SEPARATION OF URANIUM BY IMMOBILIZED INACTIVE MICROBIAL BIOMASS

By

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ABSTRACT

The ability of microorganisms to interact with and accumulate a variety of heavy metal ions from their immediate environment has been well recognized. Biosorption is termed as the sequestering of metal ions by live or dead microorganisms and their derivatives. Native microorganisms however are not rigid enough to be used as technical biosorbents in large scale operations such as a packed bed adsorption reactor.

A new immobilization technique has been developed using a spouted bed reactor that produces encapsulated R. arrhizus biomass which can be used as a technical adsorbent in the same way as an ion exchange resin or activated carbon. The immobilized biomass has 10 to 15 wt% of inert medium that is porous polymeric membrane. The overall mechanical strength of the immobilized biomass was evaluated by measuring the pressure drop at various water flow rates in a packed bed filled with immobilized biomass particles. The structure of the encapsulation membrane of the immobilized biomass particles was examined under electron microscope and appeared to be very porous. A batch kinetic mass transfer model was developed for the immobilized R. arrhizus and was numerically solved by collocation methods. Batch kinetic experiments for mass transfer rate of uranium were carried out with the immobilized R. arrhizus using a modified liquid Carberry reactor. The effective diffusivities of uranyl ion through the wall membrane and in the biomass core were estimated by fitting the kinetic data to the mass transfer model using a non-linear regression analysis.

The new immobilization technique developed in this study can produce biosorbents with desirable technical properties without affecting the biosorptive capacity of inactive R. arrhizus. This technique is very versatile and can be applied to other biomass types. The mechanical strength of the immobilized biomass and mass transfer rate through the wall
membrane can be easily controlled by adjusting the membrane properties during the immobilization process.
IN MEMORY OF MY BROTHER

SOO-IK
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NOMENCLATURE AND SYMBOLS

- $A_{ij}$: first derivative matrix
- $B_{ij}$: second derivative matrix
- $b$: Langmuir isotherm parameter
- $c$: solution concentration, mg/L
- $c_b$: bulk solution concentration, mg/L
- $c_{b, KR}$: bulk solution concentration at the outer surface of coating membrane, mg/L
- $c_{bo}$: initial bulk solution concentration, mg/L
- $c_m$: solution concentration in the coating membrane, mg/L
- $c_{m, KR}$: solution concentration at the outer surface of the coating membrane, mg/L
- $c_{m, R}$: solution concentration at the interface of coating membrane and biomass core, mg/L
- $c_0$: initial solution concentration, mg/L
- $c_r$: solution concentration in the biomass pore at the radial position, r, mg/L
- $d_p$: particle diameter, mm
- $D_m$: effective solute diffusivity in the coating membrane, cm$^2$/s
- $D_p$: effective pore diffusivity, cm$^2$/s
- $f_c$: mass fraction of polymer in the coating membrane
- $f_s$: mass fraction of additive in the coating membrane
- $k_f$: external fluid film mass transfer coefficient, cm/s
- $k_o$: overall mass transfer coefficient, cm/s
- $K$: Freundlich isotherm parameter
- $K_m$: solute partition coefficient at the interface of the bulk solution and outer coating membrane surface ($c_{m, KR}/c_{b, KR}$)
NOMENCLATURE AND SYMBOLS (continued)

\( K_p \) : solute partition coefficient at the interface between the biomass core and the coating membrane \((c_{m,R/c_r}=R)\)

\( KR \) : radius of immobilized biomass particle, cm

\( M_b \) : mass of the biomass core of the coated biomass particle, g

\( M_{bc} \) : mass of \( \text{CaCO}_3 \) in the biomass core, g

\( M_{bi} \) : mass of the insoluble cell fraction of biomass core, g

\( M_{bs} \) : mass of the soluble cell fraction of biomass core, g

\( M_{ct} \) : total mass of \( \text{CaCO}_3 \) leached out during the leaching, g

\( M_w \) : weight loss of the coated particle after the leaching, g

\( M_m \) : mass of the coating membrane of the coated biomass particle, g

\( M_{ma} \) : mass of the additive in the coating membrane, g

\( M_{mp} \) : mass of the polymer in the coating membrane, g

\( M_t \) : total mass of the coated biomass particle, g

\( n \) : Freundlich isotherm parameter

\( N_t \) : total number of particles counted

\( q \) : solid phase concentration, mg/g

\( q_{m} \) : maximum solid phase concentration, mg/g

\( r \) : radial variable, cm

\( R \) : radius of the biomass core, cm

\( Sh_p \) : modified Sherwood number \((k_{o} 2R/\lambda D_p)\)

\( u \) : dimensionless symmetric radial variable

\( u_i \) : dimensionless symmetric radial variable vector

\( v_p \) : the total volume of immobilized biomass particles charged to the kinetic reactor, cm³

\( V \) : the total bulk solution volume in the kinetic reactor, cm³
NOMENCLATURE AND SYMBOLS (continued)

\( w \): weight of coated biomass particle, g

\( w_w \): weight of coating membrane, g

\( W \): the total mass of immobilized biomass particles charged to the kinetic reactor, g

\( x \): dimensionless solid phase concentration \((q/q_m)\)

\( x_o \): dimensionless initial solid phase concentration \((q_o/q_m)\)

\( \bar{x} \): dimensionless mean solid phase concentration

\( X \): adsorbent mass concentration \((mg/L)\)

\( y \): dimensionless solution concentration \((c/c_{bo})\)

\( y_b \): dimensionless bulk solution concentration \((c_y/c_{bo})\)

\( y_j \): dimensionless solution concentration vector

\( z \): dimensionless radial variable \((r/R)\)

Greek Letters

\( \alpha \): dimensionless Langmuir isotherm parameter

\( \lambda \): biomass core porosity

\( \rho \): apparent biomass core density, g/L

\( \rho_{ap} \): apparent particle density, g/cm\(^3\)

\( \rho_a \): coating additive density, g/cm\(^3\)

\( \rho_c \): polymer density, g/cm\(^3\)

\( \rho_m \): coating membrane density \((\rho_c \times f_c + \rho_a \times f_a)\), g/cm\(^3\)

\( \delta \): coating membrane thickness \((KR-R)\), cm

\( \sigma \): geometric standard deviation
CHAPTER ONE
INTRODUCTION

1-1 The Phenomena of Biosorption and Its Applications

The growing scarcity and increasing value of some metals, and a greater awareness of the ecological effects of toxic metals released into the environment, give significance to the study of metal accumulation by microorganisms from the viewpoints of metal recovery and removal from the aquatic environment. The ability of microorganisms to interact with and accumulate a variety of metal ions from their immediate environment has been well documented (Polikarpov, 1966; Nakajima et al., 1981). The metal uptake by microorganisms is often considerable and frequently selective. Some microorganisms have been shown to concentrate metal ions as high as 60% by dry cell weight (Brierley et al. 1985). There are, however, significant variations in metal uptake capacity among different genera, different species, and also different strains within a species.

Biosorption is the sequestering of metals by live or dead microorganisms and their derivatives. The phenomenon could take place by any of the following processes, singly or in combination;

a) Ingestion or entrapment of particulates (e.g. metal hydroxide precipitate) by flagellae or extracellular filament.

b) Active transport of metal ions across the cell membrane.

c) Cation exchange or/and complexation.

d) Adsorption or/and precipitation of adsorbed metal ions.

The first two mechanisms are associated only with metabolically active cells and the others with live or dead microorganisms as well as their cellular components. The
retained metals could be located anywhere from extracellular polymers to cytoplasmic granules depending on the uptake mechanism involved.

Biosorption of metals could find its potential applications in the following areas:

a) Recovery of metal ions from mining and milling process water

b) Decontamination of mine tailings.

c) Recovery of valuable elements from natural waters (e.g. uranium from sea water)

d) Decontamination of radioactive wastewater from processes in the nuclear fuel cycle.

Actively growing cultures as well as inactive dead microorganisms have been used to separate heavy metals from various waters. The use of live microorganisms is more compatible with processes based on large storage area or lagoons (e.g. algal blooms) which are exposed to the natural elements. However, low temperatures reduce the growth rate during winter. Metal toxicity often inhibits microbial growth. Where nutrient supplements are required, the cost of nutrients will render the use of growing culture uneconomical unless the nutrients are available as by-products (e.g. municipal activated sludge process). Finally the processing of excess microorganisms and the removal of metals from the microorganisms would be expensive.

These limitations are overcome if the microorganism production is separated from the process application. The use of by-product microorganisms (e.g. activated sludge from wastewater treatment plant or waste biomass from fermentation industries) provides an inexpensive alternative. However, other factors such as their availability, their reproducibility from batch to batch and the cost of collecting and shipping should be considered.

Another approach to the use of biosorption would be to extract microbial polymers which are often released to the culture solution by certain cells, or are present in the capsules
surrounding cells, or are cellular materials responsible for metal uptake. These compounds are very specialized and could be expensive to produce.

Native biomass is not rigid enough to be used in downflow packed bed column operations because small cells and cellular debris tend to plug the bed causing a large pressure drop. They also have low density and small size so that the flow rate will be limited in the case of upflow operation. Separation of uranium from sea water has been investigated by Tsezos and Noh (1984) using a downflow packed bed column charged with various native microorganisms. In order to obtain normal flow rate through the column, the biomass particles were mixed with a large quantity of fine silica and the mixture was then charged to the column resulting in relatively small biomass charge based on overall packing volume in the column. Loss of biomass particles from the bed was observed after the column experiment mainly because fine biomass particles were washed out by the water flow. Thus the need for immobilization of the native biomass was demonstrated for the case where the biomass needs to be applied to a packed bed column operation.

Native biosorbents could be contacted with processing waters in a CSTR (continuous stirred tank reactor) and separated in the subsequent step by filtration, centrifugation, or sedimentation. However, this approach may require a series of tanks in order to recover the desired portion of metal ions or to meet a certain effluent standard.

In the present study the inactive mycelia of *R. arrhizus* is immobilized and used as technical biosorbent which has the following properties while its biosorptive capacity is maintained:

1. particle size of immobilized *R. arrhizus* ranging from 0.5 to 1.2 mm to provide easy handling and low pressure drop in a column system.
2. good resistance to compression and abrasion.
3. high porosity and good water affinity or wetting ability to facilitate sorption kinetics.
4. low mass content of inert material for immobilization to maximize biosorption capacity of immobilized R. arrhizus.
5. strong resistance to chemicals (acids, bases, and hydrolysis, etc.).
6. favorable equilibrium and kinetics.

The immobilized biomass could be used as a technical adsorbent in the same way as granular activated carbon and ion exchange resin in packed bed operation to separate metal ions from the aqueous solutions.

1-2 Overall Objectives.

The main objective of this study is to develop and evaluate a new immobilization method for microbial biomass. The immobilized biomass thus could be used as a technical biosorbent in large scale operation. Detailed objectives are as follows;

1. To develop a suitable immobilization technique for inactive dead microbial biomass for separation of uranium from aqueous solution.
2. To investigate the effects of immobilization variables on the properties of immobilized biomass.
3. To establish a base for the physical characterization of the immobilized biomass.
4. To examine uranium uptake and elution equilibrium properties of the immobilized biomass.
5. To study uranium uptake and elution mass transfer rate in a kinetic reactor.
6. To develop a kinetic mass transfer model for the immobilized biomass in the kinetic reactor.

7. To apply the mass transfer model to kinetic data and to estimate mass transfer parameters.
CHAPTER TWO
LITERATURE SURVEY

2.1 Biosorption of Metals

Industries such as energy production, electroplating and metal finishing generate wastewaters containing various heavy metals which are potentially hazardous to the environment due to their chemical and radiological properties. In a uranium mining and milling process, for example, about 15% of radioactive material in the ore is recovered as final products and the rest is discharged resulting in potential long term environmental problems (Moffett, 1976; Goldsmith, 1976).

Conventional methods of separating metals from solutions include chemical precipitation, ion-exchange, solvent extraction, electrochemical treatment, and evaporation. Such processes may be ineffective or expensive when the initial metal concentrations are in the range of 10-100 mg/L and the discharge concentrations are required to be less than 1.0 mg/L. The use of microorganisms as adsorbents for separating heavy metals offers a potential alternative to conventional methods in decontamination and/or recovery of metals from a variety of industrial process streams.

The literature on metal uptake by microorganisms is very broad, with citations including toxicity, carcinogenicity, environmental pollution. Microorganisms which have been studied for their metal uptake capabilities can be classified as follows: mixed culture, defined culture and cellular biopolymers.
2-1-1 Mixed Culture

Activated sludge, a mixed culture of mainly bacteria and some other microorganisms, has been used to remove dissolved organic material from the municipal sewage. It has been recognized that activated sludge could accumulate heavy metals from the wastewaters. As early as 1949, Ruchhoft (1949) studied removal of plutonium from wastewater by activated sludge and observed that 90% removal efficiency could be achieved in a single stage treatment. He described the uptake of plutonium as due to the propagation of a microbial population having a gelatinous matrix with tremendous surface area for adsorption of radioactive materials.

Some of the floc forming bacteria have a characteristic growth habitat known as zoogloal or gelatinous matrix in which cells are embedded. Zoogloea-producing bacteria such as the genus Zoogloea can take up high level of metal ions of copper, cobalt, iron, and zinc in the zoogloal matrix (Friedman and Dugan, 1968). Tezuka (1969) suggested that the presence of cations such as Ca²⁺ and Mg²⁺ help the activated sludge bacteria to form flocs by ionic bridges formed between negatively charged cell surfaces and cations in solution. In their study of cadmium removal by activated sludge, Cheng and coworkers (1975) found that cadmium added to activated sludge culture in synthetic medium was rapidly adsorbed (within 10-30 min.) at pH 6.7 and similar patterns of uptake for copper, cobalt, lead, and nickel were observed. Heat-sterilization of the sludge did not significantly alter the adsorption characteristics except that a secondary slower accumulative stage in the case of nickel did not appear in the heat killed sludge. It was also noted that the presence of complexing agents such as nitrioltriacetate and ethylenediaminetetraacetic acid (EDTA) interfered with the adsorption of copper by the sludge.

Casey and Wu (1977) observed that the uptake of cadmium and uranium by activated sludge was complete within 10 minutes of addition of the metal solution and the
adsorption obeyed closely the Freundlich and Langmuir isotherm models. Tsezos (1982A) also showed that heat sterilized activated sludge could sequester uranium and thorium from solution and the Freundlich isotherm model represented the adsorption data fairly well.

Denitrifying bacteria attached to 30-60 mesh anthracite coal were used to remove uranium, radium and cesium in a liquid fluidized bed system by Strandberg and coworkers (1980,1981). They noted that the uptake of uranium by the mixed culture of actively growing denitrifying bacteria was rapid with the saturation level for the biosorbent being about 14% uranium by dry cell weight and was independent of temperature in the range of 25°C – 50°C. They also found that the reactor operated in the countercurrent mode could reduce the uranium concentration in the feed solution from 25 mg/L to 0.5 mg/L in a mean residence time of 8 minutes.

2-1-2 Defined Culture

The concept of using defined culture for separating metal ions evolved from isolating a certain microrganism from mixed culture such as activated sludge and metal polluted soil where a natural selection process favoring metal-tolerant strains has been carried out. Several microrganism isolated from activated sludge have been studied for their selective uptake of heavy metals.

Chiu (1972) isolated from a sewage a certain fungus (Penicillium strain) that could take up uranium from solution. He observed that uranium was taken up equally well by the microorganism whether it is live or dead and thereby suggested a physical-chemical mechanism of uranium retention by the cell. In their study of Z. ramigera 115 isolated from activated sludge, Friedman and Dugan (1968) examined the uptake of copper, cobalt, iron, and nickel in solution by Z. ramigera. They noted that the age of cells influenced the
adsorption capacity significantly and this appeared to be related to the extent of development of the gelatinous zoogal matrix surrounding the cells.

Rothstein and coworkers (1951) studied the inhibition of glucose metabolism in baker's yeast by uranyl ion and observed that uranyl ion formed a very stable complex with certain surface active groups of the yeast cell which are responsible of glucose metabolism. They further investigated the complex formation between uranyl ion and yeast cells in the presence of various organic complexing agents which might be attracted to the cell surface, and suggested that the polyphosphate group at the cell surface was responsible for the uranyl ion complex formation. The complex formation of other cations with the yeast cell in the presence of uranyl ion was also examined in various concentration ranges and the uranyl ion yeast cell complex was found to be selective and very strong.

The accumulation of a variety of metals including uranium and nickel in the some species of bacteria and yeast (Bacillus, Escherichia, Saccharomyces, and Canandida) was investigated by Norris and Kelly (1979) who found that the accumulation of metal ions was generally greater and more efficient in terms of gross metal removal from solution in yeasts than in bacteria. They suggested that the metal uptake by the microrganism might be due to the apparent passive retention of metal ions at cell surfaces and metabolism-dependent intracellular uptake.

In his study of the staining response of Bacillus subtilis cell wall for electron microscope, Beveridge (1978) examined the uptake of more than 40 metals by the B. subtilis and found that reactions of individual metal with the cell were specific in term of both uptake capacity and staining response.

Tsezos (1981, 1985) examined the uptake of uranium, thorium and radium by various inactive dead microorganisms including activated sludge, denitrifying bacteria and
molds, *P. chrysogenum* and *R. arrhizus*. Of all these microorganisms, *R. arrhizus* was found to exhibit much higher uptake even from very dilute solutions.

Horikoshi and his coworkers (1981) screened 52 microorganisms (10 species of bacteria, 13 actinomycetes, 11 yeast, and 18 fungi) for their uptakes of uranium from solution and found that the ability of the micrororganism to accumulate uranium from dilute uranium solution (10 ppm) are generally in the following order:

$$\text{actinomycetes} > \text{bacteria, yeast} > \text{fungi}$$

Two microorganisms, *Actinomyces levoris* and *Streptomyces viridochromogenes*, were selected for further investigation. The uptakes of uranium by these two actinomycetes were rapid (less than 10 minutes) and were affected by pH of the solution and the presence of carbonate in the solution but not by the age of the cells, temperature and the presence of metabolic inhibitors in the solution. The majority of the adsorbed uranium was released by washing the cells with EDTA. It was suggested that the accumulation of uranium in the *Actinomyces levoris* depend on physico-chemical adsorption at the cell surface and not on the biological activity. The fact that the uranium uptake by the micrororganism obeys the Freundlich isotherm model supported the suggestion.

Strandberg et al. (1980) investigated the uptake of uranium, radium, and cesium by *Saccharomyces cerevisiae*, *Pseudomonas aeruginosa*, and a mixed culture of denitrifying bacteria. They observed that uranium was accumulated slowly (hours) on the surface of *S. cerevisiae*, but in contrast, *P. aeruginosa* and the denitrifying bacteria accumulated uranium rapidly (minutes) as dense apparently random intracellular deposits. It was interesting that not all of cells possessed visible uranium deposits. Those cells which had uranium deposits (32% and 44% of 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. They noted that the uranium uptake by the micrororganism
was subject to environmental factors (i.e. temperature, pH, interfering cations and anions) and cesium and radium were concentrated to a considerably lesser extent than uranium by the microrganisms tested.

Algae and other aquatic plants have been known to bind, sediment, and localize heavy metals from natural ecosystems. Increased surface areas of small algal cells exposed to metal-contaminated streams may effectively bind vagrant metals, removing potential toxicants from solution or suspension, thereby protecting aquatic life and domestic water supplies. Becker (1983) examined the limitation of heavy metal removal from wastewater by means of algae.

Jilek et al (1978) examined the retention of uranium and radium by Aspergilli and Penicillia genus. The mycelia of P. crysogenum were stiffened by cross-linking their macromolecular structure or were entrapped within the high molecular weight polymeric matrix, and the treated mycelia were claimed to have uranium uptake capacities up to 100 mg/g from 1000 ppm solution.

Galun et al (1983) studied the uranium uptake of Penicillium digitatum and observed that uranium uptake was not changed between pH 5.5 to 7.5. Boiling pasteurization and trichloroacetic acid pretreatment of the cells did not reduce the uranium uptake by the P. digitatum but generally increased the uptake. The uranium adsorbed in the cells was effectively extracted by alkali carbonates.

The mechanism of uranium biosorption by chitin and R. arrhizus has been extensively studied by Tsezos (1982, 1983) by applying various analytical techniques such as electron microscope, infrared spectroscopy, x-ray energy dispersion analysis, mass spectroscopy, and electron paramagnetic resonance spectroscopy. It was proposed on the basis of direct and indirect experimental evidence that uranium uptake by R. arrhizus takes place through three processes as follows:
Process A — involves a complex formation between dissolved uranium ionic species and the chitin chains inside the *R. arrhizus* cell wall. Uranium coordinates to the amine nitrogen of the chitin crystallites and is retained within the cell wall of the mycelium.

Process B — involves the adsorption of additional uranium by the chitin network, close to the areas where uranium is complexed by chitin nitrogen.

Process C — involves the hydrolysis of the uranium-chitin complex formed during process A and the precipitation of the hydrolysis product (uranyl hydroxide) in the cell wall. Upon hydrolysis, the freed chitin nitrogen may re-engage in uranium complexation until the accumulation of hydrolysis product inhibits the complexation-hydrolysis-precipitation cycle, when the biosorption system arrives at final equilibrium.

The process A appears to contribute the least (<3%). The significance of process A, however, could be realized by the close relation with processes B and C. Complexation of uranium by chitin triggers process C and assists process B. On the other hand, the accumulation of the adsorbed uranium by process B affects the equilibrium of process C. Thus all three processes are important as they are interrelated and affect the overall equilibrium uranium uptake capacity of *R. arrhizus*.

In a recent study, Friis et al (1986) reported a very high uranium uptake of *S. longewood* (about 450 mg/g) at the pH between 4 and 5. It was postulated by analysing the phosphorous in the cell mass and cytoplasmic fractions that the phosphorus content of the cell be responsible for the uranium uptake and one uranyl ion was taken up by one phosphorus which forms ester linkage. However, it was not explained why the other microorganism having phosphorus content did not take up uranyl ions to the same extent. If the uranium uptake mechanism is a straight ion exchange there should be much less uranium uptake by
the bacterium in the presence of other cations such as calcium, lead and copper at high concentrations.

2-1.3 Cellular Biopolymers

There have been continuous research activities in an effort to identify and subsequently isolate cellular biopolymers which are responsible for the metal uptake from aqueous solution. The biopolymers could be extracellular polymers released into the solution, polymers present in the capsule surrounding the cell, cell wall materials, or cytoplasmic components.

Norberg and Persson (1984) investigated the uptake of metals including uranium by an extracellular polymer which was produced from glucose using the microorganism _Z. ramigera._ Over 50% of uranium by biomass weight was accumulated at pH 4 and the adsorbed uranium was released by mild acid treatment that did not affect the uptake capacity of the biomass.

Brown and Lester (1979) reviewed metal removal in the activated sludge process and noted that bacterial extracellular polymers played an important role in flocculation with help of metal cations. Extracellular polymers in activated sludge are mainly of a polysaccharide nature. Metals present in ionic form may be removed from solution by adsorption to active sites on the bacterial extracellular polymers. The researchers demonstrated that extracellular polymers, extracted from the culture of _K. aerogenes_ and activated sludge, formed complexes with metal ions including cadmium, nickel, and cobalt. Adsorption and complexation of metals with the extracellular polymers followed closely the Freundlich model (Brown and Lester, 1982A and 1982B).

Jackson and coworkers (1985) isolated from a monkey flower and a jimson weed an identified peptide which has high affinity for zinc, cadmium, mercury and copper. The com-
pound is apparently manufactured when a dormant gene is activated by the presence of a toxic metal. This boosts the production of an existing enzyme that stimulates the peptide output.

2.2 Immobilization of Microbial Biomass

The immobilization of whole cells has become of increasing interest in the fermentation industry over last decade and was reviewed by others (Chibata and Wingard, 1983; Karel et al, 1985). The advantages of such an approach are; the enzymes and cofactors in cells are maintained in their natural environment, neither extraction nor purification of the enzymes from microbial cells is necessary, and the preparation and utilization of microbial cells is easier than that of enzymes. The immobilization of cells is generally practiced by following four methods; immobilization without support, adsorption of cells onto inert medium, covalent cross-linking, and entrapment of whole cells.

