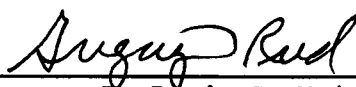
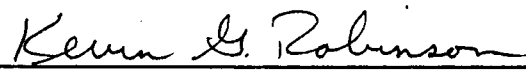


To the Graduate Council:

I am submitting herewith a thesis written by Daniel A. Kucsmas entitled "Reduction of Uranium by *Desulfovibrio Desulfuricans* in High Sulfate and Nitrate Solutions." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Environmental Engineering.



Gregory D. Reed, Co-Major Professor



Kevin Robinson, Co-Major Professor

I have read this thesis
and recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor
and Dean of the Graduate School

REDUCTION OF URANIUM BY *DESULFOVIBRIO DESULFURICANS*
IN HIGH SULFATE AND NITRATE CONTAINING SOLUTIONS

A Thesis

Presented for the

Master of Science

Degree

The University of Tennessee, Knoxville

Daniel A. Kucsmas

May 1994

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I hereby acknowledge with thanks the permission granted by Prentice-Hall, Englewood Cliffs, N.J. and the Director Copyrights & Permissions, Emily McGee, to copy Figures 5.4 and 20.8 from The Microbial World Fifth Edition, by Roger Y. Stainer, John L. Ingraham, Mark L. Wheelis, and Page R. Painter, (1986).

ABSTRACT

Biological reduction of uranium has been found to be important in the formation of iron and uranium ore deposits in anaerobic sediments. This discovery forms the basis of a new treatment technology that may be capable of selectively removing uranium from wastewaters. This study evaluated the potential for biological removal of uranium in surrogate solutions containing constituents similar to those found in actual wastewaters.

Batch laboratory experiments were performed to determine the effect of high concentrations of sulfate and nitrate on the removal of uranium by *D. desulfuricans*. The experiments were performed with sulfate concentrations up to 10,000 mg/L, nitrate concentrations up to 50,000 mg/L, and with uranium concentrations at 100 mg/L. All of the experiments were run in a bicarbonate solution with lactic acid as the carbon source. The reactors were sampled by filtering through a 0.2 micron filter. The filtrate was stored in a 4°C cooler until it was analyzed. The samples were analyzed for uranium, sulfate, and/or nitrate as required. Combined nitrate/sulfate solutions were tested for any significant changes in the rate of uranium removal.

In test reactors containing 100 mg/L uranium and <5,000 mg/L sulfate, uranium was removed to <0.1 mg/L in approximately one day. Results of a 10,000 mg/L sulfate solution experiment demonstrated a slight drop in the rate of uranium removal. A more significant decline in the rate of removal was observed in all of the sulfate reactors containing

lower initial concentration of uranium. In test reactors containing <10,000 mg/L nitrates, uranium was removed to <0.1 mg/L within one day. At 50,000 mg/L nitrate, uranium removal occurred more slowly. In the combined nitrate/sulfate solutions, the *D. desulfuricans* removed uranium from 100 mg/L to <0.1 mg/L within one day in the reactors containing <5,000 mg/L nitrate. In the reactors containing 50,000 mg/L nitrate, uranium removal to <1 mg/L occurred over 10 days. These results were independent of the sulfate concentrations. Possible explanations for the decline in the rates of uranium removal include competitive interference between the uranyl and sulfate ions, sodium inhibition, and ionic strength inhibition.

A computer model was utilized to identify the potential uranium species that would be present in the test solutions. These data were compared to data gathered for actual nitrate-containing wastes. Modeling of waste indicates this stream may not be suitable for biological uranium removal under current conditions. The model predicted most of the waste streams would have very little soluble uranium, if any. The phosphate concentration in these wastewaters were relatively small; however, it seems to have sufficient affinity to complex with uranium and precipitate. If, however, the wastewater does not contain phosphate nor significant quantities of calcium, it may be possible to selectively remove the uranium from the wastewater using *D. desulfuricans*.

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CHAPTER I

INTRODUCTION AND SCOPE

Biological removal of metals such as iron and uranium has been found to be important in the formation of ore deposits in anaerobic sediments (Lovley and Phillips, 1986). These discoveries have suggested the basis for a new treatment technology which may be capable of selectively removing aqueous uranium from wastewaters via microbially mediated precipitation. The purpose of this study was to evaluate the potential for biological removal of uranium in surrogate solutions containing constituents similar to those found in actual wastewaters. Additionally, a wastewater was evaluated through computer modeling for potential application of biological removal technology. Information obtained from these studies will be utilized to develop and evaluate the next stage of the technology investigation. These evaluations may include more focused research on biological removal and/or bench-scale tests with actual wastewaters.

