained in suspension. R. arrhizus grew as small, uniform pellets of significant mechanical stability.

The optimum growth medium is difficult to define, much less find, as one is interested not only in the growth characteristics of the biomass, but the shape of the metal uptake isotherm, pH/ uptake behaviour and specificity of uptake, to mention a few parameters. Although a medium was used in which all the species grew in some manner, it was probably not optimum for any of them.

Reproducible uptake of uranyl ion was observed for biomass of R. arrhizus, R. oligosporus and R. oryzea grown in the standard medium. Substitution of peptone for neopeptone in the fermentor media, however yielded R. oligosporus with a 32% lower uptake of the uranyl ion. It is known that cell walls of Mucorales, of which Rhizopus is a genus, are very sensitive to the medium composition and conditions of growth.[40] It is therefore reasonable that the organic nitrogen source would influence cell wall composition and structure and profoundly affect, for instance, the number of nitrogen-containing coordination sites to which Tsezos[11] ascribes the uptake of the uranyl ion.

As suggested by a knowledge of the chemistry of the uranyl ion (section 1.2) there must be a role played by oxygen-containing ligands arising from the media and/or known to be present in the cell wall such as sulfate, phosphate, carboxylate and hydroxyl, and possibly ester, ketone or aldehyde oxygens. Beveridge[5] ascribes the uptake of uranyl ion by cell walls of Bacillus subtilis to phosphodiester and glutamic carboxyl groups rather than to free amino functions. The quantities and appearance of these will be affected by media composition and conditions of growth such as oxygen tension.

To complicate matters further the antifoam agent, which was necessary to overcome otherwise catastrophic foaming of the contents of the fermentor, caused a large inhibition of the uptake of the uranyl ion. The uptake by R. arrhizus grown in the standard medium was 56% less than that by material from shake-flasks grown in the absence of the antifoam agent. The decrease was logarithmic during the logarithmic growth period.

R. oligosporus grown in a broth in which peptone was substituted for neopeptone took up 29% less uranium than the shake-flask inoculum did. R. oligosporus biomass from shake-flasks with antifoam in the medium exhibited the same uptake as the material obtained from the fermentor did, so this phenomenon must be attributed to the effect of the antifoam itself rather than different conditions of growth in the fermentor.

The role of the antifoam agent would be a non-trivial problem to resolve. Either the cells are developing a wall of different chemical structure in response to the different properties of the medium/ cell interface or the incorporation of a component of the antifoam is causing blockage of possible sites of biosorption of the uranyl ion. Once incorporated into the cell wall such hydrophobic materials would not be

removed by washing with distilled water, which was the only treatment of the biomass used. The antifoam agent also affected the growth as it greatly reduced the lag-time in shake-flask cultivation of R. oligosporus, while in all cases uptake of the uranyl ion increased with the specific growth constant. The role of the antifoam probably involves both possible modes of action.

Fermentations of different species required the addition of varying quantities of antifoam. There is no correlation between the quantity of antifoam agent added and the ability to sequester the uranyl ion, but different degrees of reduction of uptake are incorporated in the various isotherms.

6.2 Effects of Anions in Solution and pH on Uptake of the Uranyl Ion

Figure 5-3 illustrates a sharp drop in the uptake of the uranyl ion from non-complexing media between pH 3 and 2. This implies that sequestering is occurring at sites in the biomass which are sensitive to the hydrogen ion concentration. The existence of such titratable sites is demonstrated in Figures 5-5 and 5-6, while the comparison of the observed pK's for R. arrhizus and R. oligosporus is presented in Table 5-3. Similarities in the titratable sites suggest the existence of similar chemical groups or sites of uptake on the cell walls of the two species.

Detailed chemical analysis of the cell wall would be necessary before one could interpret the inflections in such titration curves in terms of the actual sites. Some observations should be highlighted at this point, however:

1. Rhizopus cell walls may contain significant quantities of phosphate[44], such groups would be titrated between pH 4 and 8[5], and phosphate is an excellent ligand for the uranyl ion.[37] This anionic phosphate is associated with the cationic polysaccharide chitosan.[44]

2. Amine groups such as would be found on chitosan

would be titrated at higher pH. Tsezos[11] has put forward evidence that some of the uranyl ion is complexed by nitrogen at pH 4. The amine function is a poorer ligand for the uranyl ion than phosphate.[37]

3. In the case of R. oligosporus 39% of the uranyl ion taken up at pH 4 is retained at pH 1, while 40% of the hydroxide equivalents required to titrate the biomass sample from pH 7 to 9.2 are expended between pH 7 and 8.

Although this does not constitute proof, these observations certainly point to the possibility of the existence of a variety of sites of uptake on the biomass with different strengths of binding with the uranyl ion and sensitivity to pH.

As well as the effect of pH on the sites of uptake of the uranyl ion, the state of ions in solution must be considered. As outlined and modelled in Chapter 3 this involves many simultaneous equilibria between hydrated and complexed species in solution and on biomass sites. A partial list of uranyl complex stability constants given in Appendix I indicates relative ligand strengths, although they are unfortunately sensitive to both the concentration of the uranyl salt and the ionic strength so are not appropriate for detailed calculations. In the presence of large excesses of chloride, nitrate, sulfate, acetate and ethylene diamine-tetraacetate (E.D.T.A.) as well as at various values of pH there is straight-forward inhibition of uptake corresponding to the order of stability of the uranyl complexes.[37,45]

$$Cl^- \leq NO_3^- < SO_4^{2-} < SCN^- < C_2O_4^{2-} < CH_3CO_2^- < E.D.T.A.^{2-}$$

Large inhibition occurred in the presence of the ligands to the right of nitrate, with the exception of thiocyanate and oxalate. As described in sections 3.1 and 3.2 such inhibition of a constant fraction of uptake occurs with simultaneous equilibria of mononuclear complexes and competition of hydrogen ion for sites on the cell wall. The presence of one ligand results in a species which is no longer of the appropriate charge or is too large or saturated with strongly bound ligands to be taken up by the biomass. It should be noted that E.D.T.A. and sulfate both form bidentate, closed-ring complexes.[37] The reversibility observed at higher pH indicates that such closed ring complexes are not stericly hindered within the cellular matrix.

The effects of large excesses of thiocyanate and oxalate in solution on the uptake of the uranyl ion are not as straight-forward. Thiocyanate enhances uptake at higher pH while oxalate does so at low pH. As there is little reason to expect these chemicals to alter the chemical make-up of the biomass, complexation of the uranyl ion by the ligand must not necessarily mean it cannot be complexed by the cell wall site. In fact, both are known to exist in many mixed uranyl compounds and form bridges in polynuclear species.[46] As a polyelectrolyte the cell wall material would tend to isolate such species from solution but such isotherms would not parallel the more straight-forward ones above. Section 3.3 describes the modelling of such a situation.

At low pH the enhanced uptake in the presence of thiocyanate was reversed as the thiocyanate was neutralized. In the presence of oxalate, uptake was enhanced at low pH where there is less likelihood of the formation of closed-ring complexes so that greater bridging possibly led to increased rather than decreased uptake.

The roles of anions in solution is not always straight-forward and a good deal of experimental experience must be built up before one may confidently predict their behaviour. These results highlight the necessity of a thorough understanding of such interactions in any application of a biosorbent.