2.2.1 Immobilization without Support

The first method, immobilization without support, can only be applied to a certain microbial cells which grow as strong pellets during fermentation. Some fungi including R. arrhizus form relatively strong pellets during fermentation but the pellets are not strong enough to be applied to practical operations like packed bed processes mainly because the entangled mycelia are subject to compression causing high pressure drop, and shear forces of passing fluid tend to tear off the cells from the pellet surface under normal operating conditions.

Treen (1981) applied R. arrhizus particles which were formed during fermentation as biosorbent in a packed column for separation of uranium. The uranium taken up by the R. arrhizus granules was eluted by acid solution. However, the flow rate in the packed column
was limited to superficial velocities of 0.7 mm/s owing to the compression of loosened biomass granules resulting in high pressure drop.

2-2-2 Adsorption of Cells onto Inert Medium

The second method, adsorption of cells onto inert medium, could be applied to soluble enzymes and the cells which strongly attach to a certain inert surface. This method is dependent on the strength with which the cells or the enzymes are attached to the active sites of the inert material. It is therefore very important to find the appropriate inert medium. The cells immobilized by this method could be released from the inert medium by the changes in solution pH and temperature. One major disadvantage of this method is the limited cell mass content of the final product, usually less than 20% by weight. Strandberg and Shumate (1981) attached P. aeruginosa and denitrifying bacteria onto porous anthracite and the immobilized biomass was used as biosorbent in a fluidized bed for removal of uranium from aqueous solution.

Hollo et al (1979), also immobilized P. aeruginosa onto a plastic carrier. The microbe utilized the softeners in polyvinylchloride as a carbon source and thereby penetrated into the plastic. The melt blown polypropylene has a very large surface which after plasma treatment has a large capacity for cell loading and very stable retention of the bacteria. The researchers were able to load up to 0.3 g of biomass per g of treated polypropylene.

2-2-3 Covalent Cross-linking

The third method, covalent cross-linking of cells, produces a stronger final product than the first method. Jilek et al (1978) immobilized P. chrysogenum mycelia by stiffening them with cross-linking agents. When particles of biomass immobilized by this method are subject to shear forces as in a packed column, the cells near the surface of the particle tend to
disintegrate resulting in loss of biomass and clogging of the column. One drawback of this method is that modification of the cell structure may occur during production because of solvent contact and this may in turn affect adversely the biosorptive characteristics of the biomass.

2-2-4 Entrapment of Whole Cells

The last method, entrapment of microbial cells, can be done either by fixation of cells into a cross-linked gel structure (Yasushi et al., 1979; Chibita et al., 1979), or by containment of cells within a semi-permeable membrane (Mohan and Li, 1975).

Polyacrylamide gel has been the most common immobilization medium for entrapment of microbial cells and enzymes in fermentation industries. Chibata in 1969 immobilized aminoaclase enzyme and succeeded in the first commercial production of L-amino acid using a packed column with enzyme immobilized by polyacrylamide gel. Other gel forming polymers, such as alginate, collagen, agar and carrageenan have been used for immobilization of cells and enzymes with the help of hardening agents including glutaldehyde (Chibata, 1979; Durand and Navarro, 1978).

Bihari et al. (1984) investigated the steroid transformation by immobilized fungal spores. Spores of Aspergillus ochraceus were entrapped in various polymer matrices including epoxy resin and polyacrylamide. The maximum cell content of the immobilized spores was 30% by weight and at higher contents, spores leaked out of the immobilized matrix.

The main drawback of this method is the low cell mass content of immobilized product which is normally less than 25%. Cells are often released to solution from the gel structure after use for some time.
In the pharmaceutical industry, the use of semi-permeable membranes has been common practice for masking drugs and for controlled release of drugs. The containment of cells or enzymes can be achieved either by microencapsulation or by coating.

Microcapsules, ranging in size from 0.1 μm to 5000 μm, of cells or small particles can be prepared in various ways including phase-separation, in-situ polymerization, and interfacial polymerization which have been developed over several decades in the field of surface, colloid and polymer science. The resulting microcapsule walls can be either microporous or freely permeable, semi-permeable or a consolidated impermeable barrier. A suitable microencapsulation method may be selected based on the properties of core material, size of capsules, membrane properties, and production rate (Hopfenberg, 1978; Kondo, 1979; Lim et al, 1980).

An early contribution to the development of microcapsules was made by Green and Schleicher (1957) who prepared microcapsules of dye for carbon-less papers by phase-separation. The method was based on the principle of purification of polymers. Core materials are first suspended in a polymer dissolved solvent. Temperature change or addition of nonsolvent causes the dissolved polymer to coacervate and aggregate onto the surface of the core material due to the decrease in polymer solubility, thus forming a polymeric membrane around the core. The strength of the membrane is dependent of the adhesion property of the polymer aggregates because the membrane is formed by a build-up of polymer coacervate particles (Senjikovic and Jalsenjak, 1982).

Chang et al (1967, 1971, 1984) developed an interfacial polymerization technique for preparation of microcapsules for the removal of metabolic wastes from the blood of uremic patients. They encapsulated urease and a complexing agent, e.g. zirconium phosphate, for ammonium ion inside a cationic polymer wall. When urea diffused into the microcapsules, it was hydrolyzed by the urease into ammonia and carbon dioxide and the resulting ammonium
ions combined with the complexing agent, remaining inside the microcapsules, while carbon dioxide diffused out and back to the blood. Chang (1984) also coated activated carbon with nitrocellulose and serum albumine for the removal of creatinine and various poisons from the blood.

Coating of particles is generally carried out in a rotating coating pan or by an air-suspension technique. The air-suspension coating process is considered a sophisticated variation of spray-drying and fluidized-bed technology. The process utilizes a vertical turbulent stream of air that suspends particulate entities (solid core material) in a coating chamber. The coating material, dissolved in a solvent, is sprayed onto the air-suspended particles, and the heated air drives off the solvent, thereby yielding the coated particles.

The Wurster process is of wide use to the pharmaceutical industry because the apparatus is capable of depositing a variety of coating materials on to solid core materials whose sizes range from about 50 µm in diameter to the size of pharmaceutical tablets (Wurster, 1959).

Mann (1983) developed a mathematical model to analyze the performance of coating in a modified Wurster unit. The amount of coating material deposited on the particles during the coating process was expressed in terms of the cycle time distribution of the particles and the distribution of the coating amount deposited on a particle in one cycle.

Particle attrition and breakage are the major problems associated with this type of coating equipment. The air velocity is so high as the particles travel up the center of the coating column, that collisions between particles and the wall of the column may produce chipping or excessive particle breakage. It is therefore essential that particles to be coated in the air-suspension coater be formulated for minimum friability and be strong enough to withstand the harsh coating conditions (Mathur and Epstein, 1974).
2-3 Uranium Chemistry

Uranium occurs in nature as a mixture of three isotopes (U$^{238}$, U$^{235}$ and U$^{234}$) in proportions 99.28%, 0.71% and 0.006%, respectively. In solution, uranium can be present as ions corresponding to four states of oxidation; +3, +4, +5 and +6. Hexavalent uranium is the most stable oxidation state. Uranium is a fairly strong reducing agent in general, and has a strong complex formation ability with a variety of organic and inorganic ligands. The uranyl ion, UO$_2^{2+}$, is the basic form in which U(VI) exists in solution or even in crystal lattices. It possesses a linear configuration; O = U = O. The stability of the configuration is so great that hydroxide ions cannot be displaced from the uranyl ions even by concentrated HF. Numerous oxides and hydroxides of uranium are known and UO$_2$(OH)$^+\cdot$H$_2$O is the most stable phase at 25°C.

Hydrolysis of uranium is complicated and the available information in the literature is not in complete agreement. However, below pH 2.5 uranium (VI) exists in solution exclusively in the form of the uranyl ion, UO$_2^{2+}$. At higher pH values, complex simultaneous equilibria are established. Mononuclear and polynuclear ions appear as hydrolysis products. UO$_2$(OH)$^+$ is the most probable mononuclear hydrolysis species of UO$_2^{2+}$. Two uranium atoms joined by two hydroxy bridges form the dinuclear complex ion. In the trinuclear complex ion, the uranium atoms form an equilateral triangle.

The monomer UO$_2$(OH)$^+$ tends to dimerize to (UO$_2$)$_2$(OH)$_2$$^{2+}$ and the trinuclear (UO$_2$)$_3$(OH)$_5$$^+$ is another important hydrolysis product. Other species such as (UO$_2$)$_3$(OH)$_4$$^{2+}$ and (UO$_2$)$_4$(OH)$_6$$^{2+}$ have been proposed. The hydrolysis products distribution of UO$_2^{2+}$ suggests that the uranyl ion UO$_2^{2+}$ is one of the major uranium ionic species in dilute solutions, below pH 5. The following equilibria have been suggested as describing hydrolysis of uranyl ion in non-complexing media (Palei, 1970 and Chernyaev, 1966);
\[ UO_2^{2+} + H_2O \rightleftharpoons UO_2(OH)^+ + H^+ \quad (\log k_1 = -5.8) \]

\[ 2UO_2^{2+} + 2H_2O \rightleftharpoons (UO_2)_2(OH)_2^{2+} + 2H^+ \quad (\log k_2 = -5.62) \]

\[ 3UO_2^{2+} + 5H_2O \rightleftharpoons (UO_2)_3(OH)_5^{2+} + 5H^+ \quad (\log k_3 = -15.63) \]

The equilibrium concentration of uranyl ion and hydrolysis species can be estimated by the equations and a uranium mass balance. For example, about 80% of the total U\(^{6+}\) in solution exists in the form of UO\(_2\)^{2+} at pH 4, while it drops to about 9% at pH 5. The uranyl ion is the only uranium species present at pH 2.

Biosorption of uranium is strongly dependent on the solution pH as well as the chemical species of U(VI) ions especially in the presence of other anions such as carbonate and phosphate. The chemical species of the U(VI) ions in the presence of sodium bicarbonate are of particular interest in view of its strong uranium elution property from uranium loaded R. arrhizus. Nakajima et al (1979) calculated the chemical species of U(VI) in the solution containing various concentrations of sodium bicarbonate at different pH values and they are listed in tables 2.1 and 2.2. It should be noted that UO\(_2\)^{2+} and UO\(_2\) OH\(^+\) are the main species at pH 5 while the uranyl ion is major one at pH 4. Comprehensive lists of equilibrium constants of uranyl complexes for other ions were compiled by Palei (1970) and Chernyaev (1966).
Table 2-1  Composition of the Chemical Species of U(VI) Ion in the Solution Containing Various Concentrations of NaHCO₃

<table>
<thead>
<tr>
<th>[NaHCO₃] (10⁻³ mol/L)</th>
<th>0.196</th>
<th>0.206</th>
<th>0.296</th>
<th>1.196</th>
<th>10.20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical Species</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>[UO₂²⁺]</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td>[UO₂ OH⁺]</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>[UO₂ (OH)₂]</td>
<td>0.78</td>
<td>0.78</td>
<td>0.77</td>
<td>0.34</td>
<td>0.00</td>
</tr>
<tr>
<td>[UO₂ (OH)₃⁻]</td>
<td>98.60</td>
<td>98.50</td>
<td>97.21</td>
<td>42.88</td>
<td>0.12</td>
</tr>
<tr>
<td>[UO₂ CO₃]</td>
<td>0.01</td>
<td>0.01</td>
<td>0.02</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>[UO₂ (CO₃)₂²⁻]</td>
<td>0.04</td>
<td>0.04</td>
<td>0.08</td>
<td>0.60</td>
<td>0.12</td>
</tr>
<tr>
<td>[UO₂ (CO₃)₃⁴⁻]</td>
<td>0.50</td>
<td>0.65</td>
<td>1.90</td>
<td>56.13</td>
<td>99.76</td>
</tr>
</tbody>
</table>
Table 2-2  Composition of the Chemical Species of U(VI) Ion in the Solution Containing $1.196 \times 10^{-3}$ mol/L of NaHCO$_3$ at the Various pH

<table>
<thead>
<tr>
<th>pH Values</th>
<th>Chemical Species</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>[UO$_2^{2+}$]</td>
<td>90.57%</td>
<td>40.94%</td>
<td>1.93%</td>
<td>0.00%</td>
<td>0.00%</td>
<td>0.00%</td>
</tr>
<tr>
<td></td>
<td>[UO$_2$OH$^+$]</td>
<td>9.05</td>
<td>40.94</td>
<td>19.32</td>
<td>0.61</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>[UO$_2$(OH)$_2$]</td>
<td>0.06</td>
<td>2.58</td>
<td>12.19</td>
<td>3.86</td>
<td>0.34</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>[UO$_2$(OH)$_3$]</td>
<td>0.00</td>
<td>0.32</td>
<td>15.34</td>
<td>48.64</td>
<td>42.88</td>
<td>45.42</td>
</tr>
<tr>
<td></td>
<td>[(UO$_2$)$_2$(OH)$_2^{2+}$]</td>
<td>0.00</td>
<td>1.54</td>
<td>0.34</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>[(UO$_2$)$_3$(OH)$_5$]</td>
<td>0.00</td>
<td>0.14</td>
<td>2.36</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>[UO$_2$CO$_3$]</td>
<td>0.31</td>
<td>13.54</td>
<td>45.39</td>
<td>3.65</td>
<td>0.04</td>
<td>0.00</td>
</tr>
<tr>
<td></td>
<td>[UO$_2$(CO$_3$)$_2^{2-}$]</td>
<td>0.00</td>
<td>0.00</td>
<td>2.39</td>
<td>4.87</td>
<td>0.60</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>[UO$_2$(CO$_3$)$_3^{4-}$]</td>
<td>0.00</td>
<td>0.00</td>
<td>0.74</td>
<td>38.37</td>
<td>56.13</td>
<td>54.47</td>
</tr>
</tbody>
</table>
CHAPTER THREE
DEVELOPMENT OF IMMOBILIZATION OF MICROBIAL BIOMASS

3-1 Selection of Immobilization Techniques

The first method, immobilization without support, discussed in the previous chapter, would be most ideal if the immobilized biomass is able to maintain proper mechanical strength properties over a life time of the immobilized biomass. The use of this method depends on whether cells grow in form of particles that have proper strength and size. R. arrhizus mycelia tend to form pellets when growing under certain fermentation conditions. The formation of the pellet strongly depends on agitation conditions in the fermentor as well as air-supply and nutrient composition. In a packed bed application of native R. arrhizus particles, high pressure drop, loss of biomass and limited flow rate prohibited their uses on a large scale. It is also very difficult to produce uniform biomass particles from batch to batch.

The second method, adsorption of cells on inert medium was not considered to be a suitable immobilization method because some cells, including R. arrhizus, do not strongly attach on an inert medium and the maximum biomass content is generally limited to about 30% of the inert material.

The third, covalent cross-linking, method has been used for immobilization of P. chrysogenum in order to improve mechanical strength properties of the biomass. This method involves in general several steps of chemical treatment which could impair the biosorptive property of the biomass.

The last method, entrapment of whole cells is generally achieved either by fixation of cells on a cross-linked gel structure or by containment of cells within a semipermeable membrane. Fixation of cells on gel matrix is often limited by cell loading generally being less
than 25% of gel material. At higher loading, the immobilized biomass tends to disintegrate, and the cells often leak out. Containment of cells inside a semipermeable membrane can be done by microencapsulation or by coating.

Microencapsulation is generally carried out in liquid solution and a considerable time period of contact of the cells with the solvents could impair the biosorption capacity of the cells. A rotating pan or air suspension has been a general coating method. A rotating pan is not suitable for particles less than 1 mm in diameter. Air suspension coating is extensively used for small particle coating in the pharmaceutical industry and carried out in a fluidized or spouted bed reactor. One of main advantages of the air suspension coating is the minimal solvent contact of the biomass during coating so that the biosorption property of the cells may not be affected by the solvents which are required in the polymeric film coating process.

Another merit of the coating process is the relatively easy control of the amount of the membrane material which can range from 0% to over 100% of the cell weight.

Since it was not possible to locate any literature related closely to the immobilization of whole cells which could meet the objectives of this study stated in chapter 1, it was, therefore, decided to use an air suspension coating method for the immobilization of the R. arrhizus microbial cells based on the above discussion. The spouted bed coating system was selected over the fluidized bed because of better control potential of the coating process at smaller scale.

3.2 Coating Membrane Materials

The mechanical strength of the polymeric membrane is a strong function of the chemical structure of the polymer, its molecular weight, solvents and other additives (i.e. plasticizers, swelling or pore forming agents). In general, polymers with higher crystallinity are stronger than amorphous polymers and those of higher molecular weight are stronger. A
good solvent will extend the polymer molecules to the maximum degree and hence make a stronger membrane. The strength of membrane is also closely related to its porosity which depends on polymer concentration in the coating solution, solvent evaporation rate, and the amount and types of pore forming agents. The more porous a polymer membrane is, the less strength and the higher mass transfer rate it will have. It is necessary to optimize the mechanical strength and mass transfer rate of the immobilized biomass.

Hydrolysis of polymers can affect the membrane characteristics in various ways and is closely related to the ageing of the membrane. If hydrolysis takes place rapidly, permeability of the membrane may increase owing to an increase in the number of hydrophilic hydroxyl groups. If it occurs slowly, the increase in hydrophilicity may be overshadowed by increased compression due to the fact that the hydrolysed polymer is more readily plasticized by water. Hydrolysis of the polymeric membrane generally leads to decreasing strength and gradual degradation (Kesting, 1971).

Polymers that have been extensively used in production of reverse osmosis and ultrafiltration membranes were initially selected for the coating membrane materials. The polymers tested in the preliminary study include cellulose acetate, polysulfone and polyvinylformal. Strong coating membranes around biomass particles were produced by using polysulfone. However, the polysulfone membrane cracked when the coated biomass was contacted with water mainly due to the swelling of the biomass core. Coating membranes produced by cellulose acetate maintained their integrity when contacted with water but the use of cellulose acetate membrane is limited to a narrow pH range due to its hydrolysis characteristics. Therefore, polyvinyl formal was selected for subsequent coating experiments because it produced strong hydrophilic coating membrane and is resistant to chemical degradation.
3.3 Spouted Bed Coating System

An extensive literature search on whether there were any biomass particle coating practices using a spouted bed coater did not locate any such information. It was therefore decided to design and fabricate a laboratory scale spouted bed based on the general characteristics of the spouted bed reactor. The spouted bed column used in this study evolved from various prototype columns. The design and operation of the spouted bed coating relies heavily on experience and is still considered to be an art.

Ghosh (1965) was able to obtain stable spouting motion of glass beads as fine as 80 – 100 mesh using a small spout bed (~1 cm diameter). He suggested that spouting action could be achieved for fine material as long as the gas inlet diameter exceeded 30 times the particle diameter. For a spouted bed coater, however, scaledown of the bed size is often limited by the size of the atomizer which sprays coating solution normally from the bottom of the bed. Diameter of the atomizer should be much smaller than that of the gas inlet orifice.

The orifice and column diameter ratio and the cone angle are the most important design variables in column geometry. In a given column, the maximum spoutable bed depth decreases with increasing the inlet orifice diameter (Di) until a limiting value is reached beyond which spouting no longer occurs. Becker (1961) suggested that the critical value of the inlet orifice to column diameter ratio (Di/Dc) equals 0.35, but the critical value for finer particles is considerably smaller, being 0.1 for sand of 0.6 mm diameter as reported by Németh and Pallai (1970). The lower conical section of the bed facilitates the flow of solid particles from the annulus into the gas jet region. The limiting cone angle depends to some extent on the internal friction characteristics and the density of the particles, but for most materials its value appears to be in the region of 40° (Hunt and Brennan, 1965).

During coating of particles in a spouted bed, the solvent should be dried fast in order to prevent particles from sticking together which often results in unstable spouting.
motion. In relatively shallow beds ($H/Dc < 3$), an increase in gas flow much above that required for minimum spouting causes the spout above the bed surface to lose its well-defined shape, and though the movement of particles in the region above the bed becomes more violent, the regular downward motion of particles in the annulus remains intact. In deep beds, on the other hand, the particles movement in the bed itself is disrupted at high gas flow rates. In general, spouting stability with respect to gas flow rate increases with increasing particle size, increasing column diameter, decreasing orifice to column diameter ratio and decreasing bed depth (Mathur and Epstein, 1974).

In the process of development of a spouted bed reactor, the cone angle of $40^\circ$ and column diameter of 52 mm were not varied. The inlet orifice diameter was determined by trial and error such that the stable spouting of the biomass particles could be obtained at $D/Dc = 0.144$ even with wide range of gas flow rates during the coating process.

As shown in figure 3-1, the spouted bed consists of three sections. The main body section was made of aluminium and has a $40^\circ$ angle conical base. A stainless steel screen (100 mesh) was placed at the inlet orifice with an opening for the atomizer tip. The bottom inlet section was also fabricated of aluminium and has a gas inlet port and a thermocouple port. A long round atomizer was inserted from the bottom through the hole in the bottom inlet section. The top glass column has an aluminium ring fringe at the bottom and a thermocouple port 10 cm from the top. All three sections were made concentric and could be assembled easily as shown in figure 3-2.

Due to the small size of the spouted bed coater, the atomizer must be small enough to be fit to the bottom orifice of the bed. The atomizer should be able to spray viscous polymer solutions up to 100 cP at a relatively low flow rate about 1 to 4 cm$^3$/min. without plugging the nozzle.
Figure 3.1  Spouted Bed Column (dimensions in mm)
There is no commercially available atomizer which could satisfy the requirements mentioned above. It was therefore, necessary to fabricate an atomizer with special design. A hydraulic atomizer sprays solution by means of sudden pressure release. The spray rate and the degree of atomization depend on the upstream pressure. Atomization of viscous solutions at low flow rate is very difficult to be achieved by a hydraulic atomizer. An air atomizer was therefore chosen over the hydraulic atomizer to be designed and fabricated.

Coating of *R. arrhizus* particles was conducted in a spouted bed coating system, the flow diagram of which is shown schematically in figure 3-2.

Air flow for suspension of the particles in the bed was supplied by a laboratory air tap (20 psig). The air from the tap was passed through an air filter and its flow rate was measured by a pre-calibrated rotameter. The air was then heated to required inlet temperature by a heater before it entered the bottom of the column. The air temperature was varied by adjusting the voltage to the heater via a variable transformer. Air flow for the atomizer was provided in the same way as for the suspension of particles except that the air was not heated.

Coating solution was sprayed into the column by means of an air atomizer. The coating solution in the feed bottle was continuously mixed by a magnetic stirrer and was fed into the atomizer by pressurizing the feed bottle with nitrogen at 20—40 kPa depending on the viscosity and the flow rate of the coating solution. The inlet and outlet air temperatures were monitored by a digital thermometer with a double-pen chart recorder.
CHAPTER FOUR
EXPERIMENTAL PROCEDURE

4-1 Production of Immobilized \textit{R. arrhizus} Particles

\textit{R. arrhizus} mycelia were produced using a fermentor with two 14 L jars. After fermentation, the mycelia were separated from the broth by filtration. The recovered biomass was freeze dried and ground to a certain size. Immobilization of the \textit{R. arrhizus} particles was carried out in a spouted bed coating system. The coated biomass particles were subsequently treated to leach out the pore forming additives from the coating membrane in a modified Carberry liquid reactor. Equilibrium biosorption properties of the immobilized \textit{R. arrhizus} particle (IRP) were determined by following the same procedure used for native biomass as discussed in section 4-4-1. Mechanical strength of IRP was indirectly evaluated by measuring the pressure drop in an IRP packed column under various flow conditions. The structure of the coating membrane of the IRP was examined using a scanning electron microscope equipped with x-ray analysis. The mass transfer rate of uranium in IRP was investigated by batch kinetic experiments using a modified Carberry reactor.

4-1-1 Fermentation of \textit{R. arrhizus}

A strain of \textit{R. arrhizus} was obtained from Canada Packers Ltd. (Ontario, Canada) and has been maintained in morphologically stable form on an agar slant by routine transfer every four months. On an agar slant \textit{R. arrhizus} grew as white, fine, fluffy mycelia which spreads over the surface of the agar before filling the tube. The fine spores matured into larger, darker clumps.
Fermentation of *R. arrhizus* was carried out on a glucose medium in a Microferm Fermentor (New Brunswick Scientific, New Jersey) which has two 14-L jars and a pH controller.