The development of biological removal technology could result in a decrease in the amount of mixed waste sludges generated at United States Department of Energy (DOE) wastewater treatment facilities. Mixed wastes are co-contaminated with Resource Conservation and Recovery Act (RCRA) hazardous and radioactive materials. Currently there are no permanent disposal options available for these sludges; therefore, the only option available for this material is storage. By selectively separating the uranium, the sludge could be concentrated into a smaller waste volume requiring storage. The remaining sludges may then contain low enough

levels of uranium to allow disposal in commercial RCRA-permitted facilities.

The wastewater selected as the basis for evaluating biological removal of uranium is treated at the DOE Y-12 Plant West End Treatment Facility (WETF). The WETF collects and treats concentrated nitrate-containing wastewaters primarily generated from uranium purification processes, plating processes, the nitrate treatment facility, and other miscellaneous sources (Hale, 1988). Analytical data on the Y-12 West End wastewaters indicate two of the major constituents are sulfate and nitrate.

This wastewater is currently stored and treated in 500,000 gallon tanks at the Y-12 West End Tank Farm. The 500,000 gallon storage tanks are fitted with mixers to allow the tanks to serve as reactors for batch suspended-growth biodenitrification. Operation of these reactors results in the generation of significant amounts of uranium contaminated metal hydroxide sludges, formed as a result of conventional coagulation/filtration treatment methods. No permanent disposal option is available for the sludges currently because they are considered mixed waste, so that these mixed-waste sludges must be stored on-site (Hale, 1988).

The use of other conventional treatment technologies, such as ion exchange and reverse osmosis, to replace the current coagulation/filtration is not considered practical. These technologies are generally utilized in water polishing operations, and do not have the capability to segregate the uranium. In addition, the feed water quality

to such systems needs to be at a level normally obtained by coagulation/filtration techniques.

Biological removal technology is based on the use of naturally occurring microorganisms to selectively remove soluble uranium directly from wastewaters. A literature search has been conducted to investigate the feasibility of utilizing microorganisms to remove uranium from wastewaters through biochemical removal. Data found in the literature indicated the most common means of biological removal of metals from wastewater was biosorption, rather than removal. One researcher has done studies which suggest iron deposits in coastal sediments could have been formed by direct biological removal (Lovley and Phillips, 1986). Experiments on sediments in the region of these deposits resulted in the isolation of a microorganism, *Geobacter metallireducans*, capable of growth through the dissimilatory removal of iron (Lovley and Phillips, 1988). Dissimilatory removal is the utilization of iron as a terminal electron acceptor, rather than for intracellular assimilation to be used as a nutrient or other cell material building block. Additional studies showed that this microorganism could also utilize uranium as a terminal electron acceptor and change it from soluble U(VI) (UO_2^{+2}) to insoluble U(IV) (UO_2) (Lovley, et.al., 1991). *G. metallireducans* is an obligatory anaerobic bacteria; therefore, culturing and utilizing it in a laboratory is difficult, if not impossible, without the use of glove boxes or other equipment with purgable atmospheres. Other studies have indicated the anaerobic sulfate reducing bacteria *Desulfovibrio desulfuricans* is also capable of dissimilatory removal of uranium (Lovley and Phillips, 1992). In addition, short term exposure to oxygen does not have a detrimental

effect on this microorganism. *D. desulfuricans* is a marine microorganism, while *G. metallireducans* was isolated in freshwater sediments. Since wastewaters may contain high concentrations of salts, marine microorganisms may be expected to survive more effectively than freshwater microorganisms. As a result, *D. desulfuricans* was selected as the microorganism for evaluation in these experiments, given these conditions.

Previous uranium removal studies utilizing *D. desulfuricans* have been conducted in relatively simple solutions, primarily natural waters and buffer solutions where U(VI) was the sole electron acceptor. In only one study was the removal of uranium in the presence of another potential electron acceptor (sulfate) evaluated. This study was performed in a solution containing 2.0 mM (192 mg/L) sulfate, and showed concurrent removal of uranium and sulfate (Lovley and Phillips, 1992).