6.3 Study of Uptake of the Uranyl Ion by a Packed Column of Beads of R.arrhizus

6.3.1 Uptake from an Unbuffered Uranium Solution

Figures 5-7 to 5-8 illustrate clearly that in an unbuffered dilute solution of uranyl nitrate, when well-washed biomass of R. arrhizus takes up a mole of uranyl ion, two moles of hydrogen ion are evolved. This indicates that the (hydrated) uranyl ion itself, and not a hydroxylated one nor one complexed with nitrate is the species being taken up by the biomass. This does not mean, however, that all sites must include this many hydrogen ions as the reaction

$$[L_5UO_2H_2O]^{n-} \rightleftharpoons [L_5UO_2OH]^{(n+1)-} + H^+$$

where L is any ligand, occurs readily[45], particularly in the presence of electron-withdrawing ligands. This type of reaction also accounts for the dramatic effect of pH on the uptake.

6.3.2 'Sorption / Desorption Cycling of R.arrhizus Beads

After the column of R. arrhizus beads was saturated the first time with 93ppm U , ~40% of it was not recovered with 0.1 M H_2SO_4. The remaining~60% of the original binding capacity was consistently freely reversible during the following seven 'sorption/desorption cycles, even when 1.0 M HNO_3 was used to desorb the bound uranium.

The large proportion of irreversible uptake by R. arrhizus sets this biomass apart from the sample of R. oligosporus which exhibited very nearly total reversibility. Tsezos[4] postulated inorganic deposition of hydrous oxides as one mechanism of uptake, and one would not expect this to be totally reversible. In fact this may not even be necessary, as irreversibility of some strong complexes ('crypt' complexes) of the uranyl ion have been reported, the simplest example being the observation that it is "impossible to cause the decomposition of $UO_2(CO_3)_3^{4-}$ ion by any excess whatsoever of oxalate, acetate or sulfate ions in aqueous solution."[45] While one might hypothesize that phosphate-bound uranium would not be as freely exchanged as that which is nitrogen-bound, the biomass from the column exhibited strong buffering action between pH 5 and 8.

Clearly not only the uptake capacity but the degree of reversibility must be factors in screening biosorbents. While an understanding of the chemistry of reversible sites may lead to a commercial metal-concentrating biosorbent, the irreversible binding may be of interest in the production of non-leachable solid concentrated wastes, for instance from nuclear installations' waste-water.

6.3.3 Elution of Uranium with 0.2 N and 1.0 N Acids

A direct consequence of the roles of the hydrogen ion, both in titrating the ionizable sites of uptake and in reactions with the hydroxylated uranyl ion, is that the stronger the acid used for eluting the column is, the more concentrated the product will be. Elution also occurs more rapidly (within limitations of mass transfer) and tailing as the last traces are washed from the column is shorter. This is demonstrated in Figure 5-9.

All break-through curves for the desorption of uranyl ion with 0.1 M H_2SO_4 were similar, even as the column was compressed. This indicates that at a flowrate of 0.83 mL/min in the 0.8 cm diameter column, mass transfer within the pellet was not limiting.

7 CONCLUSIONS

1. All nine species of Rhizopus assayed exhibited high uptake of the uranyl ion at pH 4, 25°C and 100-300 ppm U equilibrium concentration; thus this characteristic appears to be common to the genus.

2. The medium influences both the growth of the microbe and the cell wall chemistry. Because the medium was a constant in this study it is not possible to pinpoint on an absolute scale the best biosorbent. Under the conditions of growth used, however, some stood out. R. oligosporus, R. oryzea and R. javanicus took up at least 250 mg U/g cells at 300 ppm equilibrium concentration while these species as well as R. formosaensis, R. chinensis saito and R. japonicus sequestered 160 mgU/g cells at 100 ppm U equilibrium concentration.

3. The physical properties and growth behaviour of the biomass must be considered in the assessment of a biosorbent. Of the first three species listed in #2 above, only R. oligosporus grew in a disperse form that was easily washed and dried. R. arrhizus exhibited lower uptake of the uranyl ion but grew as small (<1 mm diameter) strong spheres which may be packed directly in a column.

4. A qualitative correlation exists between the measured specific growth constant and uranium uptake ability; one increased with the other.

5. Biomass of R. arrhizus and R. oligosporus possess titratable sites, exhibiting similar buffering between pH 5 and pH 9.5. These sites are involved in uptake of the uranyl ion and account for the dramatic drop in uptake by R. oligosporus between pH 3 and 2. Direct competition by hydrogen ion for sites on the biomass or uranyl complexation by sulfate, acetate or E.D.T.A. resulted in proportional inhibition of uptake.

6. Thiocyanate and oxalate in large excess resulted in enhanced uptake at high (>3) pH and low (<2) pH respectively. This is due to the formation of polynuclear bridged species.
7. Uptake by a packed column of beads of R. arrhizus involved the release of two moles of hydrogen ion per mole of uranyl ion. This may have been via ion-exchange or displacement of a hydrogen ion from a hydrated uranyl species.
8. When beads of R. arrhizus were repeatedly exposed to uranium solution then eluted with acid, 40% of the uranium originally taken up was never re-eluted. The remaining 60% was 'sorbed and desorbed 8 times with no loss of capacity nor degradation of the beads, although the beads were weaker and more compressible at low pH. This lack of reversibility contrasts with the reversibility of uptake observed for R. oligosporus.
9. Elution of a column of beads of R. arrhizus with acid was affected by acid strength as any cation exchange resin. Increased acid strength increased the concentration of the effluent and decreased tailing as the column was washed of the last uranium.

8 RECOMMENDATIONS FOR FUTURE RESEARCH

Broad areas of research in this field were outlined in Section 1. Some interesting possibilities arising from this work are:

1. Growth of a finished-product sequestering agent. The beads of R. arrhizus are very nearly a 'self-immobilizing' cell, and if the uptake capacity could be improved without loss of this remarkable form of growth the product may be quite valuable as a substitute for ion-exchange resins.
2. With reference to #1, the medium as well as the species influence not only the growth but the uptake capacity, pH behaviour, shape of the isotherm, and possibly the degree of reversibility of uptake and selectivity. Nitrogen and phosphate are good elements around which to start such an investigation.
3. To carry the field of biosorption forward in a practical sense, specific applications of a material must be considered, both in waste-water treatment and processing of minerals. The physical configuration of the contacting system as well as the feasibility of uptake should be investigated.
4. Much more rapid screening procedures to look at the uptake of a variety of metals must be developed, both to accelerate assessment of specificity and 'design' of biosorbents, as well as to allow assessment of practical applications.