500 ml of inoculum broth (5 wt% of nutrient broth) was sterilized in a steam autoclave for 30 minutes at 120°C and 105 kPa. The inoculum broth in the flask was inoculated with mature *R. arrhizus* spores and split into five 100 mL quantities in 500 ml Erlenmeyer flasks. The flasks were agitated at 200 r.p.m. on a rotary shaker with 2.5 cm displacement in a constant temperature room (28°C). The cultures grew to thick clumps of *R. arrhizus* mycelia within 36 hours. 10 L of nutrient broth which includes about 100 g of calcium carbonate crystals as a buffering medium was prepared in the 14 L fermentor jar and then sterilized for 40 minutes at 105 kPa and 120°C. The inoculum was then added to the fermentor jar. Air was supplied at 5 L/minute through a single sparger just beneath the impeller and the temperature was maintained at 28°C by circulating cooling water through the baffles inside the jar.

Agitation was provided by three six-blade impellers (8 cm in diameter) attached to a vertical shaft rotating at 400-800 r.p.m. The biomass was harvested at the end of the exponential growth phase.

4-1-2 Preparation of *R. arrhizus* Particles

After the fermentation, the fermentor jar was detached and the fermentation slurry was filtered through several layers of cheese cloth. Some *R. arrhizus* mycelia grew attached to the fermentor wall and the baffles inside. In order to examine any difference in uranium uptake capacity between the biomass grown in suspension and the biomass grown attached to the surface, the latter was collected separately. The filtered suspension-grown biomass was soaked in either distilled water or tap water and refiltered five times with total
20 l of wash water until the final filtrate was clear. It was observed that there was no significant difference in uranium uptake capacity between the biomass washed using distilled water or tap water. The washed biomass was squeezed to remove additional water. The harvested biomass was then broken into small pieces which were spread over a metal tray and placed in a steam sterilizer for 30 minutes at 120°C and 105 kPa. The sterilized biomass were enmeshed through a No.10 U.S. standard sieve and lyophilized at −50°C under vacuum for 36 hours in a freeze dryer (LABCONCO).

The freeze dried biomass was hard and light brown in colour. It was further carefully ground by mortar and pestle to the required particle sizes. The ground biomass was placed on a stack of U.S. standard sieves which were shaken for 15 minutes in a vibrator (SYNTRON). Biomass granules passing No.14 U.S. sieve but retained in No. 30 U.S. sieve were collected for immobilization experiments. Some of the CaCO₃ crystals in the nutrient broth were trapped within the biomass particles during the fermentation and were not removed during the harvest and granulation process.

4-1-3 Coating of R. arrhizus Particles

Coating of R. arrhizus particles was conducted in the spouted bed coating system described in section 3-3. Coating solutions were prepared one day before being used. In case of calcium carbonate additive formulation, a known amount of polymer was dissolved in 200 ml of solvent in a 500 ml Erlenmeyer flask. A homogeneous polymer solution was generally obtained within a day under good mixing conditions using a magnetic stirrer. A weighed amount of calcium carbonate was added to the polymer solution with good mixing and the mixture was further mixed for a day until the solution was used. However, in the case of glucose additive formulation, a known amount of glucose particles was added to about 200 ml of solvent in a 500 ml polypropylene bottle along with ceramic balls. The bottle was rolled for
two days in a mill in order to break down the glucose particles to smaller particles. After two days of milling, the solution containing fine glucose particles was separated from the ceramic balls. A known amount of polymer was added to the glucose-solvent solution with agitation and the mixture was mixed for about two days until it was being used for coating.

About 40 g of R. arrhizus particles was weighed and kept in an oven at the same temperature as would be used during the coating experiment in order to avoid heat loss due to warming up the particles in the coating bed. Approximately 130 mL of the coating solution was added to the coating feed bottle which has graduated volume calibration lines. The initial volume of the coating solution was recorded. The coating solution was kept well mixed by a magnetic stirrer throughout the coating experiment.

The spouted bed column was assembled and the atomizer was placed in position. The air heater was turned on and the suspension air flow started at about 600 cm³/s. The atomizer air flow was also initiated at a flow rate of 86.3 cm³/s which allowed good atomization of the polymer solutions used. The air flow rate calibration curves for the suspension and atomizer rotameters are given in appendices A-1 and A-2 respectively. The inlet and outlet air temperatures were monitored by the chart recorder. The inlet air temperature could be varied by regulating the voltage to the air heater with a variable transformer. The outlet air temperature typically reached a steady level in about 30 minutes after which the spouted bed body was warmed up and thermally equilibrated. In most cases, there was about 15°C temperature drop between the air inlet and outlet temperatures due to the heat loss to the environment. The main aluminium body of the spouted bed was insulated with a glass fiber mat but the top glass column was not insulated in order to observe the particle movement during the coating. Once the outlet temperature reached a steady state, both atomizer and suspension air flows were reduced and the biomass particles were poured into the bed from the top of the glass column. The atomizer air flow rate was then increased.
back to the previous level and the suspension air flow gradually increased to a level at which a stable spouting movement of the particles could be achieved. Immediately after the suspension air flow was set, the coating solution was sprayed to the particles by pressurizing the coating solution feed bottle at about 20 kPa with nitrogen gas. The coating solution flow rate could be varied by changing the nitrogen pressure applied to the feed bottle. Since particle attrition was encountered when the particles collided with each other during suspension, it was necessary to spray the coating solution as soon as possible. It took in most cases about 30 seconds to carry out these steps. Some broken biomass fines were observed to be carried away out of the glass column with the air flow during this period. Once the coating solution was sprayed onto the spouting particles the particle attrition was no longer observed and the outlet air temperature declined sharply because heat was removed from the hot air by evaporation of the solvent of the coating solution. The outlet air temperature was stabilized to reach a steady state in about 2 minutes. The spouting action of the particles was visually observed and any instability of the coating process could be detected by variation of the outlet air temperature. As coating of the particles progressed, the particles became heavier with coating membrane build-up. The suspension air flow rate was required to be gradually increased to maintain stable spouting movement of the particles. The overall coating period ranged from 10 to 60 minutes depending on the operating variables including particle charge, spray rate, and the total coating solution volume sprayed.

When a required volume of the coating solution had been sprayed, the vent line in the feed bottle was opened and the coating solution flow stopped. The outlet air temperature rose sharply immediately after terminating the coating solution flow and reached a steady state in about 2 to 3 minutes during which the remaining solvent in the coating membrane was driven off. At the end of the drying period, both suspension and atomizer air flows were stopped and the coated particles were recovered and weighed. The remaining volume of the
coating solution was also recorded. The coated particles were sieved and used for leaching experiments.

4-1-4 Leaching of Additives from the Coating Membrane

Leaching of additives from the membrane of the coated biomass was carried out in a modified liquid Carberry reactor as shown in figure 4-1. The main body of the reactor was fabricated with transparent acrylic plastic. Two plastic columns of different sizes were concentrically glued to the base of the reactor, and a cover lid was attached to the main body by butterfly-nuts. There were several ports in the lid including a center hole for the impeller shaft. A pH electrode, a thermocouple and a sampling tube were inserted to the reactor through the ports in the cover lid. The cooling water was circulated through the jacket formed in the annulus of the two columns. The reactor was supported on an aluminum frame.

An air motor (Fawcett Co. Inc. model 105A) was installed to provide necessary agitation in the reactor through a stainless steel shaft to which two basket impellers were attached. The baskets were made of two 100 mesh screened disks joined by threads to provide easy loading and unloading of the coated biomass and the solution could easily pass through the basket to permit rapid mass transfer between the particle surface and the bulk solution.

A copper-constantan thermocouple was placed about 30 mm above the basket impeller to measure the solution temperature in the reactor. The signal from the thermocouple passed to a chart recorder via a digital thermometer (Omega model 115 TC) and the temperature in the reactor was continuously monitored during the experiment.

A combination pH electrode (CanLab H5503-20) was inserted to the reactor and the solution pH was continuously monitored by a pH meter (Orion model 601 A) with a chart recorder. In some experiments, the solution pH was controlled manually by adding HCl solution.
Figure 4.1  Schematics of Leaching Kinetic Experiment
Sampling of the solution was done by a 10 mL disposable syringe attached to a 1.5 mm stainless steel tube whose end extended to 30 mm above the basket impeller. 1 mL to 6 mL of samples were collected from the reactor during periods of 5 to 10 seconds. Sampled solutions were analysed as described in section 4.2.

Heat generated by the agitation in the reactor was removed to maintain constant solution temperature by passing cold tap water (10 to 15°C) through the reactor jacket. The cooling water flow rate was adjusted such that the temperature of the solution could be maintained at 23 ± 1°C.

A general leaching experimental procedure is as follows;

1. Known amounts of coated biomass samples were placed in each of the baskets on the shaft.

2. The reactor was filled with 2000 mL of leaching solution which had been thermally equilibrated at room temperature (23 ± 1°C) and the cover lid was placed on the reactor along with the shaft, pH electrode, the thermocouple and the sampling tube.

3. Agitation was then initiated by opening the air valve and the agitation speed was set by adjusting the inlet air pressure to the air motor.

4. Sampling of the solution was conducted at the predetermined time intervals following the start of agitation.

5. The solution pH and the temperature in the reactor were continuously monitored during the experiment by the chart recorders.

6. Cooling water flow was initiated as the temperature in the reactor increased and adjusted manually to maintain the solution temperature at 23 ± 1°C.
7. After the last sample was withdrawn, the air supply valve was closed and the cooling water flow stopped and the chart recorders were turned off.

8. The cover lid was then dismantled and the leaching solution was filtered through a 0.45 μm membrane filter by a vacuum filtration in order to measure the suspended solid concentration of the solution.

4-2 Analytical Techniques

A spectrophotometric method developed by Marconko (1978) has been used for the determination of uranium concentration in solution. The reagent Arsenazo III (1,8-dihydroxynaphthalene-3, 6-disulphonic acid - 2,7-bis(azo) 2-phenylarsonic acid) forms 1:1 colour complex with uranium. The detection limit of the colour reaction is high (0.05 to 0.01 μg/ml of the element) when a spectrophotometer or a photocolorimeter is used. The colour complexes formed by Arsenazo III with U have a molar absorptivity of $1.27 \times 10^{5}$ liters/mol/cm at $\lambda = 665$ nm. The following procedure was adopted to determine uranium content in solution. A uranium stock solution was prepared by dissolving uranium nitrate crystals ($\text{UO}_2(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O} = \text{M.W. 502.13, BDH Chemicals Ltd.}$) using a pH 4 potassium bipthalate buffer solution which apparently did not interfere the uranium analysis and biosorption (Tsezos 1980). The sample solution containing no more than 80 μg of uranium was transferred to a 25 ml test tube and mixed with approximately 10 ml of 4 M HCl solution. Four pellets of zinc were added and allowed to reduce U(+6) to U(+4) for no more than 15 minutes. The solution was then transferred to a 50 mL volumetric flask using a glass funnel, and the zinc pellets still remaining in the funnel were rinsed with 4M HCl solution. 2.5 ml of freshly prepared 0.05% Arsenazo III (Aldrich Chemical Co. Inc. A9277-5) was added to the flask and the solution was diluted to the volume (50 mL) with 4 M HCl solution. The colour appeared instantaneously and remained stable for at least two hours. The absorbance was
measured at $\lambda = 665$ nm using a 1 cm path length quartz cell and a Spectronic 21 (Bausch and Lomb) spectrophotometer. The absorbance depends on the acidity of the medium but is constant over the acidity range of 4M to 8M HCl solution. Uranium content was calculated from a calibration curve which was determined by using standard uranium solutions which were subjected to the same procedures as above.

The uranium concentrations of selected liquid and solid samples were also determined by delayed neutron counting. Liquid or solid samples were prepared according to the specifications suggested by the Nuclear Activation Service (NAS) at McMaster University. The samples were sent to NAS for uranium analysis. Detailed information on the delayed neutron counting system at the NAS was published by Hoffman and Ernst (1982). Since the U$^{235}$ of uranium nitrate crystals obtained from BDH Chemicals Ltd. was partially depleted, it was necessary to determine a correction factor for accurate analysis of uranium. 10 samples of 1 ml uranium solution with 100.97 mgU/L were sent to the NAS and their uranium concentrations were determined based on U$^{235}$. The measured uranium concentration has a mean value of 54.14 mgU/L with relative error of 2.567%. The correction factor was calculated to be 1.865 and used to correct the uranium concentrations measured by NAS. The uranium solutions have been made of the uranium nitrate crystals from a same bottle throughout the experiments in order to use the same correction factor.

Calcium carbonate used as a pore forming additive was leached out from the coating membrane during leaching kinetic experiments. The amount of calcium ions in the leaching solution was determined by the ethylenediaminetetraacetic acid (EDTA) titration method (Standard Method, 16th Ed.).

According to this method, 5 mL of sample solution were added to 50 mL of distilled and deionized water (D.D.W.) in a 250 mL of Erlenmeyer flask and 1 mL of pH 12 buffer solution was also added. Addition of a small amount of dry powder indicator (Eriochrome T)
turned the solution wine red. Standardized EDTA titrant was slowly introduced to the solution with stirring until the solution turned to blue with marking the end point of the titration. The volume of the EDTA titrant was recorded and the concentration of calcium ion in the solution was calculated.

The glucose pore forming additive was leached out from the coating membrane using a batch kinetic reactor and the glucose concentration of the leaching solution was determined by the alkaline copper reduction method developed by Asatór and King (1954).

According to this method, 0.5 mL of a sample and 4.0 mL of isotonic sodium sulphate-copper sulphate solution were added to a 25 mL test tube and the solution was well mixed. 1.0 mL of the solution was pipetted to 1 mL of alkaline tartrate solution in a 25 mL test tube. A few glass marbles were put into the test tube and it was placed in a boiling water bath for 10 minutes. The test tube was then cooled to room temperature. 3 mL of phosphomolybdic acid solution and 3 mL of D.D.W. were added to the solution in the test tube. After 5 minutes, the absorbence of the sample was measured at λ = 630 by a spectrophotometer using a quartz tube with 1 cm path length. The glucose concentration was determined by a calibration curve prepared by following the same steps mentioned above with the glucose solutions whose concentrations were known.

4-3 Physical Characterization of Immobilized R. arrhizus
4-3-1 Particle Size Determination

The biomass particles were classified by sieving and their size distribution was analysed using a Zeiss particle size analyser.

About 100 g of biomass particles were placed on the top sieve of a stack of 4 U.S. standard sieves (Nos. 10, 12, 14, 30) with a top cover lid and a bottom tray. The stack of sieves
was shaken in a vibrator (SYNTRON) for 15 minutes. The biomass particles passing No. 14 sieve but retained in No. 30 sieve were collected for immobilization experiments.

The same sieving method was also employed to classify the immobilized biomass particles after the coating process. All the immobilized biomass particles were placed on the top sieve of a stack of 6 U.S. standard sieves (Nos. 12, 14, 16, 20, 25, 30) when a batch of coating was finished. The stack of sieves was then shaken for 15 minutes using the vibrator. The weights of immobilized biomass particles retained in each sieve were recorded.

A Zeiss particle size analyzer was used to determine the size distribution of immobilized biomass particles. A known amount of immobilized biomass particles was sprayed over a flat black felt at the bottom of a photo copy stand. Illumination was provided with two side lights at 45° angle in order to eliminate shadows of particles. A scale was placed on the felt to measure the particle size with proper magnification. Pictures were taken about 50 cm away from the particles and the film was processed and printed at about 3 times magnification of actual particle size.

There were some changes in size when the immobilized biomass particles were saturated with water. This effect was studied by suspending the immobilized biomass particles in a tray which was painted black inside. After the immobilized biomass particles were saturated with water and settled to the bottom of the tray, pictures were taken using a polarizing filter to reduce the glare caused by reflection from water.

The size distribution of the particles was determined from the pictures by means of a Zeiss particle size analyzer.

4-3-2 Microscopic Examination of Immobilized R. arrhizus

The coating membrane structure of the immobilized R. arrhizus was examined by a scanning electron microscope.
For the examination of the membrane surface structure, the immobilized \textit{R. arrhizus} particles were mounted on an aluminium stub with double-sided tape and grounded with a silver paint. The particles on the stub were sputter-coated with gold for 120 seconds in a cool sputter coater (Polaron E5100). The specimen was then viewed in the secondary electron image mode by a ISI-DS-130 Scanning Electron Microscope (SEM) at 15 kV of accelerating voltage.

In order to look at the cross-sectional view of the immobilized \textit{R. arrhizus}, the immobilized \textit{R. arrhizus} particles were embedded in a 5-minute epoxy resin forming about 150 mm long stick and the stick was hardened over night. The sample was placed into a liquid nitrogen bath for 3 minutes and snap-fractured in the bath. The fractured pieces were mounted on an aluminium stub and coated as described above. The specimen was then examined for the structure of the membrane cross section and the core of the particle with help of the SEM as described above.

Elements in the immobilized \textit{R. arrhizus} were determined by X-ray energy dispersion analysis. The specimen used for the cross sectional view was analysed by a PGT SYSTEM 4 with silicon detector having a window at 15 to 25 kV of accelerating voltages.

4-3-3 Pressure Drop in an Immobilized \textit{R. arrhizus} Packed Column

Figure 4-2 shows a schematic flow sheet of the packed bed column system. The column was fabricated with transparent acrylic plastic tube having 25.4 mm inside diameter with 6 mm thick wall. The column consisted of three sections, the main section 230 mm long and the top and bottom headers, 30 mm long each. Both headers could be bolted to the main section. Stainless steel screens (100 mesh) were placed between the headers and the main section to support the packing materials. The header sections were filled with glass balls of 1.5 mm diameter in order to ensure uniform radial distribution of the water flow in the
Figure 4.2 Schematics of a Packed Bed Column System
column. Two pressure taps, one positioned in the middle of column from the top header and another just below of the bottom screen from the bottom header, were connected to a differential pressure transducer (CELESCO 0.5 + PSID). The signal from the transducer was passed to a chart recorder via a demodulator.

Cold tap water (10-15°C) was passed through a heat exchanger to increase the temperature close to 23±1°C (room temperature) and introduced to the feed bottle (4 l) which had an overflow port and a bottom port. Heating of the cold water was needed to drive off the dissolved air in the water which would otherwise come out of solution later in the column section as the water warmed up. Air bubbles trapped between the particles can cause erroneous pressure drop readings.

A gear pump (Zenith Product Co. Newton, Mass.) was used to pump the water to the column. The water in the feed bottle was pumped to a surge tank which dampened the water flow pulsations generated by the pump and the water was then introduced to the column via a pre-calibrated rotameter by which the water flow rate was determined. The calibration curve for the rotameter is given in appendix A-3. The pressure transducer was calibrated by applying different water heads to each pressure taps. The differences in pressure between the two taps were measured in terms of water head and the chart recorder was calibrated accordingly.

The operating procedure for pressure drop measurement was as follows;

1. A known amount of immobilized biomass particles (10-15 g) was saturated with 200 ml of water in a 500 ml flask.
2. The bottom header was assembled to the main section and the slurry of immobilized biomass particles in the flask was poured into the column.
3. The top header was attached to the main section and the bottom and the top water lines were connected.
4. Water was pumped to the bottom of the column and the particles were suspended for about 30 minutes during which occasional tapping of the column was applied to remove any air bubbles entrapped between particles.

5. When there were no air bubbles present in the column, the vent knobs of the transducer were unscrewed with the water still running in the column and air pockets in the tap lines and inside the transducer were eliminated.

6. The water flow was then stopped and the column was gently tapped until the packing surface did not go down further. The height of the packing was recorded.

7. Then the water flow was introduced to the top of the column at a constant rate. The pressure drop in the column was continuously monitored by the chart recorder.

8. When one set of pressure drop measurements was finished, the immobilized biomass particles were recovered by filtration and dried to constant weight at room temperature. The weight loss of immobilized biomass particles after the experiment was recorded.

4.4 Mass Transfer Studies

4.4.1 Uranium Biosorption and Desorption Equilibria

The uranium biosorption isotherms were obtained by contacting the biomass with uranium solution (nitrate salt) at room temperature (23 ± 1°C).

Varying dosages of the biomass were added to 250 ml glass flasks containing 100 ml of the same uranium solution. The flasks were then shaken at 200 r.p.m. on a rotary shaker (LAB-LINE INSTRUMENTS INC.) to provide necessary mixing. The biomass was
separated from the solution by vacuum filtration through a 0.45 µm membrane filter (Millipore) at the end of necessary contact period.

The uranium concentration and the pH of the filtrate was determined. It was reported that the uranium solution pH increased substantially after contact of the *R. arrhizus* with uranium solution when the solution was prepared without a proper buffer solutions. The uranium solution was therefore made up in a pH 4 buffer solution of potassium biphthalate (20% V/V 0.05 M COOH₆H₄ COOK) which was known not to interfere with uranium biosorption of *R. arrhizus* and the Arsenazo III test (Tsezos, 1981). 18 hours of equilibrium contact time was sufficient enough to ensure equilibrium for the *R. arrhizus* powder. In case of immobilized biomass particles, however, the equilibrium contact time which strongly depends on the wall membrane properties such as porosity and thickness, had to be determined by batch kinetic experiments.

The desorption isotherms were carried out by contacting uranium loaded biomass with the pH 4 buffer solution. After filtration as in the biosorption experiment, the uranium loaded biomass was recovered and dried to constant weight at room temperature. The same procedure employed in the biosorption experiment was applied with the uranium loaded biomass except that the pH 4 solution without uranium was used as contact solution.

4.4.2 Uranium Uptake and Elution Kinetics

The same liquid Carberry reactor system was employed for the batch uranium uptake and elution kinetic experiments with a slight modification of the experimental procedures, as follows.

250 ml of uranium solution was prepared as described in the previous section. In the batch kinetic experiment, the reactor was filled with 1500 ml of pH buffer solution and the cover lid was assembled as before with a known amount of immobilized biomass particles in
each basket. The immobilized biomass particles were contacted with the solution for 24 hours to drive off any air inside the immobilized biomass particles. Occasional rotation of the shaft was provided to remove air bubbles formed in the baskets. After the presaturation period, the 250 ml of concentrated uranium solution was poured into the reactor and the flask was rinsed out into the reactor with 250 ml of the buffer solution making up the total initial volume 2000 ml.

Immediately after the agitation began, the samples were taken at predetermined time intervals. Temperature and pH of the uranium solution were monitored during the kinetic experiment. The uranium loaded immobilized biomass particles were recovered at end of the kinetic experiment and dried to constant weight and the changes in the weight of the immobilized biomass particles were recorded.

Elution kinetic experiments was conducted by following the same procedure used in the leaching kinetic experiments.
CHAPTER FIVE
MASS TRANSFER MODELLING

5.1 Equilibrium Isotherm Models

The adsorption isotherm is the equilibrium relationship between the concentration of the adsorbing species (adsorbate) in the fluid phase and the concentration of the same adsorbate in the adsorbent particles at a given temperature. For liquids, the concentration is often expressed in mass units such as mg/L. The concentration for the adsorbate on the solid is given as mass adsorbed per unit mass of original adsorbent.

Some common isotherm shapes for aqueous systems are shown in figure 5.1. For a linear isotherm which is the boundary between favourable and unfavourable isotherms, the amount adsorbed is proportional to the concentration in the fluid. Isotherms that are convex upward are called favourable, because a relatively high solid loading can be obtained at low concentration in the fluid and in the column adsorption operation they produce a sharp adsorption zone. The limiting case of a strong favourable isotherm is irreversible adsorption, where the amount adsorbed is independent of concentration down to very low values. An isotherm that is concave upward is called unfavourable because relatively low solid loadings are obtained at low concentration range. It leads to a continually expanding adsorption zone in the column operation and thus utilizes inefficiently the adsorption capacity of the adsorbate.