To evaluate the potential for biological removal of uranium by *D. desulfuricans* in wastewaters, a laboratory study was performed to determine the effects of the presence of high concentrations of two anionic species, sulfate and nitrate, on the removal of uranium by *D. desulfuricans*. All of the experiments were run in a bicarbonate solution with lactic acid as the carbon source. The solutions were purged with a mixture of CO₂:N₂:H₂ (5:80:15) to remove dissolved oxygen, and maintained in sealed pressure tubes. The solutions were sampled by filtering through a 0.2 micron filter. Samples were analyzed for uranium, sulfate, and/or nitrate as required. These studies were performed with sulfate concentrations as high as 10,000 mg/L and nitrate concentrations up to 50,000 mg/L. These levels were chosen to simulate what may be expected in

wastewaters treated at the Y-12 West Tank Farm biological denitrification reactors. Once the tests with the individual anionic species were completed, a combined nitrate/sulfate matrix was tested to look for any interferences or other significant changes in the rate of uranium removal.

In addition to the issue of the effects of nitrate and sulfate on uranium removal, bioavailability is a concern. In all of the known tests run to date, uranium was present in the carbonate form. A computer model (MINTEQ Version 3.1) was utilized to identify the potential uranium species which might be present in the test solutions.

To further determine the potential applicability of biological removal technology to the actual Y-12 waste stream, the computer model was used to predict uranium complexes in four waste solutions, based on analytical data obtained from the Y-12 laboratory. Data from the model were used to compare the uranium species expected to be present in the nitrate and sulfate test solutions with those predicted to be present in the wastewater. Computer modeling was performed to determine if additional, more focused, research on the waste stream is required.

CHAPTER II

LITERATURE REVIEW

Uranium is a radioactive element having three naturally occurring isotopes (0.006% U-234, 0.72% U-235, and 99.27% U-238) and two major oxidation states ($U^{+4}(IV)$ and $U^{+6}(VI)$) (Cothorn and Rebers, 1990). $U(IV)$ is normally found as the mineral uraninite (UO_2) which is insoluble ($K_{sp} \approx 10^{-9}$ mM/L) (Allison et.al., 1991). $U(VI)$ is found in aqueous solutions as the uranyl ion (UO_2^{+2}). The solubility of the uranyl ion is dependant on the anion(s) associated with it. For example, uranyl acetate ($UO_2(C_2H_3O_2)_2 \cdot 2H_2O$) has a $K_{sp} = 182$ mM/L, while the solubility of uranyl nitrate ($UO_2(NO_3)_2 \cdot 6H_2O$) is listed as $K_{sp} = \infty$ (theoretically infinite solubility) (Weast, 1974). Figure 1 shows the distribution of uranyl hydroxide and carbonate complexes versus pH in natural (ground) waters (Cothorn and Rebers, 1990; Langmuir 1978). In aerated aqueous solutions, the uranyl ion is very stable at a pH < 2.5. Near a pH of 7, the uranyl ion forms stable complexes with carbonate. In waters with pH between 7 and 10, the predominant anion species include $UO_2(CO_3)_2^{-2}$ and $UO_2(CO_3)_3^{-4}$.

Conventional treatment methods for removal of uranium from aqueous solutions focus on reducing the solubility of, or direct removal of, the uranyl ion. These technologies include Coagulation/Filtration, Reverse Osmosis, and Ion Exchange. Table I shows the effectiveness of these treatment methods for removing uranium from drinking water. The iron and alum based coagulation treatment methods highest uranium removal efficiencies are at pH 6 and 10. These studies indicate effective uranium removal was obtained at discrete pH levels. The best removals using this

Table I. Treatment Technology Efficiencies for Uranium Removal in Drinking Water.
Adapted from T. J. Sorg, (1990)

Treatment Method	Percent Removal	Special Conditions/ Notes
Coagulation		
Iron (Ferric)	85	pH 10
	80	pH 6
Iron (Ferrous)	90	pH 10
	40	pH 6
Alum	90-95	pH 10
	80-85	pH 6
Lime Softening	99	pH 10.6 w/Mg ⁺²
Reverse Osmosis	>99	
Cation Exchange		
H ⁺ form	90-95	pH 3.5
Ca ⁺ form	70	pH 4
Na ⁺ form	70-85	pH ≤7
Anion Exchange	99	10,000-50,000 Bed Volumes