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Appendix I

Stability Constants of Uranyl Complexes

compiled from Palei[37] and Chernyaev[36,46]; molar units

Cationic Complexes	stability/formation constant at 10°C	20°C	25°C	40°C	ionic strength(μ)
$UO_2^{2+} + NO_3^- \rightleftharpoons UO_2NO_3^+$	0.3		0.24	0.17	2 M^2
$UO_2^{2+} + Cl^- \rightleftharpoons UO_2Cl^+$	0.58		0.88	1.14	2
$4UO_2OH^+ + SO_4^{--} \rightleftharpoons (UO_2OH)_4SO_4^{2+}$		3.28			
$UO_2^{2+} + HF \rightleftharpoons UO_2F^+ + H^+$	5.5	3.5	2.6	2.1	2
$UO_2^{2+} + SCN^- \rightleftharpoons UO_2SCN^+$		5.7			1
$UO_2^{2+} + H_3PO_4 \rightleftharpoons UO_2H_2PO_4^+ + H^+$			15.5		1.06
$UO_2^{2+} + 2\ H_3PO_4 \rightleftharpoons UO_2H_2PO_4(H_3PO_4)^+ + H^+$			24		1 M $HClO_4$
$UO_2^{2+} + CH_2ClCO_2^- \rightleftharpoons UO_2(CH_2ClCO_2)^+$		27.5			1
$UO_2^{2+} + CH_3CO_2^- \rightleftharpoons UO_2(CH_3CO_2)^+$		240			
$UO_2^{2+} + CH_2OHCHOHCO_2^- \rightleftharpoons UO_2(\text{ascorbate})^+$		265			
$UO_2^{2+} + F^- \rightleftharpoons UO_2F^+$		3500			pH 3, perchloric acid

Hydrolysis

$UO_2^{2+} + 2H_2O \rightleftharpoons UO_2OH^+ + H_3O^+$	4×10^6 ($\mu = 0.374$)	1.5×10^6 ($\mu = 0.0347$)
$UO_2^{2+} + 2H_2O \rightleftharpoons UO_2(OH)^+ + H_3O^+$	2.17×10^3	
$2\ UO_2^{2+} + 3\ H_2O \rightleftharpoons UO_2UO_3^{2+} + 2\ H_3O^+$	10^{-6}	

Stability Constants of Neutral Uranyl Complexes

	10°C	20°C	25°C	40°C	μ (M^2)
$UO_2^{2+} + 2\ SCN^- \rightleftharpoons UO_2(SCN)_2$		5.5			1
$UO_2^{2+} + HSO_4^- \rightleftharpoons UO_2SO_4 + H^+$	6.1		6.4	6.5	2
$UO_2^{2+} + 3\ H_3PO_4 \rightleftharpoons UO_2(H_2PO_4)_2H_3PO_4 + 2\ H^+$			10.2		1.06
$2\ UO_2OH^+ + SO_4^{2-} \rightleftharpoons (UO_2OH)_2SO_4$		13.6			neutral
$UO_2^{2+} + 2\ H_3PO_4 \rightleftharpoons UO_2(H_2PO_4)_2 + 2\ H^+$			21.8		1.06
$UO_2^{2+} + SO_4^{2-} \rightleftharpoons UO_2SO_4$	63		76	96	2
$UO_2^{2+} + 2\ CH_2OHCHOHCO_2^- \rightleftharpoons UO_2(\text{ascorbate})_2$		9100			
$UO_2^{2+} + 2\ CH_2ClCO_2^- \rightleftharpoons UO_2(CH_2ClCO_2)_2$		195			1
$UO_2^{2+} + 2\ CH_3CO_2^- \rightleftharpoons UO_2(CH_3CO_2)_2$		2.3×10^4			
$2\ UO_2^{2+} + ((CO_2^-)_2N)\ C_2(N(CO_2^-)_2) \rightleftharpoons (UO_2)_2\cdot E.D.T.A$		10^{12}			

Stability Constants of Anionic Uranyl Complexes

		monovalent			
$UO_2^{2+} + 3\ SCN^- \rightleftharpoons UO_2(SCN)_3^-$		15.5			1
$3\ UO_2OH^+ + 2\ SO_4^{2-} \rightleftharpoons (UO_2OH)_2(SO_4)_2^-$		13.6			neutral
$UO_2^{2+} + 3\ CH_2ClCO_2^- \rightleftharpoons (UO_2)(CH_2ClCO_2)_3^-$		625			1
$UO_2^{2+} + 3\ CH_3OHCHOHCO_2^- \rightleftharpoons UO_2(\text{ascorbate})_3^-$		1.6×10^5			
$UO_2^{2+} + 3\ CH_3CO_2^- \rightleftharpoons UO_2(CH_3CO_2)_3^-$		2.2×10^6			1
$UO_2^{2+} + 3\ F^- \rightleftharpoons UO_2F_3^-$		2.9×10^{10}			1
		divalent			
$UO_2^{2+} + 2\ SO_4^{2-} \rightleftharpoons UO_2(SO_4)_2^{2-}$	580		710	820	2
$UO_2^{2+} + 2\ C_2O_4^{2-} \rightleftharpoons UO_2(C_2O_4)_2^{2-}$		6.5×10^{11}			1
$UO_2^{2+} + 4\ F^- \rightleftharpoons UO_2F_4^{2-}$			1.2×10^{11}		1
		quadrivalent			
$UO_2^{2+} + 3\ CO_3^{2-} \rightleftharpoons UO_2(CO_3)_3^{4-}$			6×10^{22}		1

Appendix II-1

```
10 PRINT "INPUT N"
20 INPUT N
30 PRINT "INPUT DATA POINTS"
40 S1,S2,S3,S4,S5=0
50 FOR I=1 TO N
60 INPUT X,Y
70 Y=LOG(Y)
80 S1=S1+X
90 S2=S2+Y
100 S3=S3+X^2
110 S4=S4+Y^2
120 S5=S5+X*Y
130 NEXT I
140 B=(N*S5-S2*S1)/(N*S3-S1^2)
150 A=(S2-B*S1)/N
160 PRINT
170 PRINT "A=";EXP(A)
180 PRINT "B=";B
190 S1=B*(S5-S1*S2/N)
200 S4=S4-S2^2/N
210 S2=S4-S1
220 STOP :PRINT HEX(03)
230 PRINT
240 PRINT
250 PRINT "                    REGRESSION TABLE"
260 PRINT
270 PRINT "SOURCE","SUM OF SQ.","DEG.FREEDOM","MEAN SQ."
280 PRINT "REGRESSION",S1,1,S1
290 PRINT "RESIDUAL",S2,N-2,S2/(N-2)
300 PRINT "TOTAL",S4,N-1
310 PRINT
320 PRINT "F=";S1/S2*(N-2)
330 PRINT
340 PRINT
350 S5=S1/S4
360 PRINT "COEFF. OF DETERMINATION=";S5
370 PRINT "COEFF. OF CORRELATION=";SQR(S5)
380 PRINT "STANDARD ERROR OF ESTIMATE=";SQR(S2/(N-2))
390 PRINT
400 PRINT "DO YOU WISH TO ESTIMATE VALUES OF Y FROM "
410 PRINT "THE REGRESSION CURVE? (1=YES,0=NO)"
420 INPUT X
430 IF X=0 THEN 500
440 PRINT "INPUT X"
450 INPUT X
460 PRINT "Y=";EXP(A)*EXP(B*X)
470 PRINT
480 PRINT "ANOTHER POINT? (1=YES,0=NO)"
490 GOTO 420
500 END
```

fit calibration of uranium analytical system
U conc. = A exp (B height)
by linear regression