There are many mathematical expressions to describe the different isotherms. The Langmuir and Freundlich isotherms are the most commonly used equations to represent the isotherm data for liquids.
Figure 5.1  Common Isotherm Shapes for Aqueous System
The Langmuir isotherm is derived based on the assumptions that maximum adsorption corresponds to a saturated monolayer of solute molecules on the adsorbent surface, that the energy of adsorption is constant for all the adsorption sites, and that there is no transmigration of adsorbates in the plane of the surface. The Langmuir isotherm model has the following form:

$$q = \frac{q_m bc}{1 + bc}$$  \( (5.1) \)

As long as its restrictions and limitations are clearly recognized, the Langmuir equation can be used for describing equilibrium relationship for adsorption systems and for providing parameters \( q_m \) and \( b \) with which to quantitatively compare adsorption behavior in different adsorbate-adsorbent systems or for varied conditions within any given system. However, even when adsorbate-adsorbent systems are described by the model, it does not necessarily mean that the adsorption mechanisms involved are those described by the model.

Two convenient linear forms of the Langmuir isotherm to estimate the parameters are as follows:

$$\frac{c}{q} = \frac{1}{b q_m} + \frac{c}{q_m}$$ \( (5.2) \)

$$\frac{1}{q} = \frac{1}{q_m} + \frac{1}{b q_m} \frac{1}{c}$$ \( (5.3) \)

The Freundlich isotherm is basically empirical but is often useful as means of data description. The isotherm generally agrees quite well with the Langmuir model and adsorption data over moderate ranges of concentration. The Freundlich equation is given as follows:

$$q = K c^n$$ \( (5.4) \)

The conventional way of estimating the parameters, \( q_m, b, K, \) and \( n \), was to use the linearized isotherms with least-square analysis of adsorption data. When the conventional
method was applied to evaluate these parameters, the loading, \( q \), is generally considered as the dependent variable and the equilibrium concentration, \( c \), as the independent variable. However, the term, \( q \) is a calculated value that depends on both \( c \) and \( X \) because the calculation of \( q \) is normally done by the following equation;

\[
q = \frac{c_0 - c_e}{X}
\]  

(5.5)

where \( c_0 \) is initial solute concentration, \( c_e \) equilibrium concentration, and \( X \) is adsorbent concentration. Therefore, the isotherm equations should be fitted to the experimental data in such a way that allows the concentration, \( c_e \), to be a function of the adsorbent concentration, \( X \), as Sweeney et al (1982) suggested. The substitution of equation 6 to equations 1 and 4 yields

\[
c^2 = \left( \frac{1}{b} - c_0 + q_m X \right) c - \frac{1}{b} c_0 = 0
\]  

(5.6)

\[
(c_0 - c) + X K c^3 = 0
\]  

(5.7)

The parameters could be estimated by solving the equations with a nonlinear regression analysis package.

When an isotherm model is applied to dimensionless transport equations, it is often necessary to transform the isotherm equation to a dimensionless form in the same way used in the transport equations. The Langmuir and Freundlich isotherm equations can be transformed to dimensionless forms as suggested by Neretnieks (1976).

For a given adsorption system, there will be an initial maximum liquid phase concentration used \( (c_0) \), and a corresponding maximum solid phase concentration \( (q_m) \) at the \( c_0 \). Then the Langmuir and Freundlich isotherms can be defined as follows;

\[
q_y = \frac{q y}{(1 + (\alpha - 1)y)}
\]  

(5.8)

and

\[
x = y^n
\]  

(5.9)

where
\[ x = \frac{q}{q_m}, \quad y = \frac{c}{c_o} \]

The parameter, \( n \), in the dimensionless Langmuir isotherm will be dependent on the concentration range used for a given system, whereas the parameter, \( m \), in the dimensionless Freundlich equation is independent of the concentration range. The concentration range of the dimensionless isotherms is always between 0 and 1 so that comparison of the isotherms for different systems can be simplified.

Figures 5-2 and 5-3 show isotherm curves for the dimensionless Langmuir and Freundlich models for various values of \( n \) and \( m \), respectively. Two limiting cases should be noted: one is the linear isotherm where \( n = m = 1 \) and another is the saturation isotherm where \( n = \infty \) and \( m = 0 \).

5.2 Batch Kinetic Modeling

When an immobilized biomass particle is suspended in a solution containing a solute, there are essentially four consecutive mass transport steps associated with the adsorption of the solute from the solution. As shown in figure 5-4, the first step, bulk transport of solute in the solution phase, is usually rapid because of mixing and convective flow. The second step, film transport, involves diffusion of the solute through a hypothetical "film" or hydrodynamic boundary layer. The third step, coating membrane transport, involves diffusion of the solute through the coating membrane which does not adsorb the solute as will be discussed in section 6.5.2. Except for a small amount of adsorption that occurs on the exterior of the biomass core, the solute then must diffuse within the pore volume of the biomass core. The actual adsorption of solute on the active sites of the biomass is generally considered to be very rapid, equivalent to an equilibrium reaction and thus insignificant in the context of overall adsorption rate. Film, coating membrane and intraparticle transports are thus the major factors controlling the rate of adsorption from the
Figure 5.2  Dimensionless Langmuir Isotherm Curves for Different Values of α
Figure 5.3  Dimensionless Freundlich Isotherm Curves for Different Values of n
Figure 5.4  Mass Transfer Model Schematic
solution. Because they act in series, the slowest of the three steps will be rate limiting; if the steps are comparable in rate, control might also be distributed between the steps.

External diffusion or film transport controls the transfer of solute from bulk solution through the boundary layer of fluid immediately adjacent to the external surface of the immobilized biomass particle. Film transport is governed by molecular diffusion and, in case of turbulent flow, by eddy mass diffusion, which controls the effective thickness of the boundary layer. The method used for contacting the immobilized biomass particles with a solution, together with the hydrodynamic and operational details of a particular system, control and define the magnitude of the external mass transfer coefficient.

Coating membrane transport controls the transfer of solute through the pores of the membrane. The molecular diffusivity of solute and the membrane porosity and thickness are the main factors to determine the solute transport through the membrane. In case of a non-porous membrane, the partition of solute between external membrane surface and adjacent solution, and the partition of solute between internal membrane surface and external biomass core should be considered to estimate the effective concentration driving force.

Intraparticle transport considers the solute diffusing within intraparticle pore voids to the cell wall of the biomass. The solute must move to the active sites which are located within the cell wall of R. arrhizus after being adsorbed on the cell wall surface.

In batch kinetic modeling, the immobilized biomass particles are assumed to have the following properties:

1. a uniform particle size having a spherical geometry
2. a uniform core material
3. a uniform coating membrane which is permeable to the adsorbate but has no accumulation of the adsorbate
The following additional assumptions are made for the mass transfer system in the batch reactor:

4. The bulk concentration of the solute is uniform due to high agitation in the kinetic reactor.

5. Local equilibrium exists between the entire pore solution and the adjacent biomass as well as between the internal coating membrane and the external biomass core.

6. Intraparticle mass transfer is due mainly to pore diffusion with constant diffusivity.

7. Accumulation of the pore liquid is negligible compared to the accumulation in the adsorbed phase (this assumption is valid because of the large uptake capacity).

8. Diffusion of the solute adsorbed on the cell wall surface to the active site of the cell wall is rapid compared to the pore diffusion of the solute. It was shown that the uranium was rapidly deposited on the cell wall of \textit{R. arrhizus} from uranium solution (Tsezos, 1981).

9. Biosorption rate at the active sites of immobilized biomass particle is much more rapid than mass transfer rate to the sites.

Mass balance of a solute in the biomass core of immobilized biomass particle is given as follows on the basis of Fick's law

$$\lambda \frac{\partial c}{\partial t} + \rho \frac{\partial q}{\partial t} = D_p \lambda \left( \frac{\partial^2 c}{\partial r^2} + \frac{2}{r} \frac{\partial c}{\partial r} \right)$$

The boundary condition at the center of the biomass core is

$$\frac{\partial c}{\partial r} = 0 \quad \text{at} \quad r = 0$$

Mass balance of the solute across the coating membrane is given by
\[
\frac{4nKR^2 D_m}{\delta} (c_{m,KR} - c_{m,R}) = 4nR^2 \rho \lambda \frac{\partial c}{\partial r} \bigg|_{r=R}
\]

Mass balance of the solute in the external fluid film is given by:

\[
k_f (c_b - c_{b,KR}) = \frac{D_m}{\delta} (c_{m,KR} - c_{m,R})
\]

where:
- \( c \): solute concentration in the pore of the biomass core (mg/L)
- \( c_m \): solute concentration in the coating membrane, (mg/L)
- \( c_b \): solute concentration in the bulk solution, (mg/L)
- \( r \): radial variable in the biomass core (cm)
- \( R \): radius of the biomass core (cm)
- \( KR \): radius of immobilized biomass particle (cm)
- \( q \): solute concentration in the biomass (mg/g)
- \( \lambda \): apparent biomass core porosity
- \( D_p \): effective solute diffusivity in the pore of the biomass core (cm²/s)
- \( \rho \): apparent biomass core density (g/L)
- \( D_m \): effective solute diffusivity in the coating membrane (cm²/s)
- \( \delta \): coating membrane thickness (KR - R), (cm)
- \( k_f \): external fluid film mass transfer coefficient, (cm/s)

Partition coefficients of the solute at the interface of the bulk solution and external coating membrane surface, and at the interface of the biomass core and inner coating membrane are defined as follows:

\[
K_m = \frac{c_{m,KR}}{c_{b,KR}}
\]

\[
K_p = \frac{c_{m,R}}{c_{r=R}}
\]

Equations 5.12 and 5.13 become by employing \( K_m \) and \( K_p \)
\[
\frac{4\pi(KR)^2 D_m}{\delta} (K_m c_{b,KR} - K_P c_{r=R}) = 4 \pi R^2 D_p \lambda \frac{\partial c}{\partial r} \bigg|_{r=R}
\]  
(5.16)

and

\[
k_f (c_b - c_{b,KR}) = \frac{D_m}{\delta} (K_m c_{b,KR} - K_P c_{r=R})
\]  
(5.17)

Rearrangement of the equation 5.17 gives

\[
c_{b,KR} = \frac{k_f c_b + \frac{D_m}{\delta} K_P c_{r=R}}{K_m \frac{D_m}{\delta} + k_f}
\]  
(5.18)

Substitution of \(c_{b,KR}\) to the equation 5.16 and rearrangement give if both partition coefficients were assumed to be one in this case due to the high porosity of coating membrane,

\[
4\pi(KR)^2 k_o (c_b - c_{r=R}) = 4 \pi R^2 D_p \lambda \frac{\partial c}{\partial r} \bigg|_{r=R}
\]  
(5.19)

where

\[
k_o = \frac{1}{\left( \frac{1}{k_f} + \frac{\delta}{D_m} \right)}
\]

If the coating membrane thickness is assumed to be relatively small compared to the biomass core radius, the equation 5.19 can be further simplified as follows;

\[
k_o (c_b - c_{r=R}) = D_P \lambda \frac{\partial c}{\partial r} \bigg|_{r=R}
\]  
(5.20)

The following dimensionless variables are defined to convert the above equations into the dimensionless forms;

\[
y = \frac{c}{c_{bo}} \quad x = \frac{q}{q_m} \quad z = \frac{r}{R} \quad y_b = \frac{c_b}{c_{bo}}
\]

where \(c_{bo}\): initial bulk concentration (same as co-defined in equation 5.9)

\(q_m\): maximum equilibrium solute concentration in the biomass at the initial bulk concentration
R: Radius of the biomass core

Applying the dimensionless variables to the equation 5.10 gives

\[
\frac{\partial y}{\partial t} = \frac{\lambda D_p n y^{n-1}}{n y^{n-1} \left[ \lambda + \left( \frac{\rho q_m}{c_{bo}} \right) n y^{n-1} \right]} \left( \frac{\partial^2 y}{\partial z^2} + \frac{2}{z} \frac{\partial y}{\partial z} \right)
\]

(5.21)

Equation 5.9, the dimensionless Freundlich isotherm equation, is used to express \( x \) in terms of \( y \).

Since the term,

\[
\left( \frac{\rho q_m}{c_{bo}} \right) n y^{n-1} >> \lambda
\]

in most high uptake cases, the equation 5.21 becomes

\[
\frac{\partial y}{\partial t} = \frac{G_p}{n y^{n-1}} \left( \frac{\partial^2 y}{\partial z^2} + \frac{2}{z} \frac{\partial y}{\partial z} \right)
\]

(5.22)

where

\[
G_p = \frac{c_{bo} \lambda D_p}{\rho q_m R^2}
\]

and the equation 5.20 becomes

\[
\frac{\partial y}{\partial z} \bigg|_{z=1} = \frac{k_o R}{\lambda D_p} (y_b - y_{z=1})
\]

(5.23)

\[
= \frac{1}{2} \text{Sh}_p (y_b - y_{z=1})
\]

(5.24)

where

\[
\text{Sh}_p = \frac{k_o (2R)}{\lambda D_p}
\]

For the bulk solution, a solute mass balance over a time period with the fresh immobilized biomass particle (i.e. \( x_o = 0 \)) gives mean solution concentration in the biomass as follows:

\[
\bar{x} = F(y_{bo} - y_b)
\]

(5.25)

where:
\[
F = \frac{V c_{bo}}{w q_m} \]
\[
V : \text{ total solution volume in the batch reactor}
\]
\[
w : \text{ total immobilized biomass weight in the reactor}
\]
The mean concentration, \( \bar{x} \), can be given by integration of solute concentration profile across the particle radius as follows:
\[
\bar{x} = 3 \int_0^1 z^2 x \, dz
\]  \hspace{1cm} (5.26)
Substitution of equation 5.25 into equation 5.26 and applying the dimensionless Freundlich equation yields the following bulk concentration,
\[
y_b = y_{bo} - \frac{3}{F} \int_0^1 z^2 y^n \, dz
\]  \hspace{1cm} (5.27)
In order to eliminate the center boundary condition equation 5.11 for the biomass core, a variable \( u = z^2 \) is defined and then the equation 5.22 becomes:
\[
\frac{\partial y}{\partial t} = \frac{G_p}{n y^n} \left( 4 u \frac{\partial y^2}{\partial u^2} + 6 \frac{\partial y}{\partial u} \right)
\]  \hspace{1cm} (5.28)
The equations 5.24 and 5.27 become:
\[
\left. \frac{\partial y}{\partial u} \right|_{z=1} = \frac{1}{4} S_p (y_b - y_{u=1}) \]
\hspace{1cm} (5.29)
and
\[
y_b = y_{bo} - \frac{1}{F} \frac{3}{2} \int_0^1 u^3 y^n \, du \]
\hspace{1cm} (5.30)
Discretization of the equations 5.28 to 5.30 was done by applying orthogonal collocation matrices as suggested by Villadsen and Stewart (1967). Equation 5.28 becomes:
\[
\frac{dy_i}{dt} = \frac{G_p}{n y_i^{n-1}} \left( \sum_{j=1}^N (4U_i B_{ij} + 6 A_{ij}) y_j + (4U_i B_{i,N+1} + 6 A_{i,N+1}) y_{N+1} \right)
\]  \hspace{1cm} (5.31)
and the equation 5.29 becomes:
\[
\left. \frac{dy_i}{du} \right|_{u=1} = \sum_{j=1}^{N+1} A_{N+1j} y_j = \frac{1}{4} S_p (y_b - y_{N+1})
\]  \hspace{1cm} (5.32)
The equation 5.30 becomes:

\[
y_b = y_{bo} - \frac{1}{F} \sum_{i=1}^{N} w_i y_i^n
\]  \hspace{1cm} (5.33)

Rearrangement of the equation 5.32 gives:

\[
y_{N+1} = \frac{Sh_p}{4\left(\frac{1}{A_{N+1,N+1}} + \frac{1}{4} Sh_p\right)} y_b - \frac{1}{\sum_{j=1}^{N} \left(\frac{1}{A_{N+1,N+1} + \frac{1}{4} Sh_p}\right)} y_j
\]  \hspace{1cm} (5.34)

Substitution of the equation 5.33 to the equation 5.34 gives:

\[
y_{N+1} = \frac{Sh_p}{4H} y_{bo} - \sum_{j=1}^{N} \left(\frac{Sh_p}{4H F} w_j y_j^n + \frac{1}{H A_{N+1,j}} y_j\right)
\]  \hspace{1cm} (5.35)

where

\[
H = A_{N+1,N+1} + \frac{1}{4} Sh_p
\]

Substitution of the equation 5.35 to the equation 5.31 results in:

\[
\frac{dy_i}{dt} = \frac{G_p}{n y_i^{n-1}} \left[ \sum_{j=1}^{N} \left( L_{ij} - \frac{M_i}{H A_{N+1,j}} y_j - M_i \sum_{j=1}^{N} \left( \frac{Sh_p}{4HF} w_j y_j^n + \frac{Sh_p}{4H}\right)\right) \right]
\]  \hspace{1cm} (5.36)

where

\[
L_{ij} = 4U_i B_{ij} + 4A_{ij}
\]

\[
M_i = 4U_i B_{i,N-1} + 6A_{i,N+1}
\]

The first order differential equations have been solved by a IMSL routine (GEAR) in order to determine the solute concentration profile in the biomass core and the bulk solution concentrations have been calculated using equation 5.33.

5-3 Simulation of Batch Kinetic Model

The mass transfer model developed in the previous section was solved to simulate the concentration profiles in a batch reactor under various conditions.

An orthogonal polynomial, \( P_n (0,0.5) \), with approximation order \( n = 2,4,6,8,10 \) was used. In most cases, \( n = 4 \) proved to be sufficient to obtain differences in the 3rd digit.
The following conditions were used for the simulation of bulk concentration changes with time in a batch kinetic reactor:

- bulk solution volume: 2000 cm$^3$
- initial solution concentration: 100 mg/L
- immobilized biomass particles charge: 2.0 g
- immobilized biomass particle diameter: 0.1 cm
- immobilized biomass particle apparent density: 773 g/L (obtained from experiment, as discussed section 6.4)

The effects of the following parameters on the bulk solution concentration profiles were simulated:

- maximum uptake capacity: $q_m$
- parameter, $n$, of the Freundlich isotherm
- $S_{hp}$ values

Figure 5-5 shows the effects of the maximum adsorbent uptake capacity on solution concentration history. Other conditions were held constant in the simulation. For $q_m = 50$ mg/g, the adsorbent is saturated at about 20 hours of run while for higher $q_m$ the equilibrium concentrations were not reached after 30 hours of run.

The effects of $S_{hp}$ on the bulk solution concentration history are presented for $n = 0.2$ in figure 5-6. It should be noted that the influence of membrane mass transfer resistance is negligible for $S_{hp} > 60$ but becomes significant for smaller values of $S_{hp}$.

Figure 5-7 shows the effects of the isotherm shapes (parameter $n$) on the concentration. The adsorption rates for strongly favourable isotherms are not much different from that for the saturated isotherm. In cases of strongly favourable isotherm systems, the saturated isotherm may be used for preliminary or rough calculation of the equilibrium time.
Figure 5.5  Effects of $q_m$ on the Bulk Solution Concentration Variation with Time
This is much simpler to handle computationally than the solutions for other types of nonlinear isotherms and will not give large errors for the strongly favourable isotherm cases.
CHAPTER SIX
RESULTS AND DISCUSSION

6-1 Production of \textit{R. arrhizus}

Seven batches of \textit{R. arrhizus} fermentation were carried out using the New Brunswick fermentor as described in section 4-1-1. Each of the fermentor vessels was filled with 10 liters of culture solution accounting about 70\% of the volume of the vessel. The fermentation period ranged from 30 hours to 48 hours depending on the amount of inoculum.

At the initial propagation stage of \textit{R. arrhizus}, natural surface active materials produced as metabolic by-products generated severe foaming under the conditions of high agitation and air flow. The foam height reached to 90\% of the volume of the vessel during the initial 10 hours of fermentation and then was maintained about 80\% of the volume in the final stages of fermentation even with addition of an antifoaming agent.

Culture solution pH decreased gradually from the initial pH 5.8 to pH 5.3 over 30 hours of fermentation and then increased rapidly back to the original pH. The fermentation was then terminated. Propagation state of the microorganism was indirectly observed by monitoring changes of culture solution pH. Decrease of the solution pH could be attributed to the production of various amino acids by the microorganism metabolizing the nutrients. It was reported that the culture solution pH declined to 3.2 during the growth phase of \textit{R. arrhizus} when no buffering agent was introduced to the culture solution (Schärer, 1985).

Table 6.1 summarizes the biomass yield from the fermentation of \textit{R. arrhizus}. The average yield of \textit{R. arrhizus} mycelia was 15.7 g of dry biomass based per one liter of culture solution. If the amount of calcium carbonate in the harvested biomass particles is compensated, the average net yield would be 11.0 g of dry biomass per one liter of culture solution.
Further discussion on the entrapped calcium carbonate powder in the biomass particles during the fermentation will be given in section 6-3. The variation in the amount of biomass produced in each batch might be due to the different number of spores inoculated in the fermentor vessel and the length of fermentation. Treen (1981) reported over 25 weight % yield of *R. arnhizus* on a sucrose basis and about 44 to 48 weight % yield of *R. arnhizus* on glucose basis was obtained by Scharer (1985).

The *R. arnhizus* mycelia grew forming discrete pellets ranging from 1 to 2 mm in size during fermentation. Similar pellet formation of the *R. arnhizus* mycelia was observed by Treen (1981). When an air lift fermenter was used to grow *R. arnhizus*, no pellet formation of the *R. arnhizus* mycelia was reported (Scharer, 1985). It was expected that *R. arnhizus* mycelia could not form bigger pellets due to high uniform shearing action of the air streams in the air lift fermenter. However, there exist in an impeller-agitated fermenter some dead zones where the mycelia could aggregate and form pellets.

Average uranium uptake capacity of the biomass was 354.2 mg U/g with a standard deviation of 5.2 %. The uranium uptake capacity of *R. arnhizus* produced in this study was about 60% higher than values reported by Tsezos (1980) and almost 100% higher than those obtained by Treen (1981). Another strain of *R. arnhizus* obtained from American Type Culture Collection (ATCC) was grown by Scharer (1985) with glucose medium and showed much lower uranium uptake capacity under similar conditions. It is believed that uranium uptake capacities of the *R. arnhizus* varies depending on the growth media and the fermentation conditions. Treen et al. (1984) also reported that the *R. arnhizus* mycelia harvested at different growth stages had different uranium uptake capacities.

Since there is no significant difference in the uranium uptake capacity of the biomass produced in each batch, the biomass from all seven batches was combined and mixed
uniformly. This combined homogeneous biomass was used for the subsequent immobilization and mass transfer study.

Table 6.1 Summary of \textit{R. arrhizus} Fermentation

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Fermentation Period (hrs.)</th>
<th>Biomass wt. Produced (g)</th>
<th>U Uptake (mgU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47.5</td>
<td>232.0</td>
<td>344.5</td>
</tr>
<tr>
<td>2</td>
<td>29.8</td>
<td>164.5</td>
<td>327.5</td>
</tr>
<tr>
<td>3</td>
<td>40.5</td>
<td>145.6</td>
<td>333.0</td>
</tr>
<tr>
<td>4</td>
<td>99.4</td>
<td>145.9</td>
<td>370.3</td>
</tr>
<tr>
<td>5</td>
<td>42.0</td>
<td>99.4</td>
<td>355.1</td>
</tr>
<tr>
<td>6</td>
<td>41.5</td>
<td>132.0</td>
<td>377.0</td>
</tr>
<tr>
<td>7</td>
<td>42.5</td>
<td>181.0</td>
<td>372.1</td>
</tr>
</tbody>
</table>

Note: # : the amount of calcium carbonate in the biomass particles was not compensated.

6-2 Coating of \textit{R. arrhizus} Particles in the Spouted Bed Reactor

The coating of \textit{R. arrhizus} particles was conducted using the spouted bed coating system described in chapter 3. Four different coating solutions used were presented in Table 6.2. Calcium carbonate powder with mean diameter of 6.7 \( \mu \text{m} \) and ground glucose powder were used as pore forming additives and polyvinylformal (PVF) was the polymeric membrane material and the solvent was tetrahydrofuran (THF). The weight percentages of polymer in the coating solution tested varied from 4.2 to 6.5. At the higher polymer concentrations, the coating solution was so viscous that the atomizer could not spray the solution.