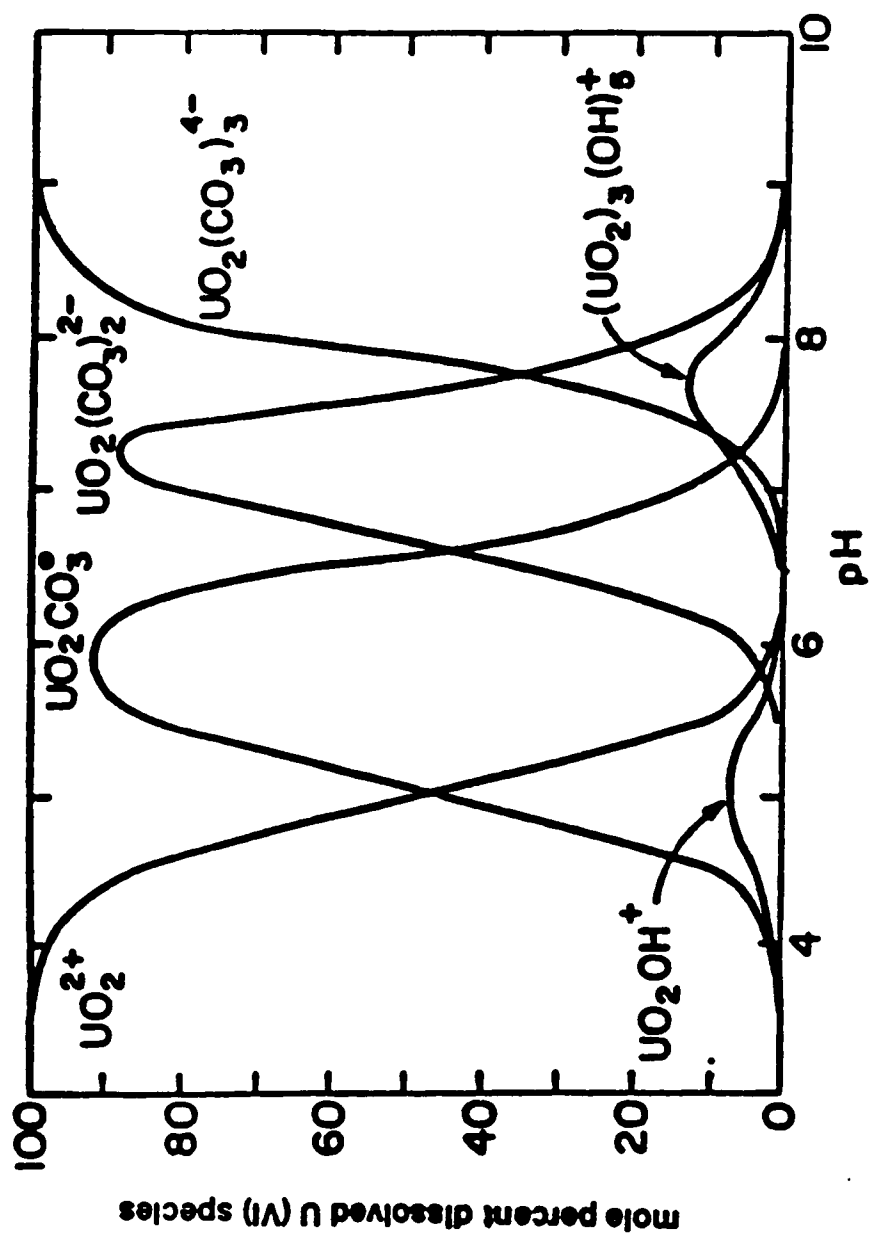


Figure 1. Distribution of Uranyl Hydroxide and Carbonate Complexes Versus pH.

Adapted from D. Langmuir, (1978)

technology (>85%) were obtained at a pH of 10, while slightly lower removal efficiencies were seen at pH 6. Studies indicated much lower removal efficiencies at pH 8, where only 15 to 40% removal was obtained. This discontinuity is believed to be due to hydroxide and carbonate complexes. At $\text{pH} > 9.5$, the positively charged $(\text{UO}_2)_3(\text{OH})_5^+$ would be formed. This species would be expected to coagulate with the negatively charged metal hydroxide and be removed. At a $\text{pH} = 6$, the primary uranyl species is the neutral UO_2CO_3 , and in this range the charge of the hydroxide precipitate would also be near zero. In the pH 7 to 9 range, the principal uranium complexes are $\text{UO}_2(\text{CO}_3)_2^{-2}$ and $\text{UO}_2(\text{CO}_3)_3^{-4}$ which are negatively charged. The metal hydroxides are also negatively charged in this pH range, so low uranium removal would be expected. A primary disadvantage to these treatments includes sensitivity to pH; that is, variations in the pH of the water to be treated may result in lower removal efficiencies. In addition, to achieve maximum uranium removal, the pH of the water must be adjusted to a range that is higher than most natural waters. Finally, the use of alum at high pH (> 10) is not recommended, due to increased solubility of aluminum hydroxides (Cothern, 1990).