adapted from Wang Computer Laboratories' Library

fit height of the U or pH curve from the baseline as a function of time to a first-sixth order equation.

```
10 COM A(13),Q(7,8),E(8),M,N
20 DIM Y1(50),J1(4)
40 J1(1),J1(2),J1(3),J1(4)=50: K=1: T2=0.: I=1
50 DEFFNC(Y)=7.1786*EXP(.67362*Y)                    U concentration from II-1
60 INPUT "DIFFERENTIAL TIME INTERVAL", TO
70 T1=TO
80 PRINT "MEASUREMENT <-100 INPUT WILL NOT COUNT, BUT WILL DOUBL
E THE TIME INCREMENT; >99 WILL END DATA INPUT"
90 PRINT "TIME=",T2: INPUT "INPUT MEASUREMENT",Y1(I)
100 IF Y1(I)>99 THEN 140
110 IF Y1(I)>-100 THEN 130
120 T2=T2+T1: T1=T1*2.: J1(K)=I-1: K=K+1: GOTO 90
130 I=I+1: T2=T2+T1: GOTO 90
140 N=I-1: T2=0: T1=TO: K=1
150 INPUT "INPUT ORDER DESIRED",M
160 FOR I=2 TO 2*M+1: A(I)=0: NEXT I
170 FOR I=1 TO M+2: E(I)=0: NEXT I
180 A(1)=N:SELECT PRINT 01C
190 FOR I=1 TO N: ON (I-J1(K)) GOTO 210
200 X=T2: Z=Y1(I): Y=FNC(Z): T2=T2+T1: PRINT "T=",X,"Y=",Y: GOTO
220
210 X=T2+T1: Y=FNC(Y1(I)): T1=T1*2: K=K+1:PRINT "T=",X,"Y=",Y: T
2=X+T1
220 FOR J=2 TO 2*M+1: A(J)=A(J)+X^(J-1): NEXT J
230 E(1),Q(1,M+2)=E(1)+Y
240 FOR J=2 TO M+1: E(J),Q(J,M+2)=E(J)+Y*X^(J-1): NEXT J
250 E(M+2)=E(M+2)+Y^2: NEXT I
260 FOR I=1 TO M+1: FOR J=1 TO M+1: Q(I,J)=A(I+J-1): NEXT J: NEX
T I
270 FOR S=1 TO M+1
280 FOR T=S TO M+1: IF Q(T,S)<>0 THEN 300: NEXT T
290 PRINT "NO UNIQUE SOLUTION": STOP
300 GOSUB 350
310 C=1/Q(S,S): GOSUB 380
320 FOR T=1 TO M+1: IF T=S THEN 340
330 C=-Q(T,S): GOSUB 390
340 NEXT T: NEXT S: GOTO 400
350 FOR J=1 TO M+2
360 B=Q(S,J): Q(S,J)=Q(T,J): Q(T,J)=B
370 NEXT J: RETURN
380 FOR J=1 TO M+2: Q(S,J)=C*Q(S,J): NEXT J: RETURN
390 FOR J=1 TO M+2: Q(T,J)=Q(T,J)+C*Q(S,J): NEXT J: RETURN
400 PRINT
410 FOR I=1 TO M+1: PRINT I-1;"DEG.COEFF.=";Q(I,M+2): NEXT I
420 STOP :PRINT HEX(03)
430 S=0
440 FOR I=2 TO M+1: S=S+Q(I,M+2)*(E(I)-A(I)*E(1)/N): NEXT I
450 T=E(M+2)-E(1)^2/N: C=T-S
460 I=N-M-1: J=S/M: K=C/I
470 PRINT : PRINT : PRINT "          REGRESSION TABLE": PRINT
```

```
470 PRINT : PRINT : PRINT "          REGRESSION TABLE": PRINT
480 PRINT "SOURCE","SUM OF SQ.","DEG. FREEDOM","MEAN SQ."
490 PRINT "REGRESSION",S,M,J
500 PRINT "RESIDUAL",C,I,K
510 PRINT "TOTAL",T,N-1: PRINT
520 PRINT "F=";J/K: PRINT : PRINT
530 J=S/T: PRINT "COEFF. OF DETERMINATION=";J
540 PRINT "COEFF. OF CORRELATION=";SQR(J)
550 PRINT "STANDARD ERROR OF ESTIMATE=";SQR(C/I): PRINT : PRINT

560 SELECT PRINT 005
570 PRINT "DO YOU WISH TO ESTIMATE VALUES OF Y FROM"
580 PRINT "THE REGRESSION CURVE? (1=YES,0=NO)"
590 INPUT I: IF I=0 THEN 650
600 PRINT : S=Q(1,M+2)
610 PRINT "INPUT X";:INPUT T
620 FOR I=1 TO M: S=S+Q(I+1,M+2)*T^I: NEXT I
630 PRINT "T=",T,"Y=",S: PRINT
640 PRINT "ANOTHER POINT? (1=YES,0=NO)": GOTO 590
650 END
```

integrate U-sorption or H-release by Gaussian quadrature

```
10 DEFFNC(X)=10^(-4-4.14*(.853889+1.6104E-02*X+4.2779E-03*X^2-6.
0901E-05*X^3+3.3391E-07*X^4-8.1717E-10*X^5+7.45546E-13*X^6))
20 INPUT "INPUT BEGINNING AND FINAL TIMES",A,B

30 INPUT "ENTER FLOWRATE (ML/MIN) AND INITIAL CONCENTRATION",F1,
U0
40 INPUT "ENTER NO. OF SUBINTERVALS",K
50 SELECT PRINT 01C: PRINT "TIME  CUM.ML  U OUT   SORBED(MG)  PA
SSED(MG)  (LN(IN/OUT))^-1.5"
60 %###.# ####.# #.###^^^^ #.###^^^^ #.###^^^^      #.####^^^^
70 C=(B-A)/K/2
80 D=A+C
90 T=0
100 FOR J=1 TO K
110 S=0
120 FOR I=1 TO 10
130 READ X,W
140 S=S+W*(FNC(D+C*X)+FNC(D-C*X))
150 NEXT I
160 RESTORE
170 T=T+S*C
180 E=D+C: F2=F1*E: T2=T*F1/1000.: T1=U0*F2/1000.-T2: U1=FNC(E):
 IF (U0/U1)<1.0000001 THEN 190:U2=(LOG(U0/U1))^(-1.5)
190 PRINTUSING 60,E,F2,U1,T1,T2,U2
200 D=D+2*C
210 NEXT J
220 SELECT PRINT 005
230 PRINT "CHANGE NUMBER OF SUBINTERVALS? ('1'--YES,'0'--NO)"

240 INPUT I
250 IF I=1 THEN 40
260 PRINT "NEW INTEGRATION LIMITS? ('1'--YES,'0'--NO)"
270 INPUT I
280 IF I=1 THEN 20
290 DATA 76526521E-9,.15275339,.22778585,.14917299,.37370609,.14
209611
300 DATA .510867,.13168864,.63605368,.11819453,.74633191,.101930
12
310 DATA .83911697,932767425-9,.91223443,626720485-9,.96397193
320 DATA 4060143E-8,.9931286,17614007E-9
330 END
```