Each of the batches that were coated was assigned a code name as summarized in Table 6.3. The coating conditions are also summarized in Table 6.3. In the batches of GCA2-1 and GCA2-2, granulated activated carbon (GAC) which passed the U.S. No. 14 sieve but
Table 6.2 Coating Solution Formulations

<table>
<thead>
<tr>
<th>Coating Solution</th>
<th>Additives</th>
<th>Mass Ratio* by wt</th>
<th>PVF wt%</th>
<th>Density g/cm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA1</td>
<td>CaCO₃</td>
<td>5.0</td>
<td>5.7</td>
<td>1.107</td>
</tr>
<tr>
<td>CA2</td>
<td>CaCO₃</td>
<td>8.0</td>
<td>4.2</td>
<td>1.203</td>
</tr>
<tr>
<td>GU1</td>
<td>Glucose</td>
<td>4.4</td>
<td>4.6</td>
<td>1.022</td>
</tr>
<tr>
<td>GU2</td>
<td>Glucose</td>
<td>2.9</td>
<td>6.5</td>
<td>1.022</td>
</tr>
</tbody>
</table>

Notes:

* mass ratio of additive to polymer (PVF)
### Table 6.3 Summary of Coating Conditions

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Core</th>
<th>Coating Solution</th>
<th>Total Solution Sprayed (mL)</th>
<th>Coating Period (min)</th>
<th>Suspension Air Flow Rate mL/s</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCA1</td>
<td>R.a.</td>
<td>CA1</td>
<td>50.0</td>
<td>19.0</td>
<td>250–320</td>
</tr>
<tr>
<td>RCA2</td>
<td>R.a.</td>
<td>CA2</td>
<td>70.0</td>
<td>24.0</td>
<td>250–405</td>
</tr>
<tr>
<td>GCA2-1</td>
<td>GAC</td>
<td>CA2</td>
<td>65.0</td>
<td>54.0</td>
<td>303–448</td>
</tr>
<tr>
<td>GCA2-2</td>
<td>GAC</td>
<td>CA2</td>
<td>35.0</td>
<td>9.0</td>
<td>303–385</td>
</tr>
<tr>
<td>RGU1</td>
<td>R.a.</td>
<td>GU1</td>
<td>80.0</td>
<td>26.0</td>
<td>250–405</td>
</tr>
<tr>
<td>RGU2</td>
<td>R.a.</td>
<td>GU2</td>
<td>88.0</td>
<td>35.0</td>
<td>250–437</td>
</tr>
<tr>
<td>HGU2</td>
<td>H.S.</td>
<td>GU2</td>
<td>70.0</td>
<td>25.0</td>
<td>250–423</td>
</tr>
</tbody>
</table>

**Notes:**

- Core Material Charge: 40.0 g
- Atomizing Air Flow Rate: 86.3 mL/s
- Inlet Air Temperature: 60–80°C
- Outlet Air Temperature: 30–45°C
retained in the U.S. No. 30 sieve was coated with the coating solution CA2. In batch HGU2, granulated HAMILTON sludge (14/30) was coated with a coating solution GU2. The minimum spouting air flow rates for R. arrhizus particles were slightly lower than those for GAC probably because of low density of the biomass. The inlet and outlet air temperatures were continuously measured in order to monitor any instability of the coating process. Typical inlet and outlet air temperature records were shown in figure 6.1. The build-up of coating material onto the surface of biomass particles made the particles heavier than the original weight resulting in gradual increase in the required suspension air flow rate. Once the outlet air temperature reached a pseudosteady state, its temperature was maintained within the range of ±1°C by adjusting the inlet air flow rate. The inlet air temperature increased gradually. When a required amount of coating solution was sprayed, the coating solution flow was stopped. Then the outlet air temperature rose sharply because the heat for vaporization of the solvent is not required but the inlet air flow rate and temperature remained constant. The outlet air temperature reached a steady point while the solvent remained in the particles was being vaporized. Once most of the solvent in the particles was removed, the air temperature rose again and then the coating was terminated. It took about 2 to 3 minutes to drive off remaining solvent after the coating solution flow stopped.

Weight changes of the particles during the coating process are summarized in Table 6.4. Weight increase of the core material was determined by subtracting the final weight of the core which was weighed after coating from the initial weight of the core material charged into the coating bed. It was found that further drying the coated particles at room temperature did not decrease weight of the coated particles. The coated particles appeared to be completely dried in the bed during the last 2 to 3 minutes of drying after stopping the coating solution flow in most coating cases. The weight gain of the core material was compared with the mass of additives and the polymer in the coating solution sprayed. The
mass balance generally agreed well within 3.6% for the biomass particle coatings. For batch GCA2, about 33% more weight gain was obtained probably due to the high coating solution flow rate resulting in the adsorption and incomplete evaporation of the solvent in the carbon particles. The coated particles were sieved using a stack of U.S. standard sieves and their size distribution is given in table 6.5. It should be noted that 15.3 wt.% of coated particles was retained on U.S. No. 14 sieve and 1.4 weight percent of them passed the U.S. No. 30 sieve. This indicated that there were little breakage of biomass particles during the coating process probably due to rapid build-up of polymer membrane onto the biomass particles which could prevent the particles from breaking even under severe particle movement in the spouted bed coater. A significant increase of particle size would be due to the build-up of the polymer and additive.

Table 6.4 Material Balance in Coating Process

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>Initial wt. charged (g)</th>
<th>Final wt. (g)</th>
<th>A wt. Gain (g)</th>
<th>B # wt. Gain expd (g)</th>
<th># # %wt.</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCA1</td>
<td>40.0</td>
<td>59.6</td>
<td>19.6</td>
<td>19.0</td>
<td>+3.5</td>
</tr>
<tr>
<td>RCA2</td>
<td>40.0</td>
<td>70.7</td>
<td>30.7</td>
<td>31.8</td>
<td>-3.6</td>
</tr>
<tr>
<td>GCA2-1</td>
<td>40.3</td>
<td>69.4</td>
<td>29.1</td>
<td>29.6</td>
<td>-1.6</td>
</tr>
<tr>
<td>GCA2-2</td>
<td>40.2</td>
<td>61.4</td>
<td>21.2</td>
<td>15.9</td>
<td>+33.2</td>
</tr>
<tr>
<td>RGU1</td>
<td>40.1</td>
<td>58.6</td>
<td>18.5</td>
<td>18.5</td>
<td>-0.2</td>
</tr>
<tr>
<td>RGU2</td>
<td>40.0</td>
<td>62.8</td>
<td>22.8</td>
<td>23.1</td>
<td>-1.2</td>
</tr>
</tbody>
</table>

Notes:

#: wt. gain B was calculated from the total coating solution volume sprayed during coating and its concentration

###: % wt. = ((A - B)/B) × 100
Table 6.5 Coated Particle Size Distribution

<table>
<thead>
<tr>
<th>Batch Size</th>
<th>RCA1 (%)a</th>
<th>RCA2 (%)</th>
<th>GCA2-A (%)</th>
<th>RGU1 (%)</th>
<th>RGU2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>+14 b</td>
<td>10.6</td>
<td>16.4</td>
<td>27.1</td>
<td>16.0</td>
<td>18.3</td>
</tr>
<tr>
<td>14/16 c</td>
<td>22.8</td>
<td>23.6</td>
<td>29.4</td>
<td>25.8</td>
<td>21.7</td>
</tr>
<tr>
<td>16/20</td>
<td>37.3</td>
<td>38.2</td>
<td>36.4</td>
<td>36.2</td>
<td>37.6</td>
</tr>
<tr>
<td>20/25</td>
<td>16.8</td>
<td>15.6</td>
<td>6.0</td>
<td>12.1</td>
<td>15.6</td>
</tr>
<tr>
<td>25/30</td>
<td>10.4</td>
<td>4.8</td>
<td>0.4</td>
<td>8.5</td>
<td>5.9</td>
</tr>
<tr>
<td>-30 d</td>
<td>2.2</td>
<td>1.0</td>
<td>0.7</td>
<td>1.4</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Notes:  

a: weight percentage  
b: particles retained in the U.S. Standard No.14 sieve  
c: particles passing the No.14 sieve but retained in the No.16  
d: particles passed the No.30 sieve

6-3 Leaching of Additives From Coating Membrane

The pore forming additives in coating membranes were leached out in the kinetic reactor. Leaching conditions and material balance of the additives are summarized in table 6.6 where sample designation indicates that the coated biomass used in the leaching experiment was obtained from the batch with the corresponding code name shown in table 6.3. A leaching time over 1400 minutes was required in most cases for the calcium carbonate additive leaching in order to insure all the additives leached out. The amount of calcium carbonate leached out was measured by titration of samples withdrawn at different time steps with standardized EDTA solution. The total weight of calcium carbonate leached out was calculated and compared with the mass of calcium carbonate expected in the coating membrane. The weight loss of the coated biomass varied from -38% to -154% while being
only – 2% for coated carbon particles. Figure 6.2 shows leaching kinetics of calcium carbonate additive from coated biomass particle. The volume of EDTA consumed was plotted against the leaching time. The initial leaching rate appeared to be very fast following the first order kinetics, and there was a secondary increase of leaching rate and then it reached plateau. The secondary increase of leaching rate might be due to leaching of calcium carbonate present inside of the biomass core. The secondary increase was not observed in the case of coated carbon particles (GCA2-1) as shown in figure 6.3.

The effects of leaching solution pH on calcium carbonate leaching rate are presented in figure 6.4. The initial leaching of calcium carbonate from the coated biomass particles (RCA2) was conducted with pH 4 leaching solution resulting in very slow leaching and then the leaching solution pH changed to pH 3 resulting in significant increase of leaching rate under the same agitation conditions. This result might suggest that dissolution rate of calcium carbonate be one of limiting steps in the leaching process.

In figure 6.5, the effect of particle size on the leaching rate is shown with samples of RCA1 and RCA1- which were coated in the same batch but have different size. The both curves show similar pattern but faster leaching rate was obtained by the smaller particles because of larger surface area for mass transfer.

The amounts of calcium carbonate and soluble material inside the biomass particle were calculated based on the volume of EDTA consumed at the end of titration and the overall weight loss of the particles after the leaching. The additional calcium carbonate leached out would be due to the presence of calcium carbonate particles inside the biomass core. Excess calcium carbonate particles were added to the fermentation broth in order to provide a buffering capacity. It was believed that the additional weight loss of the coated biomass would be attributed to the breakage of R. arrhizus mycelia during the grinding process to make pellets. The cytoplasmic materials would diffuse out through the broken mycelia. The
significant weight loss of *R. arrhizus* was observed after 2 to 3 days of the contact with solution (Seto, 1985).

Table 6.6 Overall Mass Balance of Leaching Process

<table>
<thead>
<tr>
<th>Sample</th>
<th>Initial wt. (g)</th>
<th>Final wt. (g)</th>
<th>wt. Loss (g)</th>
<th>B # wt. Loss exptd (g)</th>
<th>C ### %wt.</th>
<th>Leaching time (min.)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCA1</td>
<td>3.0023</td>
<td>1.5343</td>
<td>1.4680</td>
<td>0.6450</td>
<td>-127.6</td>
<td>1674</td>
<td>1.68</td>
</tr>
<tr>
<td>RCA1-</td>
<td>3.0062</td>
<td>1.3688</td>
<td>1.6374</td>
<td>0.6450</td>
<td>-153.9</td>
<td>1500</td>
<td>1.68</td>
</tr>
<tr>
<td>RCA2</td>
<td>3.0071</td>
<td>1.2691</td>
<td>1.7380</td>
<td>1.1620</td>
<td>-49.6</td>
<td>2100</td>
<td>1.68</td>
</tr>
<tr>
<td>RCA2-1</td>
<td>3.0008</td>
<td>1.3960</td>
<td>1.6048</td>
<td>1.1600</td>
<td>-38.3</td>
<td>1484</td>
<td>3.00</td>
</tr>
<tr>
<td>GCA2-1</td>
<td>3.0111</td>
<td>1.8776</td>
<td>1.1335</td>
<td>1.1110</td>
<td>-2.0</td>
<td>1470</td>
<td>1.68</td>
</tr>
<tr>
<td>RGU1</td>
<td>5.0133</td>
<td>3.6017</td>
<td>1.4116</td>
<td>1.2900</td>
<td>-9.4</td>
<td>180</td>
<td>7.0</td>
</tr>
<tr>
<td>RGU1-</td>
<td>5.0039</td>
<td>3.3917</td>
<td>1.6122</td>
<td>1.2880</td>
<td>-25.2</td>
<td>335</td>
<td>7.0</td>
</tr>
<tr>
<td>RGU2</td>
<td>3.0110</td>
<td>2.0735</td>
<td>0.9375</td>
<td>0.8350</td>
<td>-12.3</td>
<td>1612</td>
<td>7.0</td>
</tr>
<tr>
<td>RGU2-</td>
<td>5.0019</td>
<td>3.3113</td>
<td>1.6906</td>
<td>1.3880</td>
<td>-21.8</td>
<td>843</td>
<td>7.0</td>
</tr>
</tbody>
</table>

Notes: 
# : weight loss of additive calculated based on the total additive in the coating material sprayed during coating process
### : \((B \circ A)/B \times 100\)

- Leaching solution was 2000 mL of D.D.W
- Leaching solution temperature was maintained at 23°C ± 1°C
- R.P.M. of the impeller was 420 ± 10
- Particle size of all samples was 16/20 except the samples of RCA1- and RGU1- which were 20/25.
- coated biomass sample for RCA2-1 was obtained from the coating batch, RCA2.

A significant amount of calcium carbonate crystals present in the coated biomass particle which was not subject to acid leaching was observed by a scanning electron microscope (SEM). After the acid leaching, however, the calcium carbonate crystals could not be
Figure 6.3  Leaching Kinetics for Coated Carbon Particles with CaCO$_3$ Additive (GCA2-1)
Figure 6.4  Effect of Leaching Solution pH on the Leaching Rate
Figure 6.5  Effect of Particle Size on Leaching Rate (RCA1, RCA1-)

- RCA1 (16/20)
- RCA1- (20/25)
detected in the biomass core by SEM. The calcium carbonate mass balance of the coated carbon particles was $-2\%$ suggesting that the calcium carbonate additive in coating solution was not lost during the coating. Almost quantitative recovery of calcium carbonate was achieved by acid leaching of the coated carbon particles. This strongly indicates that the additional weight loss of coated biomass was due to the calcium carbonate crystals which were trapped within the R. arrhizus particle during the fermentation. The weight losses of the samples with glucose additive ranged from $-9.4\%$ to $-25.2\%$ which were much smaller than those with calcium carbonate additive probably because the calcium carbonate crystals inside the coated biomass were not dissolved and leached out by leaching solution of pH 7. The presence of calcium carbonate crystals was proved by the SEM examination of leached biomass particles. The leaching of glucose from the coating membrane was very rapid and completed within 2 hours in most cases. A typical leaching rate curve is presented in figure 6.6. The rapid leaching rate of glucose was probably due to the fact that dissolution rate of glucose was not a rate determining step. It was believed, however, that both diffusion and dissolution of the calcium carbonate contributed to the slow leaching rate for the samples with the calcium carbonate additives. This would be explained by examining the RCA2-1 leaching kinetic as shown in figure 6.4. The initial leaching was carried out with pH 4 solution resulting in very slow leaching rate. When the solution pH was changed to pH 3 after 100 minutes, a high leaching rate was obtained.

The overall material balance of a leaching process was attempted to estimate the amount of CaCO$_3$ inside the immobilized biomass particle.

The total mass of immobilized biomass particle ($M_t$) is sum of the mass of coating membrane ($M_m$) and biomass core ($M_b$);

$$M_t = M_m + M_b$$  \hspace{1cm} (6.1)
The coating membrane consists of polymer \( M_{mp} \) and leachable additive \( M_{ma} \) assuming that the solvent in the coating membrane was completely removed during the drying part of the coating process.

\[
M_m = M_{mp} + M_{ma}
\]  \hspace{1cm} (6.2)

The biomass core would consist of the insoluble \textit{R. arrhizus} mycelia \( M_{bi} \) and soluble fractions. The soluble fractions could be mainly trapped \( \text{CaCO}_3 \) crystals \( M_{bc} \) and soluble \textit{R. arrhizus} mycelia cytoplasmic fraction \( M_{bs} \).

\[
M_b = M_{bi} + M_{bc} + M_{bs}
\]  \hspace{1cm} (6.3)

Therefore,

\[
M_t = M_{mp} + M_{ma} + M_{bi} + M_{bc} + M_{bs}
\]  \hspace{1cm} (6.4)

The weight loss \( M_t \) of IRP after leaching and drying might be due to the loss of \( \text{CaCO}_3 \) in the coating membrane as well as inside the biomass core and the soluble \textit{R. arrhizus} fractions.

\[
M_t = M_{ma} + M_{bc} + M_{bs}
\]  \hspace{1cm} (6.5)

The mass percentage yield of dry immobilized biomass particle after leaching is given by

\[
\% M_y = (M_t - M_t)/M_t \times 100
\]  \hspace{1cm} (6.6)

The total weight \( M_{ct} \) of \( \text{CaCO}_3 \) leached out during leaching process was estimated by the \( \text{CaCO}_3 \) concentration of final leaching solution. The soluble \textit{R. arrhizus} fraction can be calculated as follows:

\[
M_{bs} = M_t - M_{ct}
\]  \hspace{1cm} (6.7)

The mass of \( \text{CaCO}_3 \) crystals present in the biomass core was estimated by subtracting the mass of \( \text{CaCO}_3 \) additive in the coating membrane from the total mass of leached \( \text{CaCO}_3 \) \( M_{ct} \).

\[
M_{bc} = M_{ct} - M_{ma}
\]  \hspace{1cm} (6.8)

The mass percentage of polymer membrane of the immobilized biomass particle is given by

\[
\% M_{mp} = M_{mp}/(M_t - M_t) \times 100
\]  \hspace{1cm} (6.9)

The mass percentage of calcium carbonate in the biomass core is given by
\[
\%M_{bc} = \frac{M_{be}}{M_b} \times 100
\]  
(6.10)

The mass percentage of soluble fraction of the biomass core is given by

\[
\%M_{bs} = \frac{M_{bs}}{(M_{bi} + M_{bs})} \times 100
\]  
(6.11)

The mass percentage of insoluble biomass fraction of the immobilized biomass particle is given by

\[
\%M_{bi} = \frac{M_{bi}}{(M_{bi} + M_{imp})} \times 100
\]  
(6.12)

The results of the overall mass balance analysis of coated particles are summarized in table 6.7. The average mass percentage of calcium carbonate inside the biomass particle was estimated to be 30% from three samples which were leached out with the pH 1.68 solution assuming that all calcium carbonate powder inside the particle was dissolved and diffused out during the acid leaching. The mass percentage of polymer membrane of the immobilized biomass particles vary from 8.4% to 14.0% based on the final immobilized biomass particles but the actual polymer mass component of immobilized biomass particles with glucose additive would be higher than those shown in table 6.7 when based on biomass mass because calcium carbonate inside of the biomass particles was not leached out by the neutral leaching solution. The overall yield of immobilized biomass for the coated biomass with glucose additive is generally higher than that with calcium carbonate additive due to the remaining calcium carbonate inside of the biomass particle with glucose additive. In case of coated carbon particles with calcium carbonate additive, it can be seen that there is slight short of calcium carbonate leached out. It should be noted that the coated biomass particles of the sample (RCA2-1) were leached out with pH 3 solution and the calcium carbonate inside the biomass particles appears not to be completely leached out at the end of leaching process.
Table 6.7 Overall Mass Balance Analysis of Coated Particles

<table>
<thead>
<tr>
<th>Samples</th>
<th>%M_{mp}</th>
<th>%M_{bc}</th>
<th>%M_{bs}</th>
<th>%M_{bi}</th>
<th>%M_{y}</th>
<th>M_{et} (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCA1</td>
<td>8.4</td>
<td>30.9</td>
<td>8.8</td>
<td>91.6</td>
<td>51.1</td>
<td>1.330</td>
</tr>
<tr>
<td>RCA2</td>
<td>9.4</td>
<td>35.1</td>
<td>14.5</td>
<td>90.6</td>
<td>45.5</td>
<td>1.428</td>
</tr>
<tr>
<td>RCA2-1</td>
<td>11.5</td>
<td>24.1</td>
<td>12.9</td>
<td>88.5</td>
<td>42.2</td>
<td>1.571</td>
</tr>
<tr>
<td>RGU1</td>
<td>10.4</td>
<td>21.1</td>
<td>6.5</td>
<td>89.6</td>
<td>46.5</td>
<td>1.518</td>
</tr>
<tr>
<td>RGU1-1</td>
<td>9.0</td>
<td>-</td>
<td>3.6</td>
<td>-</td>
<td>71.8</td>
<td>-</td>
</tr>
<tr>
<td>RGU2</td>
<td>9.5</td>
<td>-</td>
<td>9.6</td>
<td>-</td>
<td>67.8</td>
<td>-</td>
</tr>
<tr>
<td>RGU2-1</td>
<td>13.4</td>
<td>-</td>
<td>5.4</td>
<td>-</td>
<td>68.8</td>
<td>-</td>
</tr>
<tr>
<td>GCA2-1</td>
<td>14.0</td>
<td>-</td>
<td>9.6</td>
<td>-</td>
<td>66.2</td>
<td>-</td>
</tr>
</tbody>
</table>

6-4 Physical Characterization of Immobilized R. arrhizus Particles

6-4-1 Particle Size Analysis

The particle sizes of biomass particles were determined using a Zeiss particle size analyser. The particle size distributions of samples were plotted on a log-normal probability graph. Figure 6.7 shows size distribution of the R. arrhizus particles passing the U.S. No. 14 sieve but retained in the No. 30 sieve. The total number of particles counted was 910. The size distribution of the coated biomass particles (RGU1) passing the No. 15 sieve but retained in the No. 20 sieve is shown in Figure 6.8. The total number of particles counted was 940. Figure 6.9 shows size distribution of biomass particles (RGU1L) which were obtained from the leaching and subsequent drying of the coated biomass particles (RGU1). The total number of particles counted was 1240. The size distribution of the water swollen biomass particles (RGU1LW) which were prepared by soaking RGU1L in water is shown in Figure 6.10. The total number of particles counted was 575.
Figure 6.8  Particle Size Analysis for Coated Biomass
Figure 6.9 - Particle Size Analysis for Immobilized Biomass Particles
Cumulative Particle Size Distribution (%)

Figure 6.10  Particle Size Analysis for Wet Immobilized Biomass Particles
Reasonably straight lines over the 10% to 90% range were obtained from most of data plots. The geometric number weighted average diameter was obtained at 50% from the graph and the geometric standard deviation was estimated by the following equation:

\[ \sigma_g = \frac{(dp)_{94\%}/(dp)_{50\%}}{f} \]  

(6.13)

The apparent density of particle was calculated by the following equation:

\[ \rho_{ap} = \frac{\text{wt.}}{N_t \times \bar{r}^3} \]  

(6.14)

The results of particle size analysis are summarized in Table 6.8. The density of glucose leached particles (RGU1L) is 0.508 g/cm³ which is about 60% less than that of non-leached one. The diameter of the glucose leached particle was slightly bigger than that of the non-leached one. The glucose leached particles appeared to be irregular in overall shape. There was no significant change in particle size for water swollen particles probably due to the high porosity of the biomass particle as well as leaching of calcium carbonate.

<table>
<thead>
<tr>
<th>Sample</th>
<th>RA</th>
<th>RGU1</th>
<th>RGU1L</th>
<th>RGU1LW</th>
</tr>
</thead>
<tbody>
<tr>
<td>N_t</td>
<td>910</td>
<td>940</td>
<td>1240</td>
<td>575</td>
</tr>
<tr>
<td>d_p (mm)</td>
<td>0.96</td>
<td>1.03</td>
<td>1.15</td>
<td>1.00</td>
</tr>
<tr>
<td>\sigma_g</td>
<td>1.36</td>
<td>1.18</td>
<td>1.07</td>
<td>1.16</td>
</tr>
<tr>
<td>\rho_{ap} (g/cm³)</td>
<td>-</td>
<td>0.933</td>
<td>0.508</td>
<td>0.773</td>
</tr>
</tbody>
</table>

Notes: RA: *R. arrhizus* Particles (14/30)

RGU1: Coated Biomass Particles (16/20)

RGU1L: Dry Glucose Leached Particles (16/20)

RGU1LW: Water Swollen Glucose Leached Particles (16/20)
The particle size of calcium carbonate powder was measured using a Coulter counter. The mean diameter of particles was 6.7 μm and the most particles were less than 10 μm as shown in figure 6.11. The glucose particles were ground using a ball mill with tetrahydrofuran (THF) solvent. The particle size of milled glucose was examined using an optical microscope. After 24 hours of milling at 60 r.p.m., majority of particles appeared to be less than 10 μm but some were as big as 30 μm. The longer milling time did not produce smaller particles probably due to the fusion of smaller particles. The glucose particles have a broader particle size range than the calcium carbonate crystals have.