Lime softening to remove uranium is found to be most effective at a $\text{pH} > 10.7$, with efficiencies of 85 to 90%. Below this pH, very little uranium removal occurs. For example, it was found that at a $\text{pH} = 9$, only approximately 16% of the uranium was removed from solution by lime softening. To achieve removal efficiencies of 90 to 99%, magnesium would need to be added to the solution. The investigators (Cothern, 1990) suggest that, at the high pH, the water becomes depleted of carbonate so

that the uranium is converted to uranyl hydroxide, $(\text{UO}_2)_3(\text{OH})_5^+$, which would coagulate similar to the methods discussed above. To achieve maximum uranium removal with lime softening, the pH of the water needs to be adjusted to a range higher than is found in most natural waters. This treatment could generate a significant amount of uranium-contaminated calcium sludge, which might require disposal as low level radioactive waste (Cothorn, 1990).

Pilot plant reverse osmosis (RO) studies in Florida have shown a 99% removal of uranium in ground waters. Removal of ions by RO is a function of charge and size, so uranium should be removed effectively. Disadvantages of RO systems include high capital and energy costs to build and operate; these systems may generate a secondary waste brine solution which would require additional processing (i.e. drying) prior to disposal (Cothorn, 1990).

Ion exchange treatment with cation resins is most effective utilizing the hydrogen cycle at low pH. The calcium and sodium base resins do not achieve the removal efficiencies of the more standard coagulation and lime softening technologies. In addition, the capital and operating costs for these systems do not seem to make them practical for uranium removal. Anion ion exchange resins have been found to remove uranium effectively, and the tested resins had significant capacity for uranium. A range of 10,000 to 50,000 bed volumes of water was treated in these tests. Other than the initial capital costs, the major disadvantage of the system was found to be the difficulty of complete regeneration of the resin. Recoveries ranged from 5 to 86%, resulting in uranium accumulation in the columns. Regeneration studies were conducted using

sodium chloride, sodium hydroxide, sodium bicarbonate and hydrochloric acid as regenerants. The results indicated hydrochloric acid, was the most effective, while sodium hydroxide was the least effective. In general, it was concluded that uranium is difficult to remove from the resin by all regenerants tested, and that large quantities of regenerant would be needed if complete removal of uranium is required (Cothorn, 1990).

One potential technology for removal of uranium in aqueous solutions is biological or microbial removal, using bacteria to reduce enzymatically the valence state of the metal ion and decrease its solubility. Uranium VI as UO_2^{+2} would receive two electrons and be reduced to uranium IV or UO_2 , which is the insoluble mineral uraninite.

The microbial reduction of metals such as iron, magnesium, and uranium has been recognized by geologists as playing a potentially important role in the formation of ore deposits in sediments (Lovley et.al., 1989). The role of anaerobic microorganisms in the reduction of Fe(III) in organic-containing sediments of coastal waters has been investigated for several years. Fermentative bacteria, with sulfate removal or methane production as the terminal process, have been shown to be a minor pathway for Fe(III) reduction. As a result, it is thought that these processes cannot account for the complete oxidation of organic matter coupled to Fe(III) reduction because their metabolism generates a buildup of fermentation products (Lovley and Phillips, 1986). The complete metabolism of organic matter with Fe(III) as the terminal electron acceptor has been shown to be more thermodynamically favorable than mineralization of organic matter with sulfate reduction or methane production as the terminal step (Lovley and Phillips, 1986). This type of

Fe(III) reduction, coupled with complete oxidation of organic matter, is considered to be a significant process for organic matter mineralization.

Geological evidence in support of biological uranium removal is indicated in observations of U(IV) accumulation in bleached "reduction spots" of otherwise red Fe(III)-rich rock (Lovley and Phillips, 1988). Uranium reduction of organic-rich sediments in anaerobic conditions (below an anoxic water column) has shown a positive correlation between Uranium concentration and organic matter content (Cochran et.al. 1986). Two microorganisms which have been demonstrated to reduce uranium in the laboratory include *Geobacter metallireducans* and *Desulfovibrio desulfuricans*. Each of these microorganisms is discussed below.