Appendix III Biosorption by Rhizopus Biomass at pH3.6-3.9 and 25°C

Figure III-1

Uptake of the Uranyl Ion by Biomass of Rhizopus arrhizus

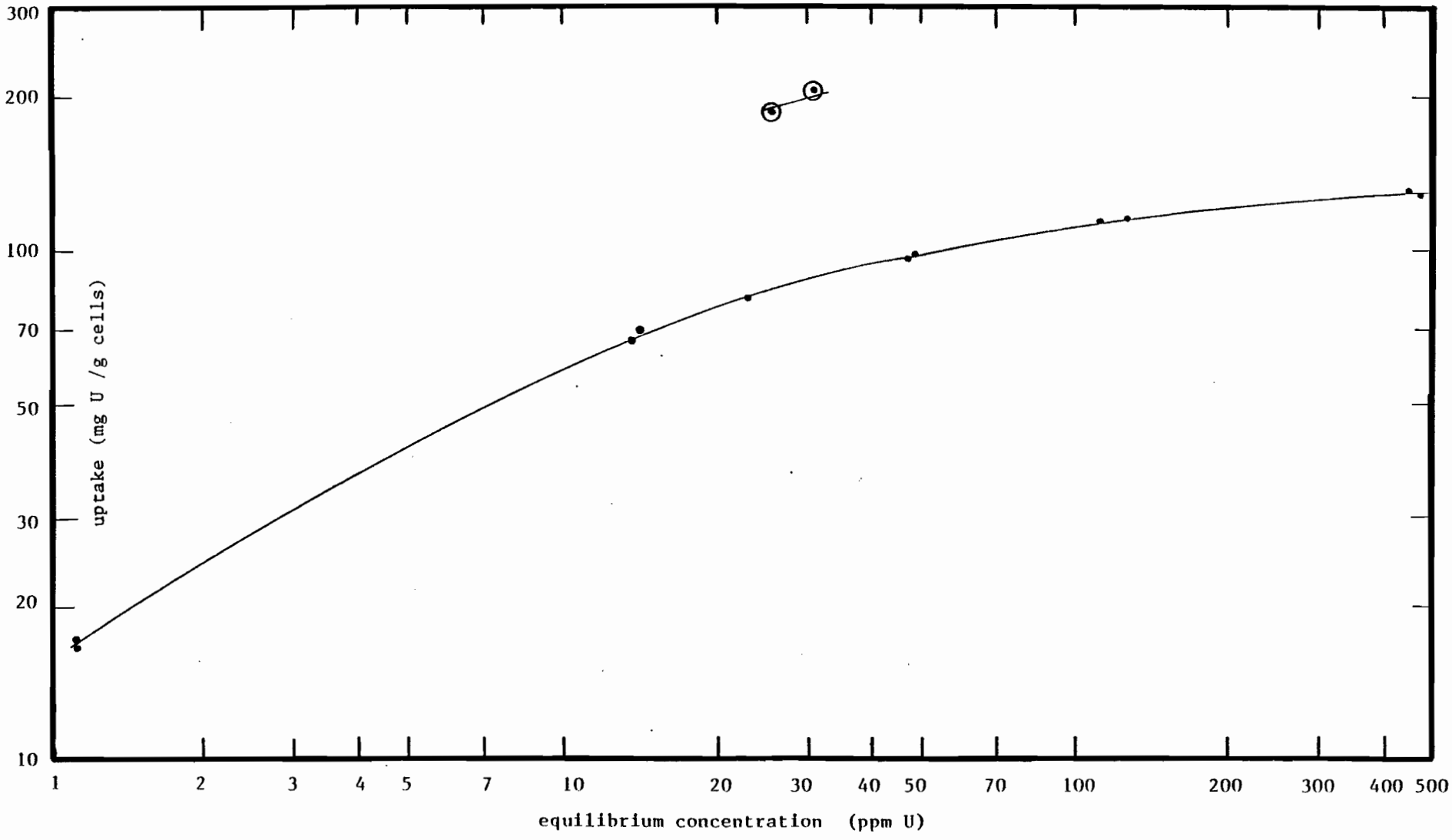


Figure III-2

Uptake of the Uranyl Ion by Biomass of Rhizopus chinensis saito

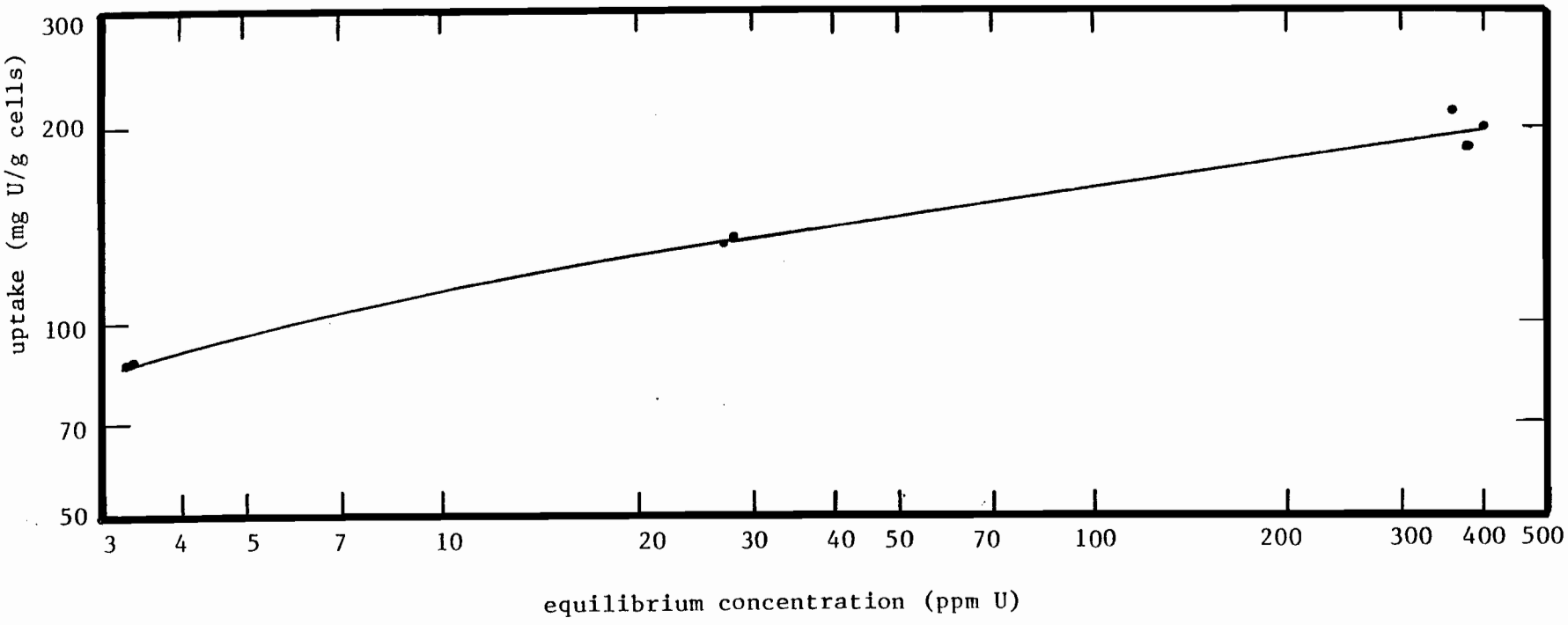


Figure III-3