6.4.2 Estimation of Coating Membrane Wall Thickness

The most direct method of determining the wall thickness of a coated particle is by measurement of sliced (by microtome) coated particle, using a microscope fitted with a micrometer. The accuracy of such lengthy and tedious determination is highly dependent on the precision of obtaining a section through the exact center of the coated particle as illustrated in figure 6.12. Any deviation from this center may result in a larger measured section of wall (t2) than exists (4). Since the microtome sections obtained are frequently at a distance from the center, these readings represent an average that may exaggerate the true value. The wall thickness determined microscopically represents an average of the many determinations representative of various sections. Therefore, the volume relationship of two concentric spheres used for the calculation of wall thickness gives a better description of the average wall thickness without being as time consuming. The wall thickness of coated biomass particles was estimated by following the model developed by Madan (1981). The equations for estimating the wall thickness by volume relationship are given as follows;
Figure 6.11
Particle Size Distribution of Calcium Carbonate Powder

MASS PERCENTAGE

PARTICLE DIAMETER (µm)
Figure 6.12  Different X-sectional Cuts of a Coated Particle
\[
\frac{KR}{R} = \left( \frac{W_w \times \rho}{\rho_w \times (W - W_w)} + 1 \right)^{1/3}
\]

\[
KR - R = R \left[ \left( \frac{W_w \times \rho}{\rho_w \times (W - W_w)} + 1 \right)^{1/3} - 1 \right]
\]

where

- \( KR \): radius of the large sphere (coated biomass particle)
- \( R \): radius of the small sphere (biomass core)
- \( W \): weight of the large sphere
- \( W_w \): weight of the coating membrane
- \( \rho \): density of the small sphere
- \( \rho_w \): density of the coating membrane (\( = \rho_p \times m_p + \rho_{ad} \times m_{ad} \))
- \( \rho_p \): density of the polymer
- \( \rho_{ad} \): density of the additive
- \( m_p \): mass fraction of the polymer in the coating membrane
- \( m_{ad} \): mass fraction of the additive in the coating membrane

The estimated membrane thickness data are tabulated in Table 6.9. The coating wall thicknesses of the coated biomass particles range from 47 \( \mu \)m to 86 \( \mu \)m depending on the amount and type of coating solutions sprayed. The coating walls of coated biomass particles with glucose additive are thicker than those particles with calcium carbonate additive due to high density of the latter additive. The estimated thicknesses of coating walls were not directly compared with those observed by SEM examinations. However, they are in the same order of magnitude.
Table 6.9 Estimated Coating Membrane Wall Thickness

<table>
<thead>
<tr>
<th>Sample</th>
<th>m_p</th>
<th>m_ad</th>
<th>(\rho_w) (g/cm³)</th>
<th>w (g)</th>
<th>(W_w) (g)</th>
<th>thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCA1</td>
<td>0.167</td>
<td>0.833</td>
<td>2.504</td>
<td>59.6</td>
<td>19.6</td>
<td>47</td>
</tr>
<tr>
<td>RCA2</td>
<td>0.111</td>
<td>0.889</td>
<td>2.590</td>
<td>70.7</td>
<td>30.7</td>
<td>68</td>
</tr>
<tr>
<td>RGU1</td>
<td>0.184</td>
<td>0.816</td>
<td>1.501</td>
<td>58.6</td>
<td>18.5</td>
<td>70</td>
</tr>
<tr>
<td>RGU2</td>
<td>0.254</td>
<td>0.746</td>
<td>1.477</td>
<td>62.8</td>
<td>22.8</td>
<td>86</td>
</tr>
<tr>
<td>GCA2-1</td>
<td>0.111</td>
<td>0.889</td>
<td>2.504</td>
<td>69.4</td>
<td>29.1</td>
<td>65</td>
</tr>
</tbody>
</table>

6-4-3 Coating Membrane Structure

A scanning electron microscope (SEM) equipped with X-ray energy dispersion analyser was used for structural examination of the immobilized biomass particles and identification of elements present in the biomass particles. In most samples examined, 15 KV of accelerating voltage provided clear identification of coating membrane pores, pore forming additives and R. arrhizus mycelium with up to 10 K magnifications. The deterioration of polymeric coating membrane was experienced in some samples when higher accelerating voltage (25 KV) was used with 25 K magnification.

Figure 6.13 shows the overall shape of a coated biomass particle with calcium carbonate additive. The surface of the particle appears to be uniformly porous and relatively smooth. A close-up picture of this coated biomass particle is shown in figure 6.14. The calcium carbonate additives are clearly shown as cubic crystals embedded in the polymer matrix and the surface appears porous. The overall shape of a coated biomass particle with glucose additive is presented in figure 6.15. The surface structure of coated biomass with glucose additive appears to be rougher than that of the particle with calcium carbonate additive. A close-up picture of this coated biomass particle is shown in figure 6.16. The glucose particles can not be clearly seen on the surface of the coating membrane. However, the particles appear
Figure 8.13  The Overall Shape of a Coated Biomass Particle with CaCO3 Additive
Figure 6.14 The close-up picture on the surface of the coated particle shown in Figure 6.13.
Figure 6.15 The Overall Shape of a Coated Biomass Particle with Glucose Additive
Figure 4.71  The Close-up Picture on the Surface of the Coated Particle Shown in Figure 6.15.
to be embedded in the polymeric matrix. The glucose particles appear to have wide particle size range while the calcium carbonate crystals appeared to be cubes with a similar size.

Figure 6.17 shows an overall shape of coated biomass particle after the calcium carbonate additive was leached out from the coating membrane. It can be seen that the calcium carbonate crystal cubes disappeared leaving many pores on the surface of the immobilized biomass particle. A close-up picture of the particle is presented in figure 6.18. Most of calcium carbonate additive were completely leached out and the surface of the membrane appears to be very porous. The overall shape of an immobilized biomass particle from which the glucose additive was leached out is shown in figure 6.19. There appear to be many pin holes on the surface of the RGU1 particle. Figure 6.20 shows the close-up picture of the surface of this particle. The surface appears to be very porous and irregular. The glucose particles were completely leached out from the wall.

The cross section view of an immobilized biomass particle out of which calcium carbonate additives were leached by the pH 1.68 solution was shown in figure 6.21. There was clearly shown the porous membrane encapsulating the biomass core. The hollow parts in the biomass core are the artifacts when the particle was snap-fractured during sample preparation. The biomass core appeared to be very porous. A close-up picture of membrane cross-section is presented in figure 6.22. The coating membrane appears to be inter-connected with pores formed at external membrane surface. Calcium carbonate crystals are not observed in the coating membrane suggesting complete leaching of the additive. In figure 6.23, the R. arrhizus mycelia are shown to be entangled in each other. It should be noted that there were no calcium carbonate crystals observed in the biomass core for this sample because all the crystals were completely dissolved and leached out by the pH 1.68 leaching solution. Figure 6.24 shows a cross-section view of biomass core of an immobilized biomass particle from which glucose additive was leached by the pH 7 leaching solution. There is significant
Figure 6.19  The Overall Shape of an Immobilized Biomass Particle from which Glucose Additive was Leached Out.
number of calcium carbonate crystals remaining in the biomass core even after leaching of glucose. These are calcium carbonate crystals trapped between the *R. arrhizus* mycelia during fermentation were not dissolved and leached out by the neutral leaching solution due to low solubility of calcium carbonate at pH 7. Figure 6.25 clearly shows the presence of calcium carbonate crystals in the core of coated biomass particle which was coated with glucose additive but was not subjected to the acid leaching.

The identification of elements present in the biomass particles was done by X-ray energy dispersion analysis using a SEM. Figure 6.26 shows X-ray spectrum of the surface of coated biomass particles with calcium carbonate additive. A distinctive calcium peak appears at about 306 (KeV) when a cubic crystal in the polymeric membrane matrix was focused on. Clear identification of calcium carbonate crystals present in the biomass core which were not subject to the acid leaching is shown in figure 6.27 where a distinctive calcium peak is obtained by analysing the cubic particles inside of the biomass core. P and K peaks are probably due to the elements in the *R. arrhizus* cell and gold coating used for sample preparation should be responsible for the Au peak. Figure 6.28 shows a X-ray spectrum for a cross-section of an immobilized biomass particle which was loaded with uranium after the glucose additive was leached out at pH 7. Cell wall of a *R. arrhizus* mycelium was focused. High U and K peaks are observed suggesting the presence of the uranium in the cell. It should be noted that Ca peak also appears probably due to some dissolved calcium on the surface of cell wall or cellular calcium. After uranium loaded immobilized biomass particle was eluted by sodium bicarbonate solution, the presence of the uranium in the cell of the immobilized biomass particle was examined by X-ray analysis focusing on a cell wall of *R. arrhizus* in the biomass core of the uranium eluted immobilized biomass particle. As shown in figure 6.29, the U peak completely disappeared implying 100% uranium recovery by the sodium bicarbonate eluant but there is still a Ca peak observed.
Figure 6.25 The Cross-section View of a Coated Biomass Particle with Glucose Additive Which Was Not Leached Out
Figure 6.26  X-ray spectrum Obtained by Focussing on the Surface of a Coated Biomass Particle with CaCO$_3$ Additive (No CaCO$_3$ Leaching)
Figure 6.27  X-ray Spectrum Obtained by Focussing on the CaCO$_3$ Crystal Present in the Biomass Core of the Coated Biomass Particle Which Was Not Subject to the Acid Leaching
Figure 6.28  X-ray Spectrum Obtained by Focussing on near a *R. arrhizus* mycelium in the Biomass Core of the Sliced Immobilized Biomass Particle Which Was Loaded with Uranium
Figure 6.29  X-ray Spectrum Obtained by Focussing on near a R. arrhizus mycelium in the Biomass Core of the Sliced Immobilized Biomass Particle Which was Loaded with Uranium and Subsequently eluted by NaHCO₃ Solution.
6-4-4 Pressure Drop in Immobilized \textit{R. arrhizus} Packed Column

The pressure drops in immobilized biomass particle packed column were measured using a down-flow packed column under various flow conditions. The compression of immobilized biomass particles was determined by measuring the changes in packing height at the end of each run. Pressure drop measurements in GAC packed column were carried out under the similar conditions in order to compare the pressure drops obtained in the IRP packed column. Three pressure drop experiments were conducted with GAC, and the immobilized biomass particles (glucose leached, calcium carbonate leached) which passed the No. 16 sieve but remained in the No. 20 sieve. The results of pressure drop measurement are shown in figure 6.30.

The pressure drop in the immobilized biomass particles (RGU1) packed column increases almost linearly up to the flow rate 16 cm/min., but at higher flow rate, it increases rapidly probably due to the compression of the immobilized biomass particles under high shear. The pressure drop in the immobilized biomass particles (RCA1) packed column is slightly lower than that in the GAC packed column at low flow rates but expected to increase rapidly at high flow rates. The immobilized biomass particle (RCA1) appears to be more compression resistant than the ones with glucose additive. The smaller pressure drop for the coated biomass with calcium carbonate additives might be attributed to more uniform pore formation of the coating membrane after leaching because of the narrow particle size distribution of calcium carbonate particles. The glucose particles with bigger and wider particle size distribution resulted in the coating membrane with non-uniform pore size after leaching. The collapse of some bigger pores might be contributed to compression of the immobilized biomass particles under a certain pressure. The change in the packing height, however, was not noticeable at the end of each run with the immobilized biomass particles.
6.5 Equilibrium Studies

6.5.1 Uranium Biosorption and Desorption by Inactive Native *R. arrhizus*

Uranium loading of biomass was calculated from biomass dosage and the initial and final uranium concentrations of solution as follows:

\[ q = \frac{(C_{in} - C_e)}{m} \quad (6.17) \]

where

- \( C_{in} \) = initial U concentration (mg/U/L)
- \( C_e \) = final U concentration (mg/U/L)
- \( m \) = biomass concentration (g/L)

The uranium loading of biomass is plotted against equilibrium uranium concentration in figure 6.31. In order to determine the effect of initial uranium concentration on the isotherm, two different initial uranium concentrations were used: 100.8 mg/L and 1000.6 mg/L. The initial uranium concentration did not appear to affect equilibrium isotherm except the two points obtained at low biomass dosages with low uranium concentrations. It should be noted that increase of solution pH was observed after the uranium loading even though pH 4 buffer solution was used for preparing the uranium solution. A similar increase in solution pH after uranium loading was reported by others (Tsezos, 1980, Treen, 1981 and Friis, 1986).

The uranium loading of *R. arrhizus* particles approaches at the solution concentration of 230 mg U/L maximum 280 mg/g which is about 30% lower than that of *R. arrhizus* powder which was several times washed and conditioned to pH 4 with dilute HCl solution before the uptake experiment. The biomass which was washed and conditioned with dilute acid solution would not contain calcium carbonate. The biomass which was not conditioned would have less cell mass such that the uranium loading will be less if the loading is calculated based on the initial biomass weight. The presence of calcium carbonate in the biomass particle as observed by SEM, and the additional leaching of calcium carbonate and
loss of biomass after leaching in the acidic solutions, indicate that some of calcium carbonate
added in the fermentation broth was trapped between the matrix of R. arrhizus mycelia and
not washed away during the washing process.

When the initial biomass dosages were less than 20 mg per 100 mL of uranium solution, the amount of biomass effectively contacted with the uranium solution would be less
than that of biomass added initially due to adherence of the biomass powder at the beaker
wall in the form of a rim when the biomass slurry rotates in the rotary shaker. The mass of
biomass adhering to the beaker would not be significant to reduce the effective biomass
contacted with the solution when the biomass dosage was large. However, its effect on the
uptake capacity was significant when the initial biomass dosage was less than 20 mg as shown
in the two data points in figure 6.31.

Five replicates of uptake runs were carried out at 100 mg/L initial concentration
with 100 mg of biomass dosage in order to determine the reproducibility of the isotherm
data. The average uptake capacity at the average equilibrium concentration of 4.7 mg U/L
and the pH of 4.0 was 96.02 mg/g with standard deviation of 0.82%.

The desorption of uranium loaded biomass was conducted and its results were
plotted along with biosorption data on figure 6.31 as well. A hysteresis of the desorption curve
was observed at uranium concentrations over 20 mg/L and below that concentration the
desorption data matched adsorption data quite well.

6-5-2 Uranium Biosorption of Immobilized R. arrhizus

The uranium biosorption isotherm of immobilized R. arrhizus particles was
obtained following the similar procedure used for native inactive R. arrhizus discussed in the
previous section except longer contact period. The equilibrium contact time for the
immobilized biomass particles was determined by a kinetic experiment and 7 days of contact
period assured the equilibrium with the immobilized biomass (RGU1). The uranium uptake capacity of polymeric membrane material (polyvinylformal) was examined to see if the coating membrane could adsorb uranium. About 120 mg of polyvinylformal powder was contacted with 150 mL of 300 mg/L solution for 7 days in a rotary shaker and its uptake capacity was determined. The polymer has negligible uranium uptake capacity. The uranium uptake by the polymer was not accounted for when estimating the uranium uptake of the immobilized \textit{R. arrhizus}.

The uranium biosorption isotherm for the immobilized biomass particles (RGU1) is presented along with those for native \textit{R. arrhizus} in figure 6.32. The overall uranium uptake of the immobilized biomass was about 40% of that of native biomass. The lower uranium uptake of immobilized biomass particles could be explained as follows:

1. about 9 weight % of the coating membrane material which has negligible uranium uptake capacity was not considered in the calculation of uranium uptake by the immobilized biomass particles and about 30% by weight of calcium carbonate is retained in the biomass core of the immobilized biomass particle resulting in reduction of effective \textit{R. arrhizus} mycelia content of the immobilized biomass particle.

2. the uranium biosorption isotherm for the native biomass was obtained with smaller particles passing No. 30 mesh than those of the immobilized biomass (16/20 mesh) and the calcium carbonate within the immobilized biomass was not removed after the glucose leaching. It is expected that some calcium carbonate would be dissolved and remained in the form of bicarbonate ion inside the biomass particle suppressing the uranium uptake by competing for active sites of the biomass.
Figure 6.32  Comparison of Biosorption Isotherms for Native R. arrhizus and Immobilized R. arrhizus
3. It was reported that the bicarbonate and carbonate ions were very effective to elute the loaded uranium from the biomass. A detailed study on the effects of these ions on elution of uranium from *R. arrhizus* was made by Tsezos (1984).

6.5.3 Application of Isotherm Models and Parameter Estimation

In general, the adsorption isotherm data were reasonably well fitted to the Freundlich and Langmuir isotherm models. Tsezos (1980) reported similar fitting of uranium adsorption data for the *R. arrhizus*. A non-linear regression analysis package (UWHAUS) was employed to estimate the parameters of the two isotherm models. The parameters were estimated by UWHAUS using isotherm data shown in appendix C and are listed for the Freundlich and Langmuir models in tables 6.10 and 6.11, respectively. The numbers in the brackets represent 95% confidence limits of each parameter.

| Table 6.10 Regressed Isotherm Parameters for the Freundlich Model |
|---|---|---|
| Sample | Parameter | Value |
| Native *R. arrhizus* | K | 111.4 (141.0) |
| | n | 0.144 (0.192) |
| Immobilized *R. arrhizus* | K | 32.5 (40.2) |
| | n | 0.194 (0.244) |

| Table 6.11 Regressed Isotherm Parameters for the Langmuir Model |
|---|---|---|
| Sample | Parameter | Value |
| Native *R. arrhizus* | q₀ | 276.3 (288.2) |
| | α | 0.0446 (0.0625) |
| | | (264.4) (0.0267) |
| Immobilized *R. arrhizus* | q₀ | 104.2 (112.0) |
| | α | 0.0337 (0.0454) |
| | | (96.3) (0.0220) |
6-6 BATCH KINETIC STUDIES

6-6-1 Effects of Coating Formulation on Mass Transfer Rate

The rates of uranium transfer for the native *R. arrhizus* particles and immobilized biomass particles were determined using a batch kinetic reactor as described in the section 4-4-2. The uranium kinetic experiments were conducted with the immobilized *R. arrhizus* particles which have different membrane structures and particle sizes in order to investigate the effects of different coating formulations on the uranium transfer rate. One kinetic experiment was carried out with native *R. arrhizus* particles which passed the No.16 sieve and were retained on the No.20 sieve.

The results of batch kinetic experiments are summarised in table 6.12, where sample designation indicates that the immobilized biomass used in the kinetic experiments was obtained from the immobilized biomass produced in the corresponding leaching batch shown in table 6.6 except RAI. For the kinetic run with native *R. arrhizus* particles (RAI), about 16% weight loss of the biomass particle was observed after the kinetic run. This might be due to the facts that some soluble fraction of the biomass was lost and *R. arrhizus* mycelia were torn off from the biomass particles when the native biomass particles were contacted with the uranium solution under high agitation. There were weight gains of 1.8 to 1.9 % in cases of RCA samples which had been leached with the acidic solutions. The weight gains were still slightly lower than those expected by the uranium uptake probably due to some loss of soluble fraction of the biomass core. Weight gains of 4% and 4.8% was obtained for the samples of RGU1- and RGU1, respectively even though their uranium uptake capacities are relatively high. This might be attributed to the further leaching of soluble fraction (mainly calcium carbonate crystals) remaining inside the biomass particle during the kinetic runs.
Table 6.12 Summary of the batch kinetic experiments

<table>
<thead>
<tr>
<th>Samples</th>
<th>Initial wt. (g)</th>
<th>wt. change %</th>
<th>Initial C. (mgU/L)</th>
<th>Final C. (mgU/L)</th>
<th>Final pH</th>
<th>Final U uptake (mgU/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RA1</td>
<td>1.1985</td>
<td>-16.0</td>
<td>78.3</td>
<td>52.5</td>
<td>4.7</td>
<td>37.6</td>
</tr>
<tr>
<td>RCA1</td>
<td>1.4935</td>
<td>1.8</td>
<td>103.1</td>
<td>79.5</td>
<td>4.2</td>
<td>43.2</td>
</tr>
<tr>
<td>RCA2</td>
<td>1.2561</td>
<td>2.0</td>
<td>101.1</td>
<td>74.0</td>
<td>4.2</td>
<td>43.2</td>
</tr>
<tr>
<td>RGU1</td>
<td>2.0951</td>
<td>4.8</td>
<td>203.9</td>
<td>105.1</td>
<td>4.7</td>
<td>94.3</td>
</tr>
<tr>
<td>RGU1-</td>
<td>2.0034</td>
<td>4.0</td>
<td>200.4</td>
<td>111.5</td>
<td>4.6</td>
<td>88.7</td>
</tr>
<tr>
<td>RGU2</td>
<td>0.5039</td>
<td>-4.6</td>
<td>101.1</td>
<td>94.9</td>
<td>4.0</td>
<td>24.5</td>
</tr>
</tbody>
</table>

Notes:

A: wt. change % = (final wt. – initial wt.)/initial wt.

B: Uranium uptake capacities were calculated by the concentration difference at the end of kinetic run based on initial mass of immobilized R. arrhizus particles.

All the batch kinetic experiments were conducted under the following conditions:

Solution volume: 2000 mL

Initial solution pH: 4.0 ± 0.1

Temperature: 23±1°C

Impeller speed: 420±10 R.P.M.

Particle size of the samples were 14/20 except the sample of RGU1- which was 20/25.

Samples of about 2.0 ml were collected from the reactor at predetermined time intervals and the total sample volume did not exceed 2% by volume of the initial bulk solution. Therefore, the bulk uranium concentrations sampled were not compensated for the
volume reduction due to the sampling. The uranium concentrations of the samples were determined by the procedures described in section 4.2.

Figure 6.33 shows the change of reduced bulk uranium concentration with time for the kinetic run with native R. arrhizus particles which passed the No. 15 sieve and remained on the No. 26 sieve. The initial bulk uranium concentration was 78.3 mgU/g.

For the kinetic runs with immobilized R. arrhizus particles that were coated with the coating solutions having different amount of calcium carbonate additive, the bulk uranium concentration decreased rapidly and reached equilibrium within about 4 hours for the kinetic run with the immobilized R. arrhizus particles which were coated with high calcium carbonate content (additive/polymer: 8/1 by weight). It took more than 24 hours with the biomass particles having lower calcium carbonate additive (additive/polymer: 5/1 by weight) to reach the equilibrium. Even though there are slight differences in membrane thickness of two immobilized biomass particle samples, the effects of amount of calcium carbonate additive in the coating membrane on the mass transfer rate can be clearly seen as shown in figure 6.34. It should be noted that their uranium uptake capacities are not much different. More dramatic effects of membrane porosity due to the amount of pore forming additives on the mass transfer rate can be seen in figure 6.35 in which the reduced bulk uranium concentrations of two kinetic runs with immobilized biomass particles having different amount of glucose in the coating membrane (by weight, glucose/polymer: 2.9/1 for RGU2, and 4.4/1 for RGU1). The kinetic of RGU2 sample is extremely slow and less than 5% reduction of the bulk uranium concentration was obtained in 25 hours of run. The uranium uptake rate for the sample RGU1 is slower than that of RCA2 even though more immobilized biomass particles were charged to the reactor. This might be due to the thicker membrane of the immobilized biomass particle of RGU1 sample.
Figure 6.33  Uranium Biosorption Kinetics for Native R. arthavis Particles (RAI)
Figure 6.34. Uranium Biosorption Kinetics for Immobilized B. arnizus Particles with CaCO₃ Additive (RCA1 and RCA2)
The effects of particle size of the immobilized biomass on the mass transfer rates are shown in figure 6.36. The uranium uptake rate of smaller particles appears to be slightly faster at the beginning of the run and remains almost same as that of larger particles. More discussion on this will be given in the next section.