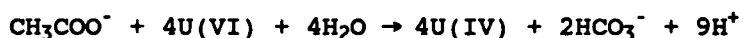
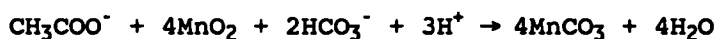
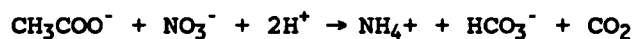
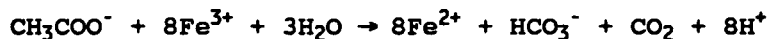
Geobacter metallireducans (GS-15)

The direct biological removal of Fe(III) to Fe(II) was first demonstrated in a fresh water isolate from sediments of the Potomac River designated as "*Geobacter metallireducans*", formerly known as GS-15 (Lovley and Phillips, 1986). Isolation of *G. metallireducans* occurred as a result of studies which suggested that sediments of the Potomac River contain microorganisms which completely oxidize organic matter to carbon dioxide with the reduction of Fe(III), Mn(IV), or NO_3^- . To isolate the microorganism, core samples of tidal sediments were collected from a site in Gunston Cove, Virginia. Observation of the purified cultures with the use of phase-contrast microscopy revealed only rod-shaped organisms roughly 2 by 0.5 μm , with an obligatory anaerobic respiratory mechanism. There have been no documented observations of motility or spores (Lovley and Phillips, 1986).

G. metallireducans reduction of iron has been termed "dissimilatory" to distinguish it from removal of iron during microbial assimilation of intracellular iron (Lovley and Phillips, 1988). There are three pieces of evidence that support dissimilatory iron reduction by *G. metallireducans*. First, *G. metallireducans* cells did not orient in response to an applied magnetic field like magnetotactic bacteria. Second, transmission electron microscopy examination of the precipitate formed during the reduction of Fe(III) to Fe(II) found the precipitate to consist of crystals ranging in size from 10 to 50 nm. The crystals were clearly external to the cells and not aligned in chains (Lovley and Phillips, 1988). These crystals were identified as magnetite. Third, *G. metallireducans* cell membrane fragments were found to actively reduce Fe(III) to Fe(II) (Gorby and Lovley, 1991; Lovley and Phillips, 1988). Washed *G. metallireducans* cells were broken by passing them through a French Pressure Cell three times at 18,000 lbs/in². The resulting suspension was centrifuged to remove unbroken cells. The resulting supernatant was centrifuged to separate the soluble proteins from the membrane fragments. Analysis of the soluble proteins showed no Fe(III) reductase activity, while the membrane fragments actively reduced Fe(III) at a rate of 280 nmol of Fe(II) produced per mg of protein.

In addition to reduction of Fe(III) to Fe(II), *G. metallireducans* has been demonstrated to utilize NO₃⁻, Mn(IV), and U(VI) as sole electron acceptors to sustain growth (Lovley and Phillips, 1986). In the experiments, NO₃⁻ was reduced to NH₄⁺, Mn(IV) was reduced to Mn(II), and U(VI) was reduced to U(IV). In all experiments designed to identify different sole electron acceptors, acetate was utilized as the sole

electron donor. Suggested stoichiometry for reduction reactions related to the metabolism of acetate are shown below (Lovley and Phillips, 1986).



Evidence for terminal electron acceptor selectivity on the part of *G. metallireducans* has been demonstrated in studies attempting to identify the electron transport mechanism(s). *G. metallireducans* cells cultured in a ferric citrate based media reduced twice as much Fe(III) as cells cultured in a sodium nitrate media. In addition, only the cells cultured in the sodium nitrate based media consumed the test solution containing nitrate as the sole electron acceptor (Gorby and Lovley, 1991). These results are interpreted as evidence of a separate enzymatic system for reduction of nitrate. In addition, evidence for multiple enzyme systems in *G. metallireducans* was found by exposing cells to four respiratory inhibitors. These inhibitors included sodium cyanide, sodium azide, rotenone, and oxygen. Sodium azide and sodium cyanide were found to completely inhibit nitrate reduction, while they had no effect on the metabolism of acetate coupled to the reduction of Fe(III) (Gorby and Lovley 1991). The rotenone inhibited iron reduction by 30% without

affecting nitrate reduction. The oxygen completely inhibited reduction of both nitrate and iron. The existence of this separate system was determined by examining the absorbance spectra of *b* cytochrome in the different cell cultures. This was possible because both the Fe(III) and nitrate reductase enzymes were found to accept electrons from the type *b* cytochrome. These spectra showed that cells cultured in the ferric citrate based media contained only the Fe(III) reductase enzyme, while the nitrate cultured cells had both Fe(III) and nitrate reductase (Lovley and Phillips, 1988).