Uptake of the Uranyl Ion by Biomass of Rhizopus delemar var multiplici-sporus

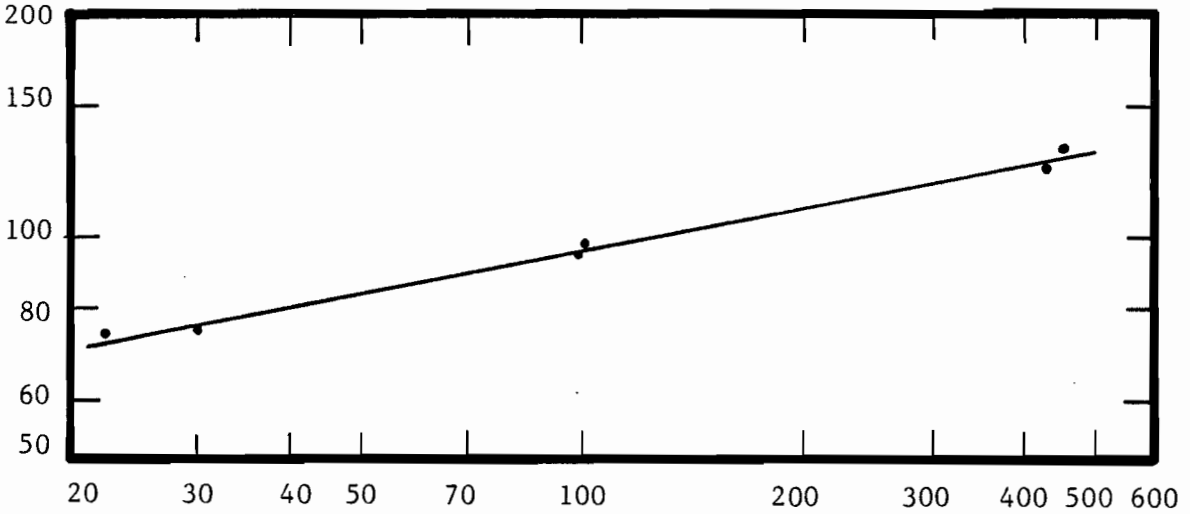


Figure III-4

Uptake of the Uranyl Ion by Biomass of Rhizopus formosaensis

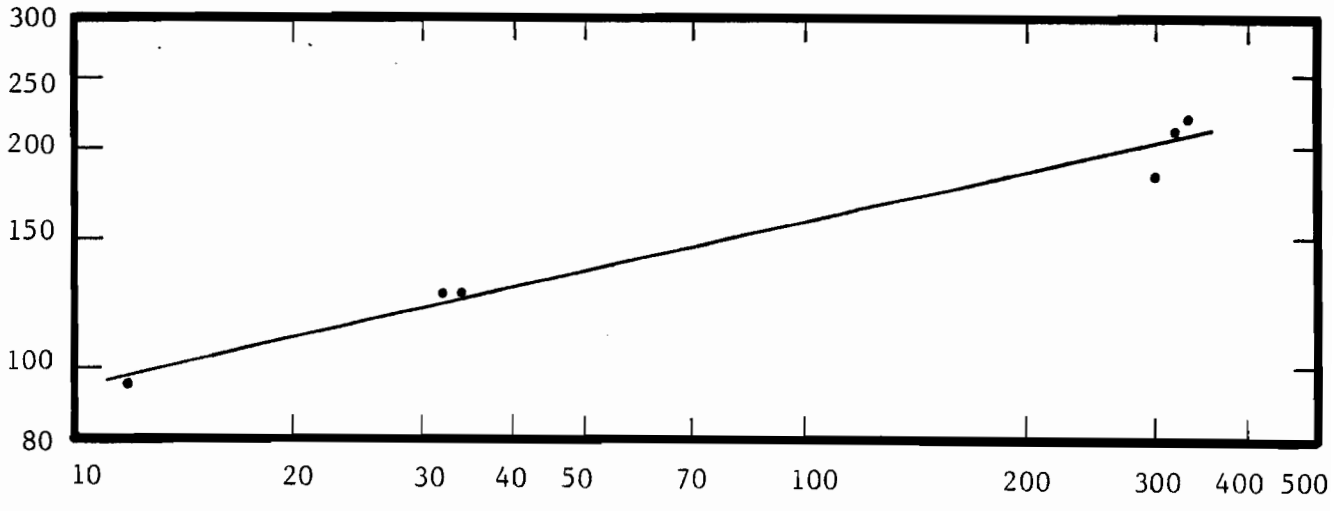


Figure III-5

Uptake of the Uranyl Ion by Biomass of Rhizopus japonicus

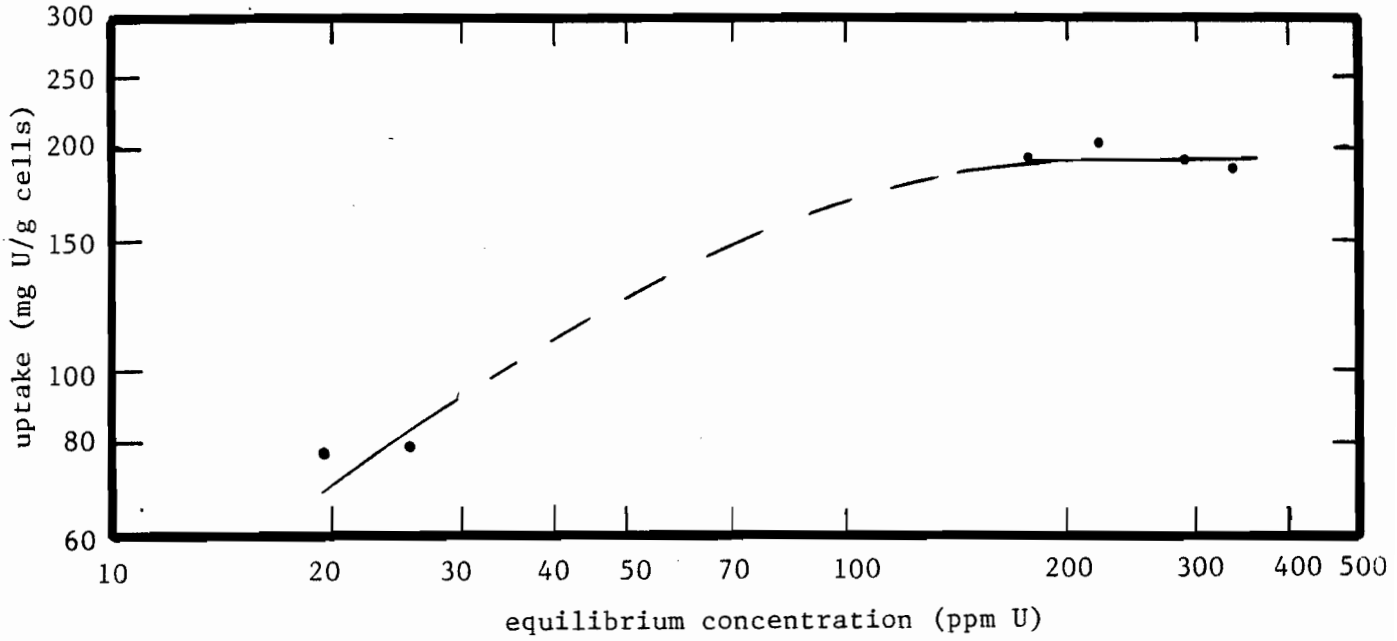


Figure III-6