6-6-2 Application of the Kinetic Model and Parameter Estimation

The mass transfer model developed in section 5-2 was applied to the results of batch kinetic experiments. Two batch kinetic data (RGU1 and RGU1-) were regressed using a non-linear least square analysis routine (UWHAUS, McMaster University Computing Center). The estimated parameters are presented on table 6.13.

<table>
<thead>
<tr>
<th>Runs Parameters</th>
<th>RGU1</th>
<th>RGU1-</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_o$ (cm/s)</td>
<td>3.24E-4 ± 14.3%</td>
<td>3.44E-4 ± 15.5%</td>
</tr>
<tr>
<td>$D_p$ (cm²/s)</td>
<td>2.22E-6 ± 10.8%</td>
<td>1.28E-6 ± 14.5%</td>
</tr>
<tr>
<td>Sum of Squares</td>
<td>5.01E-4</td>
<td>2.15E-4</td>
</tr>
<tr>
<td>effective membrane (cm²/s)</td>
<td>2.27E-6</td>
<td>2.41E-6</td>
</tr>
<tr>
<td>diffusion coefficient</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The effective membrane diffusion coefficients were estimated by the overall mass transfer coefficients ($k_o$) and the coating membrane thickness ($δ$). It was assumed in this calculation that the external fluid mass transfer resistance would be negligible compared to the resistance across the coating membrane. The overall mass transfer coefficients estimated by regression analysis appear to be several orders of magnitude smaller than the external fluid mass transfer coefficient which would be obtained for for particles without coating.
Figure 6.36  Uranium Biosorption Kinetics (RGU1 and RGU1-)
membrane in a similar reactor with high agitation. As expected, the effective membrane diffusion coefficients of the immobilized biomass particles that were produced in the same batch but have different particle size are pretty close. The effective pore diffusion coefficient of the larger sample (RGU1) is almost same as the effective membrane diffusion coefficient. However, the effective pore diffusion coefficient of the smaller particles (RGU1-) is almost half of the effective membrane diffusion coefficient. The smaller effective pore diffusivity for the RGU1- might be attributed to the denser biomass core of the smaller particle which was formed during preparation of the biomass particles.

Figure 6.37 shows comparison of experimental data with the values predicted by the model for the batch kinetic run (RGU1). The model predicted the experimental data reasonably well at the beginning of the run and underestimate the equilibrium uranium uptake capacity of the immobilized biomass particles. The model prediction of experimental data of a kinetic run for immobilized biomass particles (RGU1-) is presented in figure 6.38. The data points are quite well matched with the values predicted by the model. A slight underestimation of uptake capacity of the immobilized biomass particle is also observed. The poor prediction of the model to the data points at later part of the kinetic run might be due to the relatively small number of data points which were used in the regression analysis.

6-6-3 Uranium Elution Kinetics

The elution from the uranium loaded immobilized biomass particles was carried out in the batch kinetic reactor. The uranium loaded particles which were recovered and dried at 23±1°C after the uranium kinetic run (RGU1-) were contacted with 2000 mL of 0.1 N NaHCO₃ in the reactor. The eluant NaHCO₃ was chosen because of its effectiveness for recovery of uranium from the uranium loaded R. arrhizus. Complete elution of uranium with 0.1 N NaHCO₃ solution was reported even at high solid to liquid ratios (Tsezos, 1984). In the
Batch Kinetic Run: RGU
Model Parameters: $D_p = 2.2 \times 10^{-6} \text{cm}^2/\text{s}$
$k_0 = 3.24 \times 10^{-4} \text{cm/s}$

Figure 6.37: Comparison of Biosorption Kinetic Data with Model (RGU)
Figure 6.38  Comparison of Biosorption Kinetic Data with Model (RGU1-)

Batch Kinetic Run: RGU1-
Model Parameters: $D_p = 1.28 \times 10^{-6} \text{cm}^2/\text{s}$
$K_o = 3.44 \times 10^{-4} \text{cm/s}$
reactor 2.0794 g of uranium loaded immobilized biomass particles containing total 177.7 mg of uranium was charged at the beginning of run. The final uranium concentration of the eluant was 89.0 mg U/L resulting in recovery of total 178.0 mg of uranium. Almost 100% uranium recovery was obtained.

Figure 6.39 shows the bulk uranium concentration as a function of elution time. About 50% of uranium was eluted within 4 hours of run and complete elution of uranium took more than 48 hours. In figure 6.40, uranium biosorption kinetics (RGU1-) and elution kinetics are presented for comparison. It took approximately 30 hours for the immobilized biomass particles to be saturated with the uranium solution. Almost as twice as long a time was required to elute the uranium from the immobilized biomass particles. This might be due to the facts that the eluant must diffuse through the coating membrane and interact with adsorbed uranium and then diffuse back to the bulk solution. Uranium desorption rate was expected to be very rapid and not a limiting step.
Figure 6.40  Comparison of Uranium Biosorption and Elution Rates
CHAPTER SEVEN
CONCLUSIONS AND RECOMMENDATIONS

The following conclusions were drawn from the experimental work on the preparation of immobilized biomass particles;

1. *R. arrhizus* mycelia grew forming discrete pellets during the fermentation and the fermentor culture solution pH decreased from the initial pH 5.8 to the pH 5.3 and then increased back to the original pH at the end of fermentation.

2. The average yield of dry *R. arrhizus* mycelia was 11.0 g based per one liter of the culture solution after subtracting the mass of calcium carbonate powder which was entrapped within the mycelia particles.

3. The spouted bed coating system developed in this study has been successfully used for the immobilization of biomass particles.

4. A coating solution formulation containing polyvinylformal, tetrahydrofuran, and pore forming additives such as glucose and calcium carbonate provided a strong porous membrane around biomass particles.

5. It is believed that only the solvent was evaporated while the polymer and additive were deposited onto the particle surface since the weight gain of the biomass particles after coating agreed quite well with the mass of polymer and pore forming additive in the coating solution sprayed during the coating.

The results of characterization of immobilized biomass particles led to the following conclusions;
1. The calcium carbonate leaching kinetics of coated biomass particles showed typical secondary increase of leaching rate indicating that the calcium carbonate powder inside the biomass particle was being leached out.

2. The leaching of glucose additive from the coated biomass particles was rapid and completed within 2 hours in most cases but the calcium carbonate inside the biomass particles was not leached out at the pH 7 leaching solution.

3. The slow leaching of calcium carbonate was believed to be attributed to both diffusion and dissolution rates.

4. The mass percentage of polymeric membrane material in the immobilized biomass ranged from 8.4% to 14.0%.

5. The average mass percentage of calcium carbonate in the biomass particles was calculated to be about 30%.

6. The apparent density of wetted immobilized biomass particles (with glucose additive) was 0.773 g/cm³.

7. The coating membrane thickness of immobilized biomass particles was estimated by the two-concentric sphere model and ranged from 47 μm and 86 μm.

8. The surface of immobilized biomass particles with glucose additive appeared more rough and nonuniform than that of immobilized biomass with calcium carbonate additive and the membrane appeared to be porous on the surface as well as across it.

9. The calcium carbonate crystals inside of the immobilized biomass particles which were not subject to the acid leaching were clearly observed as cubes and identified by x-ray analysis.
10. The uranium taken up by immobilized *R. arrhizus* mycelia was identified as a clear uranium peak by X-ray analysis and its peak disappeared when uranium was eluted from the biomass by sodium bicarbonate.

11. The pressure drop in immobilized biomass packed column increased almost linearly up to the superficial velocity 16 cm/min. and comparable to that in a column packed with granulated activated carbon and the immobilized biomass particles with calcium carbonate additive were more compression resistant than those with glucose additive.

The following conclusions were drawn from the mass transfer modelling and kinetic studies;

1. The maximum uranium loading of unconditioned *R. arrhizus* particles was about 260 mgU/g which is about 30% less than that of thoroughly washed and conditioned *R. arrhizus* powder, and this is believed to be due to calcium carbonate inside the unconditioned biomass particles.

2. The uranium biosorption data were quite well fitted to the Freundlich and Langmuir isotherms.

3. Uranium uptake capacity of immobilized *R. arrhizus* with calcium carbonate additive was much less than that of native biomass mainly because of the acid conditioning of the biomass during the calcium carbonate leaching at the pH 1.67.

4. Uranium uptake capacity of immobilized *R. arrhizus* with glucose additive was about 40% of that of native biomass particle and this might be due to suppression of uranium uptake by the dissolved carbonate ions present inside immobilized biomass particles.
The uranium was completely eluted from the uranium loaded immobilized *R. arrhizus* particles by 0.1 N NaHCO₃ solution and the elution rate was approximately twice as slow compared to biosorption rate under similar conditions.

The mass transfer model developed for the immobilized biomass particle in this study was successfully used for estimation of mass transfer parameters such as effective pore diffusion coefficient and overall external mass transfer coefficient.

The estimated effective pore diffusion coefficient was close to the effective membrane diffusion coefficient calculated from the overall mass transfer coefficient and the thickness of the coating wall membrane.

The immobilization method developed in this study can be applied to various types of biomass and other adsorbents but following further studies are recommended for refinement of the method and better understanding of mass transfer mechanisms in the immobilized biomass particles.

1. *R. arrhizus* is to be grown without the calcium carbonate buffer under the controlled pH nutrient media such that adverse effects of the calcium carbonate inside biomass particle on the uranium uptake capacity of the biomass could be eliminated.

2. In order to improve the pellet formation of the *R. arrhizus* mycelia during the fermentation, a lift type fermentor is to be employed for uniform mixing of the fermentation broth which will produce more uniform and smaller biomass pellets.
3. The optimization of growth media and conditions for maximum uranium uptake capacity and their physiological mechanisms will be closely examined to understand uranium uptake mechanisms and to further lead to identification and production of synthetic adsorbents for heavy metal separation from aqueous solution.

4. Further screening of microorganisms which have higher uranium uptake capacity and selectivity in the presence of competing ions will be required in order to apply the biosorption to actual process streams. The microorganisms which are naturally mutated and/or genetically engineered could be employed for uranium and other metal separations from process waters.

5. Different polymeric materials should be tried as coating membrane to increase the mechanical strength of the immobilized biomass particles while maintaining proper mass transfer rate.

6. Different types of pore forming additives which have high solubility in neutral pH and uniform small size should be tried to improve coating membrane strength and mass transfer rate.

7. The effects of coating membrane thickness on the mass transfer rate and mechanical strength of immobilized biomass particles should be further investigated.

8. The effects of mass percentage of pore forming additive with regard to polymer in the coating solution on the mass transfer rate and mechanical strength of the immobilized biomass particles should be further investigated.

9. A mass transfer model which will take account for the elution of uranium from the loaded immobilized biomass particles is to be developed and compared with experimental data.
10. The mass transfer model for immobilized biomass particles packed bed column system is to be developed and compared with experimental data.

11. Immobilization of other microorganisms and adsorbents should be tried by the new immobilization technique developed in this study in order to verify the applicability of this method.

12. Granulation of microorganisms which do not form pellets during fermentation should be further investigated in order to apply the developed immobilization technique to those microorganisms.

13. Immobilization of live microorganisms which could be used for production of chemicals would be attempted by the developed immobilization method with some modifications.

14. A large scale production of immobilized biomass particles by this method and industrial application of immobilized biomass particles are to be tried.
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Figure A.1  Air Flow Rate Calibration Curve for the Suspension Air Rotameter
Figure A.3  Water Flow Rate Calibration Curve for the Packed Column Rotameter
APPENDIX B COMPUTER PROGRAMS

Appendix B-1 Computer Simulation Programs for Batch Kinetic Runs
PROGRAM PLSIMU(INPUT,OUTPUT,TAPE3=INPUT,TAPE6=OUTPUT)
CHARACTER DATAFIL*7
PARAMETER (ND=4,NP=NP,NPROB=1)
REAL LI(10,10),LI2(10,10)
COMMON /ALP1/ROOT(NP),LI,LI2,VECT(NP)
COMMON /ATZ/ F,BETA,MAX
COMMON /ATZ/ DIA, W, DEN3, VOL, CIN, QIN
DIMENSION TH(NP)

C
KCON = 1
NSET = 3
READ(5,22) DATAFIL
FORMAT (A7)
OPEN (UNIT=10, FILE=DATAFIL)
READ(10,*), CIN,QIN,BETA
READ(10,*), DIA,W, DEN3, VOL
READ(10,*), (TH(I), I=1,NP)

C
CALL KINPLOT(TH,NP,KCON)
KCITY = KCON + 1
IF (KCITY .LE. NSET) GO TO 33
CALL PLOT(33,0,0,0,0)

C
STOP
END

C
SUBROUTINE FCN (N,T,Y,YPREG)
PARAMETER (ND=1,NP=NP,NPROB=1,NWK=3*ND+ND*ND)
REAL LI(10,10), LI2(10,10)
COMMON /ALP1/ROOT(NP), LI, LI2, VECT(NP)
COMMON /ATZ/ F, BETA, MAX
COMMON /ATZ/ DIA, W, DEN3, VOL, CIN, QIN
DIMENSION Y(N), YPREG(N), YCHECK(N)

DO 5 K=1,N
5 IF(Y(K) .LE. 0.0) THEN
   Y(K) = 1.0E-10
   YCHECK(K) = -1.0E-10
ELSE
   YCHECK(K) = 1.0E-10
ENDIF

ALPA = LI(N,1) + SHP/4.0
ALPHA = SHP/4.0/ALPA/F

DO 10 I=1,N
10 SUMT = 0.0
   SUMT = SUMT + LI(I,1)
   SUMT = SUMT + LI2(I,1)
   SUMT = SUMT + SUMT
   SUMT2 = SUMT2 + SUMT

20 IF(YCHECK(I) .LT. 0) THEN
   IF(YCHECK(I) .LT. 0) THEN
   GO TO 10
ENDIF

CONTINUE
RETURN
END
SUBROUTINE FCMJ (N, T, Y, PD)
DIMENSION Y(N), PD(N)
RETURN
END
SUBROUTINE COLL (N, ROOT, L1, L2, VECT)
DIMENSION DIF1(10), DIF2(10), DIF3(10), ROOT(10), VECT(10), X(10)
REAL L1(10), L2(10, 10)
AL=0.0
DE=0.9
DO 10 N=1, N
EP=NO-N1
CALL JACOBI (N, T, NO, N1, AL, 8E0, DIF1, DIF2, DIF3, ROOT)
DO 150 N=1, N
150 CONTINUE
ID=1
DO 5 J=1, NT
CALL DFORP (N, T, NO, N1, J, ID, DIF1, DIF2, DIF3, ROOT, VECT)
DO 250 N=1, NT
250 L(J)=-VECT(N) &
5 CONTINUE
ID=2
DO 6 J=1, NT
CALL DFORP (N, T, NO, N1, J, ID, DIF1, DIF2, DIF3, ROOT, VECT)
DO 255 N=1, NT
255 L(J)=VECT(N) &
6 CONTINUE
ID=3
CALL DFORP (N, T, NO, N1, J, ID, DIF1, DIF2, DIF3, ROOT, VECT)
RETURN
END
SUBROUTINE MODEL (T, NP)
PARAMETER (*N=4, NP=8, NWK=18*NO+NO+NO)
REAL L1(10, 10), L2(10, 10)
COMMON /A1/ ROOT(NP1), L1, L2, VECT(NP1)
COMMON /A2/ F, G, P, Q, PE, PY, PX
COMMON /AT/ W, T, OME, VOL, CIN, QIN
COMMON /PLOT/ XMAX, XMIN, YMAX, YMIN
EXTERNAL FCN, FCMJ
DIMENSION T(NP), Y(NP)
DIMENSION Y(N), K(NWK), I+K(NO)
C
EDIFF = T(2)
COFF = T(1)
C
RTL = XKLIM + np0 + np1*EDIFF*4+G/CIA/DIA
SHP = DIA*COFF/EDIFF
F = VOL/2+CIN/QIN
TMAX = CIN/QIN
C
M=NO-4
T0 = 1.0*DE-5
W = 1.0*DE-5
METH = I
INDEX = 1
CALL COLL (N, ROOT, L1, L2, VECT)
DO 10 I=1, N
10 Y(I) = 1.0*DE-10
DO 20 K=1, 100

DELT = RTL/100*(FLAT(I))
CALL ODGEAR(X*FCN,Y*FCN*T,HEMT,INDEX,INK,SWK,IER)
IF (IER .GT. 120) GO TO 99
SUN3 = 0.0
DO 30 I=1,N
IF (Y(I) .LE. 0.0) THEN
Y(I) = 1.0E-10
ENDIF
SLY3 = VECT(I)*Y(I)*BETA
SLY3 = SUN3 + SLY3
30 CONTINUE
YP(I) = 1.0 - SLY3/F
C 20 CONTINUE
RETURN
99 WRITE(6,60) IER
60 FORMAT(1X,13ERROR PARAMETER = ,I3)
RETURN
END
SUBROUTINE KINPLOT(T,H,P,KCON)
CHARACTER XAXIS*30,YAXIS*30
DIMENSION TH(NP),P(100),P(100)
COMMON /ATZ/ E,B,A,S,P,YMAK
COMMON /A4/ DNP,DT,GEN, VOL, CIN, JIN
COMMON /PLOT/ YLIM, YLIM, P(100)
XLW,YLIM
UPPER BOUNDS ON INPUT DATA MINUS 1.0 RESPECTIVELY
TITLE LABEL FOR GRAPH 33 CHARACTERS
YAXIS,XAXIS LABELS FOR GRAPH 30 CHARACTERS EACH
XL,YL LENGTHS OF X(Y) AXIS CM.
IF (KCON .GT. 1) GOTO 43
XL = 13.5
YL = 8.5
YLIM = 1.0
YAXIS = '* REDUCED CONC. (C/CO ) !
XAXIS = '* TIME HOURS ) !
SET PLOT SCALE TO CMS AND WRITE NAME DATE
CALL PLOT(10,10,20)
CALL NEWPEN(3)
CALL DRAW THE OUTER FRAME
CALL PLOT(0.0,1.0,0.3)
CALL PLOT(6.0,25.0,0.2)
CALL PLOT(13.0,0.2,0.2)
CALL PLOT(3.0,0.1,0.2)
CALL PLOT(0.0,1.0,0.2)
MOVE AND PAN PLACE AXIS AND SET PLOTTING LIMITS
CALL PLOT(-10.0,5.0,40)
DRAW THE AXIS AND COMPLETE THE EDGES OF THE GRAPH
CALL PLOT(0.0,0.3)
CALL PLOT(XL,0.2)
CALL PLOT(XL,YL)
CALL PLOT(0.0,YL)
CALL PLOT(0.0,0.2)
INDEX THE AXIS, TEN SEGMENTS ON XAXIS, FIFTEEN SEGMENTS ON YAXIS.
DO 10 I=1,9
RI=FLOAT(I)*YL/10
CALL PLOT((I-9)*PI/2)
10

DO 11 I=1,9
RI=FLOAT(I)*XL/15
CALL PLOT((I-1)*YL/3)
11

DO 12 I=1,9
RI=XL-(I-1)*FLOAT(I)/15
CALL PLOT((R-0.3)*R)
12

COUNT=2.*XL/15.
DO 20 I=1,18
AA=FLOAT(I-1).*COUNT
YA=2.*FLOAT(I-1)*XL/15-0.6.
TAKES=8.
20 CALL NUMBER((X+YA)*0.2*AA+3.3,6H(F5,1))
COUNT=YL/10.
DO 21 I=1,11
YA=FLOAT(I-1)*YL/10-0.1
TAKES=8.
AA=FLOAT(I-1).*COUNT
21 CALL NUMBER((X+YA)*0.2*AA+0.0,6H(F3,1))

CALL LETTER(30.0,3.0,0.0,2*XL-3.0,XAXIS)
CALL LETTER(30.0,3.0,9.0,1.0,0.2*YL-YAXIS)

DO 33 1=1,18
TP=1.*XL/10C0.*FLOAT(I)
33

DO 34 1=1,5
TP=1.*YL
CONTINUE

XP(1) = 0.0
YP(1) = 1.0*YL
CALL NEWPEN(KPCON)
CALL XPLOT(XP,YPT,100)
RETURN
END

SUBROUTINE NUMBER(X,Y,HEIGHT,ANUM,THETA,FMT)
INTEGER FMT
DIMENSION BCD(11)
C ENCODE IS A COMPUTER BUILT IN FUNCTION WHICH TRANSLATES NUMBER INTO ENCODED SYMBOL WHICH CAN UNDERSTAND
C INTEGER (X,Y,HEIGHT,BCD,THETA,10)
RETURN
END

SUBROUTINE JACOBI(ND,NX,NO,N1,AL,BE,DF1,DF2,DF3,ROOT)
DIMENSION DF1(ND),DF2(ND),DF3(ND),ROOT(ND)
C EVALUATION OF ROOTS AND DERIVATIVES OF JACOBI POLYNOMIALS
C PNT (AL,BE)
C FIRST EVALUATION OF COEFFICIENTS IN RECURSION FORMULAE
C RECURSION COEFFICIENTS ARE STORED IN DF1 AND DF2

AB=AL+BE
AD=BE+AL
AP=BE+AL
DF1(I)=(AD/(AB+2.0)+1.0)/2.0
DF2(I)=0.0
IF(NL=2) GO TO 15
DO 10 I=2,N
II=I-1
Z=AB+2.0
DF1(I)=(AB+AP+II)/(Z+2.0)+1.0)/2.0
IF(I=NE.2) GO TO 11
DF2(I)=(AB+AP+II)/(Z+1.0)
CONTINUE
10 CONTINUE
Z=(AB+II)
Y=Y*(APY)
DF2(I)=Y/Z/(Z-1.0)
CONTINUE

ROOT DETERMINATION BY NEQN METHOD WITH SUPPRESSION
OF PREVIOUSLY DETERMINED ROOTS
C CONTINUE
X=0.0
DO 20 I=1,N
X=0.0
XI=R
XI=1.0
DO 30 J=1,N
XP=DF1(J-1)+X+DF2(J)*X
XP=DF1(J-1)+XN+DF2(J)*X+XN
X=XN
XI=XI+1
XI=XP
30 CONTINUE
CONTINUE
GO TO 50

CONTINUE
DO 25 J=1,MT
X=ROOT(J)
AX=X/(1-X)
IF(X.EQ.0) AX=AX/X
IF(X.LT.1.0) AX=AX/((1-X)/(1-X))
VECT(J)=AX/DIF(J)**2
Y=Y+VECT(J)

CONTINUE
DO 60 J=1,MT
VECT(J)=VECT(J)/Y

CONTINUE
RETURN
END
Appendix B-2 Computer Programs for Parameter Estimation of Mass Transfer