Biological reduction of water soluble U(VI) to insoluble U(IV) was accomplished with *G. metallireducans* cultured in ferric citrate based solutions. As a result, it can be assumed that the dissimilatory reduction of U(VI) to U(IV) by *G. metallireducans* may utilize the same enzymatic pathways. Visual examination of the uranium precipitate shows the material is external to the cells in a manner similar to the iron precipitate. This evidence indicates a mode of U(VI) to U(IV) reduction coupled to oxidation of organic matter similar to the dissimilatory reduction of iron. Experiments have demonstrated that *G. metallireducans* could obtain energy for growth by coupling oxidation of acetate to the reduction of U(VI) to U(IV) (Lovley et.al., 1991). In any case the biological reduction of uranium by *G. metallireducans* could be of special significance to locations with uranium contaminated waste streams or groundwaters.

Drawbacks to utilization of *G. metallireducans* in a bioreactor for removal of uranium include the potential for interferences with the growth solution. The soluble Fe(III) may chemically oxidize the biologically reduced U(IV) back to U(VI), thus short circuiting the process. In

addition, some of the solid Fe(III) oxides may sorb the U(VI), and form greater than expected volumes of secondary waste. (Lovley and Phillips, 1992).

Desulfovibrio desulfuricans

Traditionally, nonenzymatic removal of U(VI) to U(IV) by sulfide has been an explanation for the formation of uranium deposits in sediments and rock. Removal of mobile uranyl species to U(IV) with precipitation of highly insoluble uraninite ($\text{UO}_2(\text{s})$) must often reflect concurrent oxidation of proportionate amounts of more abundant species of iron, sulfur, and/or carbon (Langmuir, 1978). One possible stoichiometry for this abiotic reduction at a pH = 8 is shown below.



Sulfide for this reaction in the form of H_2S may be generated through biological activity through the reduction of soluble sulfates.

The production of sulfide in anaerobic sulfur reducing bacteria such as *D. desulfuricans* occurs in one of two pathways: assimilatory or dissimilatory sulfate reduction. In either process, the sulfur atom in the sulfate ion has a valence of +6 which is reduced to -2 and forms the sulfide ion S^{-2} .

The assimilatory sulfate removal pathway is shown in Figure 2. The initial two-electron reduction of sulfate ($\text{SO}_4^{-2} + 2\text{e}^- \rightarrow \text{SO}_3^{-2} + 1/2\text{O}_2$) is accomplished through a series of three enzymatic steps requiring the expense of three high-energy phosphate bonds. The final six-electron

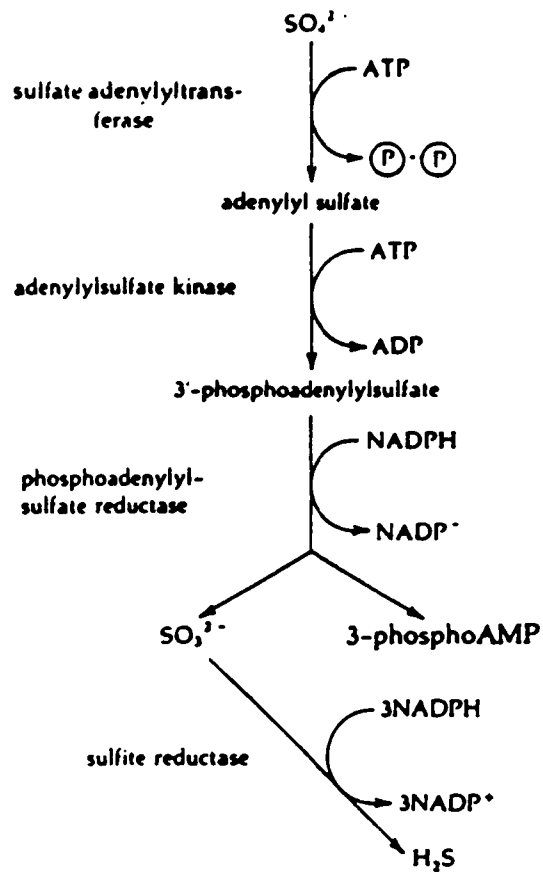


Figure 2. Assimilatory Sulfate Removal Pathway

Stainer, R.Y., Ingraham, J.L., Wheelis, M. L., and Painter P. R., The Microbial World, Fifth edition, Prentice-Hall, Inc., 1986, pg 108. Reproduced by permission of Prentice-Hall, Inc., Englewood Cliffs, NJ.

removal is accomplished through an enzyme known as sulfite reductase. Sulfite reductase is a very large and complex flavometallo-protein. In *E. Coli*, for example, sulfate reductase has a molecular weight of 750,000. Once the sulfite is reduced to sulfide it can then be incorporated into cellular organic compounds (Stainer, et.al, 1986). As a result, this process may not produce sulfite available for uranium reduction as discussed above.