Uptake of the Uranyl Ion by biomass of Rhizopus javanicus

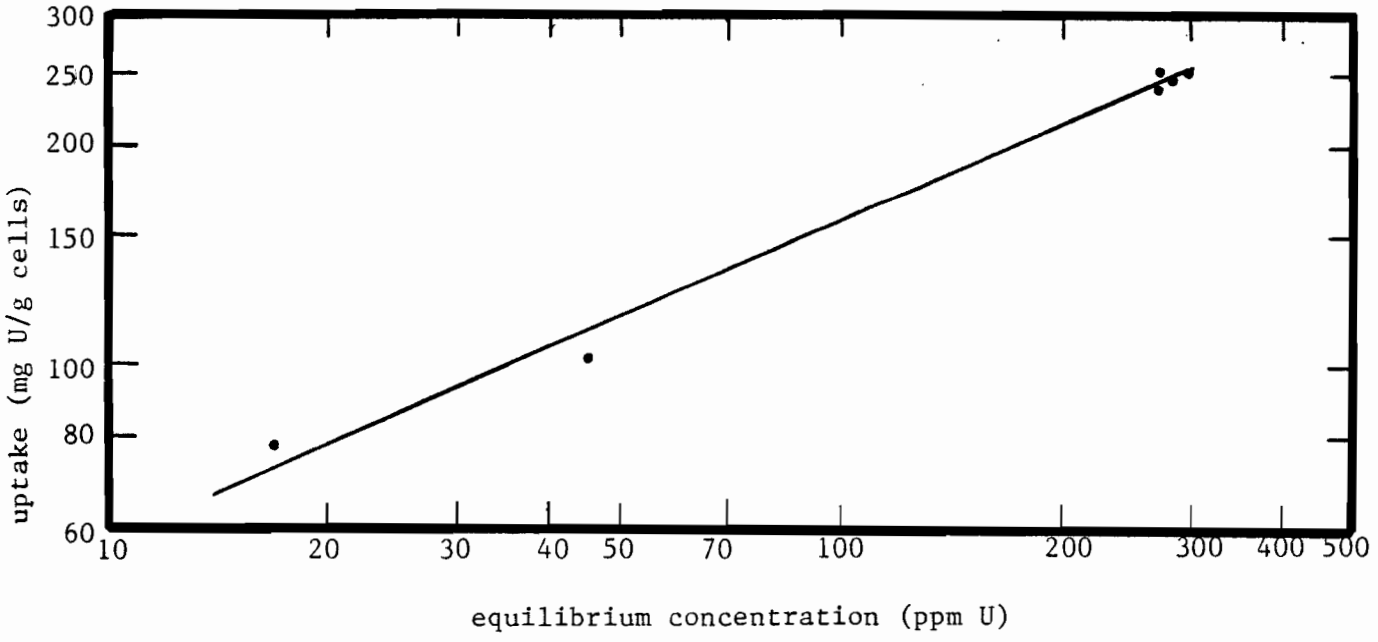


Figure III-7 Uptake by Rhizopus oligosporus

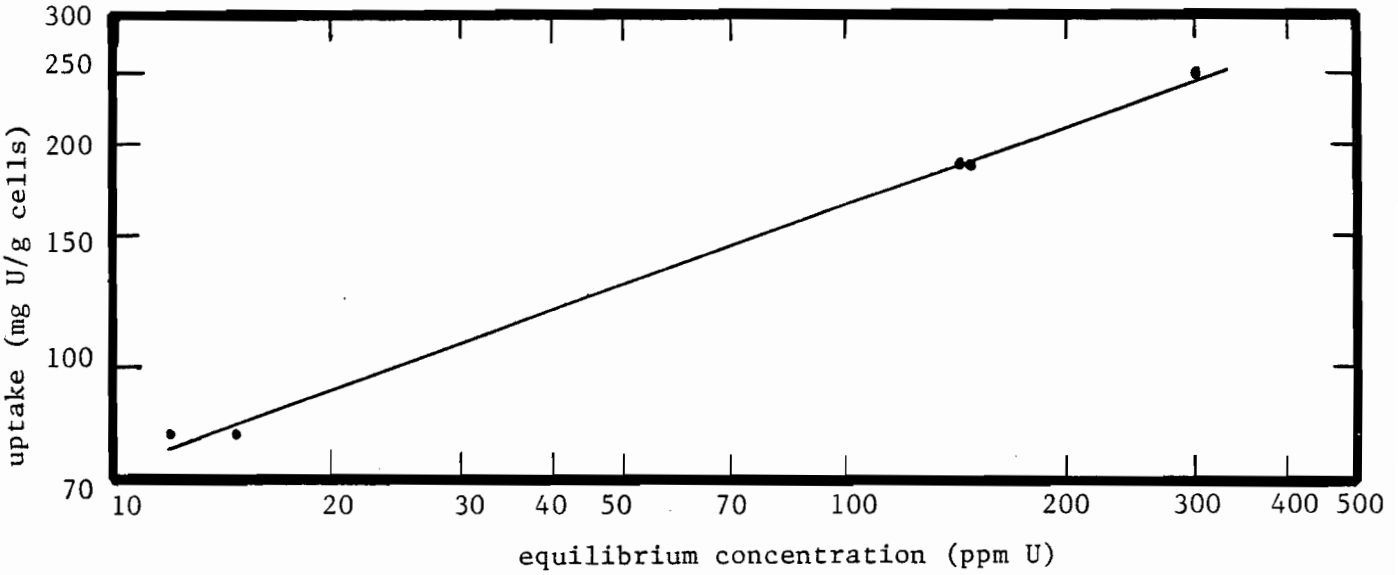


Figure III-8 Uptake by Rhizopus oryzea

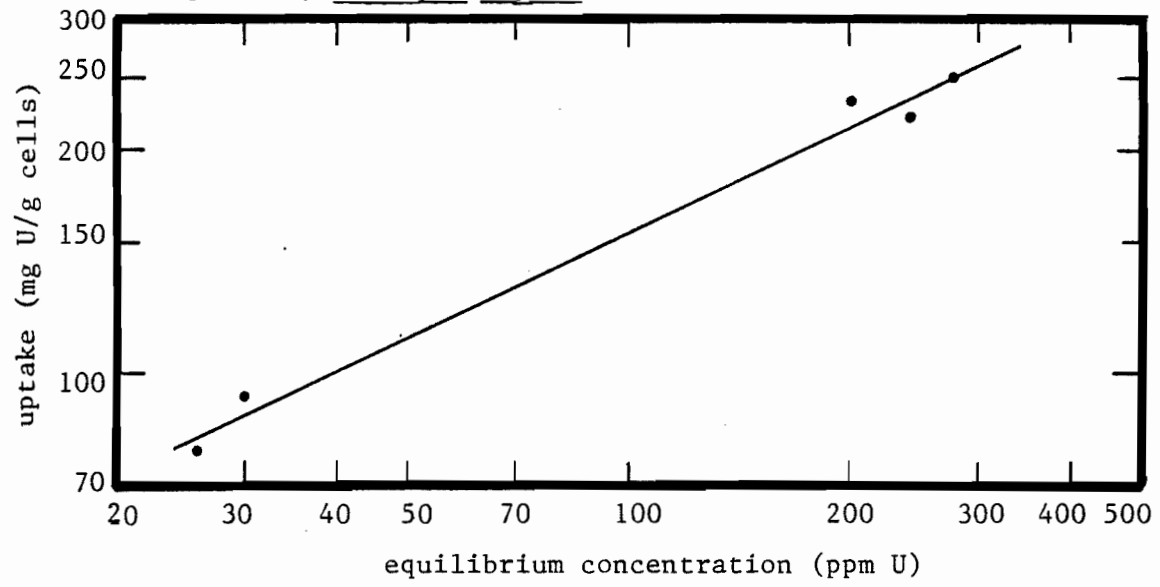
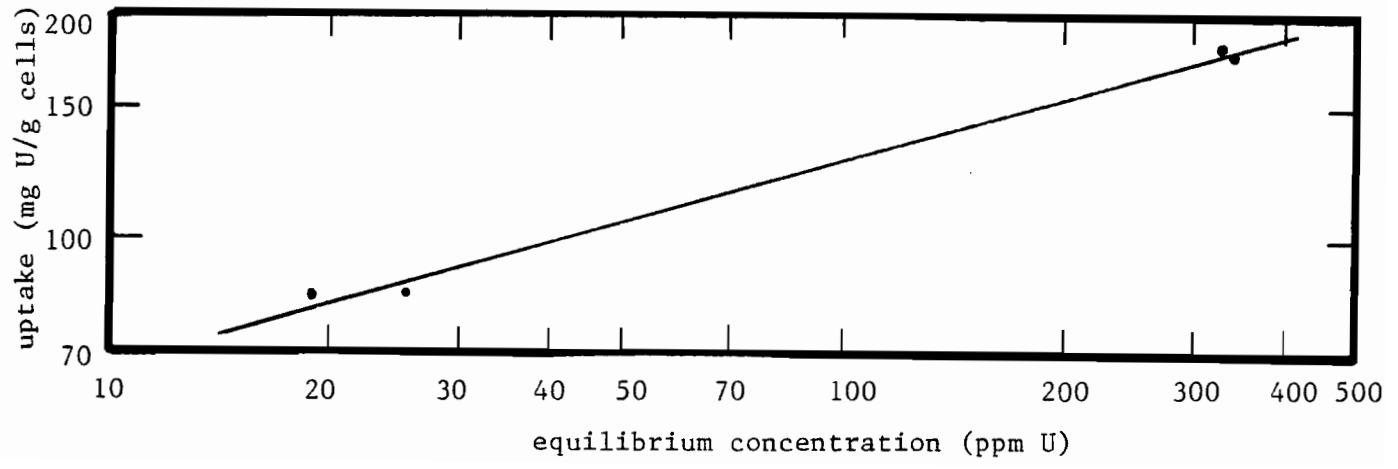
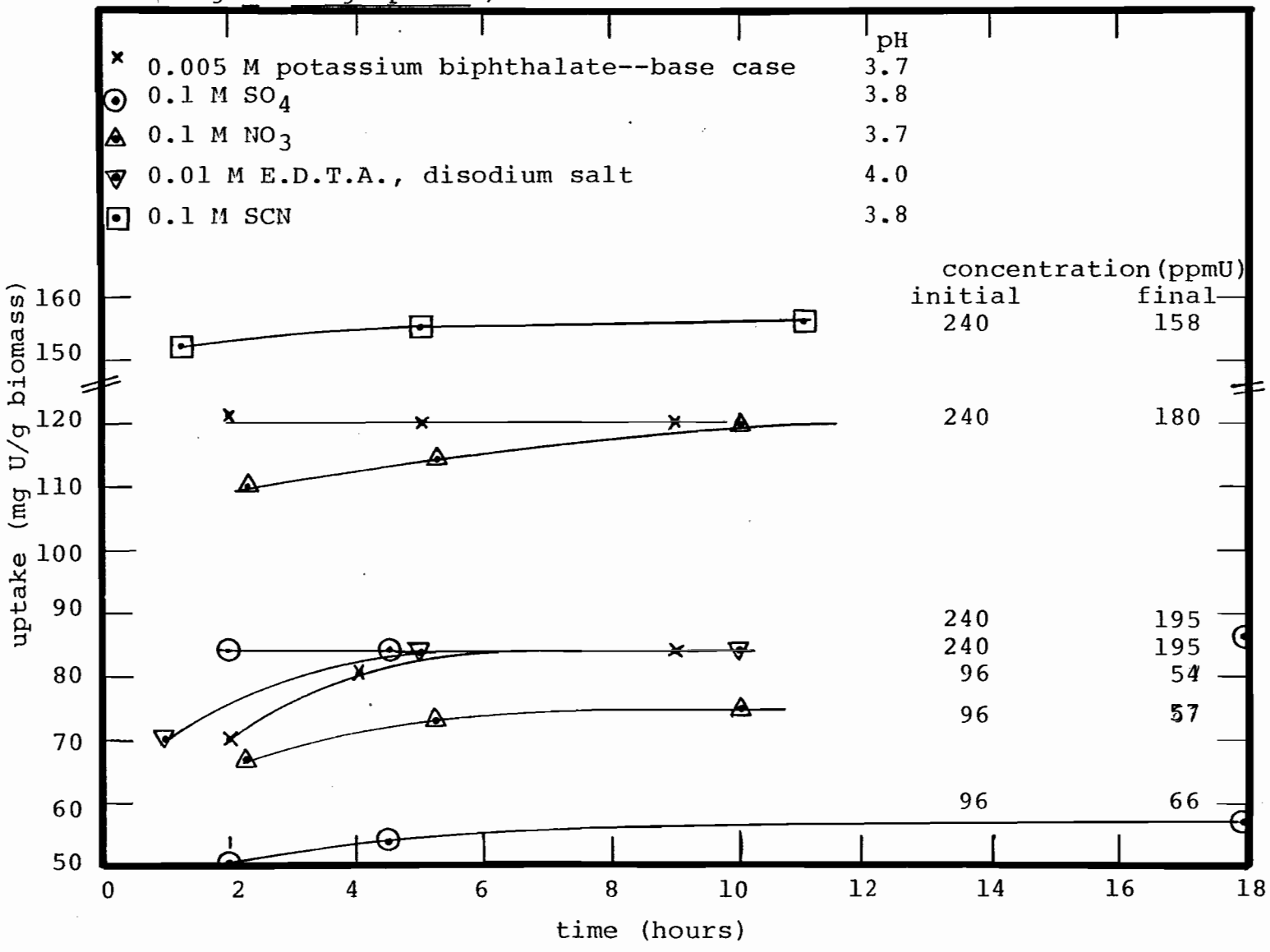


Figure III-9 Uptake by Rhizopus stolonifer



Appendix III-10 Time Dependence of U-uptake in the presence of Anions --Shake Flask Studies

0.1 g R. oligosporus / 200 mL solution



Appendix III-11 Time Dependence of U-uptake in the Presence of Anions
-- Shake Flask Studies at Low pH

0.1 g R. oligosporus / 200 mL solution