Model from Batch Kinetic Data
PROGRAM REGKINE (INPUT, OUTPUT, TAPE5=INPUT, TAPE6=OUTPUT)
CHARACTER DATAFIL(7)
PARAMETER (ND=4, NP=NO=1, WK=10+ND+NO+ND)
PARAMETER (NP=2, NPROB=1)
REAL L1(10,10), L2(10,10)
COMMON /AI1/POST(NP), L1, L2, VECT(NP)
COMMON /AI2/ F, Beta, LPHA, MAX
COMMON /AT1/TIME(30), T(30)
COMMON A14/ AT, WT, DENS, VOL, CIN, QIN
DIMENSION TH(NP), SIG(TH(NP)), DFF(NP), SCRAT(300)
DIMENSION RCON(30), CONS(30)
EXTERNAL MODL, FCN, FCNJ
DATA EPS1, EPS2, MAT, FLAM, FNU/I, OE-3, I, OE-3, 10, 0, 1, 10, 0/
READ(22) DATAFIL
22 OPEN (UNIT=10, FILE=DATAFIL)
READ(10*) NOB
READ(10*) CIN, QIN, BETA
READ(10*) DIA, WT, VOL, DENS
READ(10*) (TH(I), I=1,NP)
DO 10 I=1,NOB
READ(*), TIME(I), CONS(I)
RCON(I)=CONS(I)/CIN/I*OE-6
10 CONTINUE
DO 5 I=1,NP
DIFF(I)=0.0
SIG(I)=1.0
5 CONTINUE
CALL WMAUS (NPROB, MODEL=NP, RCON, NP, T4, DFF, 
SIG, EPS1, EPS2, MAT, FLAM, FNU, SCRAT)
STOP
END
SUBROUTINE FCN (N, T, TPRIME)
PARAMETER (ND=4, NP=NO=1, WK=10+ND+NO+ND)
REAL L1(10,10), L2(10,10)
COMMON /AI1/POST(NP), L1, L2, VECT(NP)
COMMON /AI2/ F, Beta, LPHA, MAX
COMMON /AT1/ T, WT, DENS, VOL, CIN, QIN
DIMENSION Y(NP), TPRIME(NP), YCHECK(NP)
DO 5 K=1,N
IF(Y(K) .LE. 0.0) THEN
Y(K) = 1.0E-10
YCHECK(K) = -1.0E-10
ELSE
YCHECK(K) = 10.0
END IF
CONTINUE
ALPHA = L1(N+1,N+1) + SHP*/4.0
SHP*/4.0/ALPHA
5 CONTINUE
DD 10 I=1,N
DO = 4.0*RODIT(I)*L2(I,N+1) + 6.0*L1(I,N+1)
DD 10 = 0.0
DO 20 J=1,N
C = 4.0*RODIT(I)*L2(I,J) + 4.0*L1(I,J)
SUM1 = C/ALPHA + ALPHA /Y(I)^J*J
SUM2 = 4.0*ALPHA*VEC(I)*J*W(T)*BETA
SUM1 = SUM1 + SUM2
SUM2 = SUM2 + SUM1
20 CONTINUE
IF(YCHECK(I) .LT. 0) THEN
CONTINUE
ENDIF
C YPRIME(I) = Q*C
C GOTO 10
E NDFP

C YPRIME(I) = Y(I)**(1.0 - ETA)/GAMMA*YMAX*(SUM1-SUM2*0.0*SHP**4/ALPHA)
C CONTINUE
C RETURN
E NDFP

C SUBROUTINE FCN(N,Y,P)
C DIMENSION Y(N),P(N)
C RETURN
E NDFP

C SUBROUTINE COLN(N,ROOT,LI,L2,VECT)
C DIMENSION DIF1(10), DIF2(10), DIF3(10), ROOT(10), VECT(10)
C REAL LI(10), L2(10)
C AL = 0.0
C BE = 0.5
C NO = 0
C N1 = 1
C NT = N + NO + N1
C CALL JACOB(Y,NT,N,N0,N1,AL,BE,DIF1,DIF2,DIF3,ROOT)
C DO 150 I = L1,NT
C X(I) = SQRT(ROOT(I))
C CONTINUE
C ID = 1
C DO 250 J = L1,NT
C CALL DIFPR(N,N0,N1,N,J,10,DIF1,DIF2,DIF3,ROOT,VECT)
C CONTINUE
C ID = 2
C DO 255 J = L1,NT
C CALL DIFPR(N,N0,N1,N,J,10,DIF1,DIF2,DIF3,ROOT,VECT)
C CONTINUE
C ID = 3
C CALL DIFPR(N,N0,N1,N,J,10,DIF1,DIF2,DIF3,ROOT,VECT)
C RETURN
E NDFP

C SUBROUTINE MODEL(NP,TH, Y,B,DATA,NP,DATA)
C REAL L1(10), L2(10)
C COMMON /A1/ F, ETA, SHP, YMAX
C COMMON /A3/TIME(50), RT(50)
C COMMON /A4/ DIA, DF, DENS, VOL, CIN, QIN
C EXTERNAL FCN, FCN'
C DIMENSION TH(NP), Y(NP)
C DIMENSION Y(NH), K(NW), K(N)
C EDITF = T + (2)
C COFF = T + (1)

C DO 15 I = 1,NOB
C EDITF = TIME(I)**B0*EDITF**B1*DIA/DIA
C CONTINUE
C NP = DIA*COFF/EDITF-J
C VOL/DF*QIN/G
C YMAX = CIN/G
C N = NO
C I = 0.0
C TOL = 1.0E-6
C N = 1.0E-8
METH = 1
MITER = 0
INDEX = 1
CALL COLLN, ROOT, L1, L2, VECT
DO 10 I = 1, N
10 Y(I) = 1.0E-10
DO 20 K = 1, N0B
DEL(1, K) = AT(I, K)
CALL DGEAR(N, FCN, FCN, T, Y, DEL, TOL, METH, MITER, INDEX, IVK,
   + C, IEPR)
IF(IEPR .GT. 128) GO TO 99
SUMT3 = 0.0
DO 30 I = 1, N
IF( Y(I) .LE. 0.0 ) THEN
   Y(I) = 1.0E-10
ENDIF
SUMT3 = VECT(I) .times. Y(I) .times. BETA
SUMT3 = -SUMT3 + SUM3
30 CONTINUE
YB(I) = 1.0 - SUM13/3
CONTINUE
RETURN
99 WRITE(6, 60) IEPR
60 FORMAT(*10X, 10X ERROR PARAMETER = *, 13)
RETURN
END

SUBROUTINE JACOBI(N, N, NG, N1, AL, BE, DIF1, DIF2, DIF3, ROOT)
DIMENSION DIF1(N), DIF2(N), DIF3(N), ROOT(N)

EVALUATION OF ROOTS AND DERIVATIVES OF JACOBI POLYNOMIALS

FIRST EVALUATION OF COEFFICIENTS IN RECURSION FORMULAE.
RECURSION COEFFICIENTS ARE STORED IN DIF1 AND DIF2.

A2 = AL + BE
AD = BE + AL
AP = S2 = AL
DIF1(I) = (AD/(A2+Z2)+1)/Z
DIF2(I) = 0
IF(N .LT. 2) GO TO 15
DO 10 I = 2, N
Z = 1
Z = AB .times. Z
DIF3(I) = (AB .times. AD/((A2+Z2)+1)/Z
IF(N .LT. 2) GO TO 11
DIF2(I) = (AB .times. AP+Z2)/Z
11 CONTINUE
Z = 1
Z = (AB+Z1)
Y(Y) = AP(Y)
DIF3(I) = Y/Z/(Z-1)
10 CONTINUE

ROOT DETERMINATION BY NEWTON METHOD WITH SUPPRESSION
OF PREVIOUSLY DETERMINED ROOTS

CONTINUE
IN = 1
DO 20 IN = 1, N
CONTINUE
A0 = 0
XN = 1
X01=0.
XN1=0.
DO 30 J=1,N
XP=(DIF1(J)-X)*XN-DIF2(J)*X01+X01
XPI=(DIF1(J)-X)*XN1-DIF2(J)*X01-XN
X01=XN
XN1=XP
XN=XP
CONTINUE
30
IF(I.EQ.1) GO TO 21
DO 21 J=1,N
Z=Z'-1/(X-ROOT(J-1))
CONTINUE
21
IF(CABS(Z)GE1.E-9) GO TO 25
ROOT(J)=Z
X01=0.001
CONTINUE
25
ADD EVENTUAL INTERPOLATION POINTS AT X=0 OR X=1
NT=N+NO+1
IF(NO.EQ.0) GO TO 35
DO 31 J=1,NO
JN=1
ROOT(J+1)=ROOT(J)
CONTINUE
31
ROOT(1)=0.
35
CONTINUE
IF(N1.EQ.1) ROOT(1)=1.
NOW EVALUATE DERIVATIVES OF POLYNOMIAL
DO 40 I=1,NT
X=ROOT(I)
DIF1(I)=0.
DIF2(I)=0.
DIF3(I)=0.
DO 40 J=1,NT
IF(I.EQ.J) GO TO 40
Y=X-ROOT(J)
DIF3(I)=Y*DIF3(I)+2.*DIF2(I)
DIF2(I)=Y*DIF2(I)+2.*DIF1(I)
DIF1(I)=Y*DIF1(I)
CONTINUE
RETURN
END
SUBROUTINE DIFPR(NO,N,NO,N,1,0,DIF1,DIF2,DIF3,ROOT,VECT)
DIMENSION DIF1(NO),DIF2(NO),DIF3(NO),ROOT(NO),VECT(NO)
SUBROUTINE EVALUATE DISCRETIZATION MATRICES AND
GAUSSIAN QUADRATURE WEIGHTS AND NORMALIZED TO SUM 1
ID=1: DISCRETIZATION MATRIX FOR T(i) (X)
ID=2: DISCRETIZATION MATRIX FOR Y(z) (X)
ID=3: GAUSSIAN QUADRATURE WEIGHTS
NT=N+NO+1
IF(10.EQ.1) GO TO 10
DO 20 J=1,NT
IF(J.NE.1) GO TO 21
20
CONTINUE
21
21
IF(I0 NE 1) GO TO 5
    VECT(1) = DIF2(1)/DIF1(1)/Z1
    GO TO 20
20 CONTINUE
    VECT(1) = DIF3(1)/DIF1(1)/Z1
    GO TO 20
21 CONTINUE
    T = ROOT(J)
    VECT(J) = DIF1(J)/DIF1(J)*Y1
    IF(I0 EQ 2) VECT(J) = VECT(J)*DIF2(1)/DIF1(1)-Z1/Y1
    GO TO 20
10 CONTINUE
    Y = 0
    DO 25 J = 1, NT
        X = ROOT(J)
        AX = X(1, X)
        IF(AX EQ 0) AX = AX/Y1
        IF(AX EQ 0) AX = AX/(1-X1)/(1-X1)
        Y = Y + VECT(J)
    25 CONTINUE
    DO 60 J = 1, NT
        VECT(J) = VECT(J)/Y1
    60 CONTINUE
    RETURN
END

SUBROUTINE HAUS(NP, PROB, MODE, NOB, Y, NP, TH, DIFF, SIGNS,
                 EPS1, EPS2, MI, FNU, SCRAT)
DIMENSION SCRAT(I)

A = 1
B = A + NP
C = B + NP
D = C + NP
E = E + NP
F = F + NP
G = G + NOB
H = H + NOB
I = I + NP + NOB
J = I + NP + NOB

RETURN
END

SUBROUTINE HAUSQ(NP, PROB, MODE, NOB, Y, NP, TH, DIFF, SIGNS,
                 EPS1, EPS2, MI, FNU, GPRM, PHM, SPM, FPRM, DELZ)
DIMENSION TH(1), DIFF(1), SIGNS(1), Y(1), A(1), P(1), E(1),
        Q(1), O(1), T(1), F(1), R(1), X(1), DELZ(1)
OACOS(X) = ATAN10QRT(-1.0000+X**2-1.0000)**
NP = NP + NP + NP + NP + NP + NP + NP + NP + NP + NP
    eps1 = eps1 + eps1
    eps2 = eps2 + eps2
    npsq = npsq + npsq
    scrac = scrac + scrac
    wri = wri + wri
    npsq = npsq + npsq
    scrac = scrac + scrac
    write '16610410' npsq, npsq, scrac, scrac, write '16610410'
call gauss(1, np, temp, temp)
WRITE (06, 1002)
CALL GASS80 (I, NP, DIFZ, TEMP, TEMP)
IF (FNU - 1) 100, 400, 99, 99, 15
15
CONTINUE
DO 19 I = 1, NP
TEMP = DABS (DIFZ (I))
19 CONTINUE
IF (DOMINI (1.0D0 - TEMP, DABS (TH(I)))) 99, 99, 19
573
CALL MODQ (NPROB, TH, F, NOB, NP)
DO 90 I = 1, NOB
90 SSO = SSO + (I) * R(I)
WRITE (06, 1003) SSO
100 GA = GA + FNU
IMINT = 0
WRITE (06, 1004) NIT
101 JS = JS + NOB
DO 120 J = 1, NP
TEMP = TH(J)
P(I) = DIFZ (I) + TH(J)
TH(I) = TH(I) + P(I)
GUJ = 0.
JS = JS + NOB
120 CALL MODEQ (NPROB, TH, DELZ (JS), NOB, NP)
JS = JS + 1
DO 130 J = 1, NOB
130 UN = UN + 1
SUM = SUM + NOB
KJ = NOB * (J - 1)
KI = NOB * (J - 1)
DO 140 K = 1, NOB
140 XJ = KJ + 1
SUM = SUM + DELZ (KJ) * DELZ (KJ)
TEMP = SUM / (PI (I) * PJ (I))
DO 150 I = 1, NP
150 DJ = DJ + 1
CONTINUE
DO 193 J = 1, NP
193 CONTINUE
AI(J) = (I) / (I) * E(J))
J = J + NP
AI(J) = A(J)
II = II + NP
DO 193 J = 1, NP
A = SCALED MOMENT MATRIX
P(I)*O(I)/E(I)
P(I)*P(I)
II = NP + 1 + II
A(I) = A(I) + GA
I = 1
CALL MATIN(A, NP, P, I, DET)
STEP = 1.0
SUM0 = 0
SUM2 = 0
DO I = 1, NP
SUM = P(I) + SUM
SUM2 = SUM + SUM2
PHI(I) = PHI(I) + SUM
231 TEMP = 1/SUM
TEMP = D3RT(SUM2, T3)
TEMP = 0.01/TEMP, I = 10000
TEMP = 0.72550014DAOSI/TEMP
WRITE(0, 164) DET, TEMP
170 DO 220 I = 1, NP
P(I) = PHI(I) * STEP / E(I)
T(I) = TH(I) + P(I)
220 CONTINUE
WRITE(0, 7000)
7000 FORMAT ('SOLUTIONS POINT PARAMETER VALUES')
WRITE(0, 2000) (T(B(I), I = 1, NP)
DO 222 I = 1, NP
IF (SIGN(I)) 221, 222, 222
221 CONTINUE
SUM9 = 0
CALL MODPL(NP, NP, TB, F, X0B, NP)
DO 230 I = 1, NP
R(I) = Y(I) - F(I)
SUM = SUM + R(I) * R(I)
WRITE(0, 1043) SUM
IF (SUM > (1.00 + EPS1) * SUM) 662, 662, 663
663 CONTINUE
WRITE(0, 1043) SUM
IF (SUM > (1.00 + EPS1) * SUM) 662, 662, 663
662 WRITE(0, 1043) SUM
DO 230 I = 1, NP
TH(I) = TB(I)
CALL GAS5001(I, NP, T, TEMP, TEMP)
WRITE(0, 1040) GA, SUM
629 IF (EPS1) 230, 230, 239
230 DO 240 I = 1, NP
TH(I) = TH(I) + TH(I) + EPS2
240 CONTINUE
WRITE(0, 1040) EPS1, EPS2
GO TO 280
IF (EPS1) 241, 241, 241
241 GO TO 280
255 IF (EPS1 < EPS2) 256, 256, 257
256 WRITE(0, 1010) EPS1
GO TO 280
270 EPS1 = SUM
NIT = NIT + 1
IF (NIT = MIT) 100, 100, 280
2790 WRITE(6,1011)
2791 WRITE(6,2001) (F(I),I=1,NB)
2792 WRITE(6,2012)
2793 WRITE(6,2001) (*1I=1,NB)
2794 WRITE(6,1017)
2800 FORMAT(/11H XPRIME-K MATRIX/1)
2801 CALL GASS6G(*NP,TEMP,TEMP,D)
2802 SSO=SSO-IND
call MATIN(D, NP, P, I, DET)
2803 DO 7692 I=1,NP
2804 7692 E(I)=OSQRT(D(I))
2805 DO 340 J=1,NP
2806 J=J+1
2807 IF(J.EQ.1) THEN
2808 CALL GASS6G(*NP, TEMP, TEMP, A)
2809 IF(IF.DF) THEN
2810 SDEV=SSQ/OSQRT(IF)
2811 WRITE(6,1016)
2812 DO 341 I=1,NP
2813 P(I)=TH(I)+E(I)*SDEV
2814 WRITE(6,1039)
2815 CALL GASS6G(*NP, TEMP, T8, P, TEMP)
2816 GO TO 101
2817 DO 414 K=1,NCR
2818 TEMP=0.0
2819 DO 420 J=1,NP
2820 DEBUG2=DEBUG3=DEBUG4=DELZ(1,1)
2821 DEBUG1=DELZ(K+NOB*J-1)
2822 DEBUG2=DELZ(K+NOB*J-1)
2823 DEBUG3=D(I,J)+(DEBUG1*DEBUG2*DEBUG3)/SDEV
2824 TEMP=TEMP+DEBUG3
2825 WRITE(6,1000)
2826 IF(NOB-IE) 430,435,436
2828 IF(IE) 430,435,436
2829 WRITE(6,2001) (F(J),J=1,NB)
2830 WRITE(6,2001) (F(J),J=1,IE)
WRITE(C0,1033) NPROB
RETURN
GO TO 430
1000 FORMAT(36HNON-LINEAR ESTIMATION, PROBLEM NUMBER 1))
1001 FORMAT(36H0 OBSERVATIONS, I5, 11H PARAMETERS, I4, 17H SCRATCH REQUIRED)
1002 FORMAT(36H0 HOPROPORTION, USED IN CALCULATING DIFFERENCE QUOTIENT, I0)
1003 FORMAT(36H0 HINITIAL SUM OF SQUARES = E12.4)
1004 FORMAT(36H0 HINITIAL ITERATION NO. = I4)
1007 FORMAT(36H0 HOPARAMETER VALUES VIA REGRESSION)
1008 FORMAT(36H0 HAPPROXIMATE CONFIDENCE LIMITS FOR EACH FUNCTION VAL
1009 FORMAT(36H0 HINITIATION STOPS - RELATIVE CHANGE IN EACH PARAMETER LE
1010 FORMAT(36H0 HINITIATION STOPS - RELATIVE CHANGE IN SUM OF SQUARES LE
1011 FORMAT(36H0 HFINAL FUNCTION VALUES)
1012 FORMAT(36H0 H0 RESIDUALS)
1013 FORMAT(36H0 H0 VARIANCE OF RESIDUALS = E12.4, 1H, 14H, 0)
1014 FORMAT(36H0 HDegrees OF FREEDOM)
1015 FORMAT(36H0 HCCORRELATION MATRIX)
1016 FORMAT(36H0 HEND OF PROBLEM NO. 13)
1034 FORMAT(36H0 HOMICPARAMETER ERROR)
1039 FORMAT(36H0 HINDIVIDUAL CONFIDENCE LIMITS FOR EACH PARAMETER (ON LI
1040 FORMAT(36H0 H10000000A = E10.3, 40X, 33HSUM OF SQUARES AFTER REGRESSION = 1E5.
1041 FORMAT(36H0 HDETERMINANT = E12.4, 6X, 25H ANGLE IN SCALED COCO.
1043 FORMAT(36H0 H0TEST POINT SUM OF SQUARES = E12.4)
1045 FORMAT(36H0 H0RESIDUE)
0 subroutine matm(a, nvar, b, nb, det)
0 dimension a(nvar, 1), b(nvar, 1)
0 pivot = a(1,1)
0 det = 1.00
0 do 350 icol = 1, nvar
0 pivot = a(icol, icol)
0 det = det * pivot
0 do 350 l = 1, nvar
0 a(icol, l) = a(icol, l) * pivot
0 do 372 l = 1, nvar
0 a(l, l) = b(l, b(l) * pivot
0 reduce non-pivot 371
0 do 550 l = 1, nvar
0 if (l-icol) < 1, 550, 551
0 t = a(l, icol)
0 a(l, l) = a(l, l) / t
0 do 450 l = 1, nvar
0 a(l, l) = a(l, l) * t
0 do 552 l = 1, nvar

500 8(L1, L) = 8(L1, L) - 8(ICOL, L)*T
530 CONTINUE
535 RETURN
540 END
550 SUBROUTINE GAS(SG, ITYPE, NQ, A, B, C)
555 IMPLICIT REAL*8(A-H, O-Z)
560 DIMENSION A(NQ), B(NQ), C(NQ, NQ)
565 NQ = NO
570 NR = NP/10
575 LOW = 1
580 LUP = 10
585 IF (NR .GT. 15, 20, 30)
590 RETURN
595 IF (LUP) 30, 30, 15
600 WRITE(6, 500) (J, J = LOW, LUP)
610 GO TO 40
620 WRITE(6, 600) (A(J), J = LOW, LUP)
630 GO TO 100
640 WRITE(6, 600) (B(J), J = LOW, LUP)
650 GO TO 40
660 IF (ITYPE .GT. 4) GO TO 70
670 DO 90 I = LOW, LUP
680 WRITE(6, 70) (I, J, J = I, L)
690 END DO 90
700 DO 70 I = 1, LDB
710 WRITE(6, 721) I, (C(I, J), J = LOW, L)
720 CONTINUE
730 LOW = LUP + 1
740 IF (LOW .GT. NP) 96, 95, 100
750 CONTINUE
760 WRITE(6, 720) I, (C(I, J), J = LOW, LUP)
770 GO TO 100
780 DO 98 I = 1, LDB
790 WRITE(6, 721) I, (C(I, J), J = LOW, LUP)
800 END DO 98
810 LOW = LUP + 10
820 LUP = LUP + 10
830 NR = NR - 1
840 GO TO 10
850 FORMAT(18E11.2)
860 FORMAT(10E12.4)
870 FORMAT(10E13.4, 6E12.4)
880 CONTINUE
890 RETURN
900 END
Table C.1 Uranium Biosorption Isotherm data for Native "arrhizus" Particles

<table>
<thead>
<tr>
<th>Ce (mg U/L)</th>
<th>qe (mg U/g)</th>
<th>Ce (mg U/L)</th>
<th>qe (mg U/g)</th>
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</thead>
<tbody>
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<td>4.5</td>
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<td>1.7</td>
<td>66.7</td>
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<tr>
<td>5.8</td>
<td>94.9</td>
<td>56.5</td>
<td>187.1</td>
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<td>8.0</td>
<td>95.8</td>
<td>228.5</td>
<td>253.8</td>
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<td>737.8</td>
<td>260.7</td>
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<td>145.9</td>
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<tr>
<td>90.5</td>
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Table C.2 Uranium Desorption Isotherm Data for Native "arrhizus" Particles

<table>
<thead>
<tr>
<th>Ce (mg U/L)</th>
<th>qe (mg U/g)</th>
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<tbody>
<tr>
<td>1.7</td>
<td>96.6</td>
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<tr>
<td>1.5</td>
<td>130.5</td>
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<tr>
<td>10.8</td>
<td>175.1</td>
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Table C-3  Uranium Biosorption Isotherm Data for Immobilized *R. arrhizus* Particles

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<td>79.0</td>
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<td>211.7</td>
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<td>253.1</td>
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<td>261.1</td>
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<tr>
<td>270.4</td>
<td>97.6</td>
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APPENDIX D URANIUM UPTAKE AND ELUTION KINETIC DATA
### Table D-1  Uranium Uptake Batch Kinetic Data for Native \textit{R. arrhizus} Particles (14/30)

<table>
<thead>
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<th>TIME (min.)</th>
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<tr>
<td>180.0</td>
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<tr>
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### Table D-2  Uranium Uptake Batch Kinetic Data for Immobilized \textit{R. arrhizus} Particles (RCA1,16/20)

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<th>TIME (min.)</th>
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Table D-3  Uranium Uptake Batch Kinetic Data for Immobilized *R. arrhizus* Particles

(RCA2,16/20)

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<th>TIME (min.)</th>
<th>$C_b$ (mgU/L)</th>
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<tbody>
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Table D-4  Uranium Uptake Batch Kinetic Data for Immobilized *R. arrhizus* Particles

(RGU1,16/20)

<table>
<thead>
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<th>TIME (min.)</th>
<th>$C_b$ (mgU/L)</th>
<th>TIME (min.)</th>
<th>$C_b$ (mgU/L)</th>
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Table D-5 Uranium Uptake Batch Kinetic Data for Immobilized *R. arrhizus* Particles (RGU1-, 20/25)

<table>
<thead>
<tr>
<th>TIME (min.)</th>
<th>$C_b$ (mgU/L)</th>
<th>TIME (min.)</th>
<th>$C_b$ (mg/L)</th>
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<td>200.0</td>
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Table D-6 Uranium Elution Batch Kinetic Data for Uranium Loaded Immobilized *R. arrhizus* Particles (RGU1-, 20/25)

<table>
<thead>
<tr>
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<th>$C_b$ (mgU/L)</th>
<th>TIME (min.)</th>
<th>$C_b$ (mgU/L)</th>
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<tbody>
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