Dissimilatory sulfate reduction involves a process where sulfate is utilized as a terminal electron acceptor. The enzymatic pathway for dissimilatory sulfate reduction is not completely understood. One possible mechanism is shown in Figure 3. The first few steps of the reaction are relatively well established. Sulfate is activated through a sulfur based ester-forming reaction, generating adenosylphosphosulfate and pyrophosphate from an adenyl group by the enzyme ATP sulfurylase. The Gibbs free energy of this reaction strongly favors the reactants (SO_4^{-2} and ATP) so the isolated reaction does not occur to any significant extent. However, inside the living cell, the pyrophosphate is rapidly hydrolyzed through the action of pyrophosphatase allowing adenosylphosphosulfate to accumulate in significant quantities. Removal of the adenosylphosphosulfate occurs to yield AMP and bisulfite. The remaining steps of the pathway are incompletely understood. This part of Figure 3 shows the most widely accepted scheme for the sequential removal of bisulfite. In this scheme, bisulfite is reduced to trithionite, thiosulfate, and finally sulfide. An alternative involves the reduction of bisulfite in three successive two-electron transfers to enzyme bound

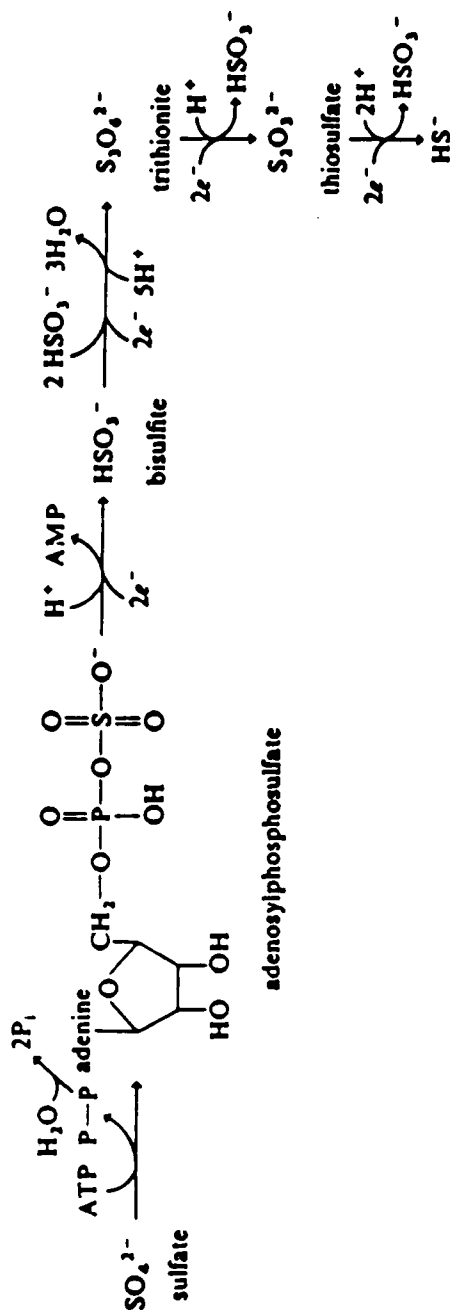


Figure 3. Dissimilatory Sulfate Removal Pathway

Stalner, R.Y., Ingraham, J.L., Wheelis, M. L., and Painter P. R., The Microbial World, Fifth edition, Prentice-Hall, Inc., 1986, pg 460. Reproduced by permission of Prentice-Hall, Inc., Englewood Cliffs, NJ.

intermediates. This process is analogous to the mechanism for assimilatory sulfate reduction discussed above. In this method, the trithionite and thiosulfite would be byproducts rather than intermediates in the pathway (Stainer et.al., 1986).

Lovley (Lovley and Phillips, 1991) showed that sulfate reducing microorganisms are able to use U(VI) as the sole terminal electron acceptor in much the same way as Fe(III)-reducing microorganisms such as *G. metallireducans*. The results of this study indicated *D. desulfuricans* is one such sulfate reducing microorganism. Cell suspensions containing *D. desulfuricans* showed a rapid loss of U(VI) and corresponding increase of in the amount of U(IV). A black precipitate formed in the bottom of the test cultures. When the precipitate was analyzed through X-ray diffraction, it was found to be the mineral uraninite (UO_2). When examined by transmission electron microscopy, the precipitate was found to be extracellular (Lovley and Phillips, 1991), indicating a dissimilatory pathway for enzymatic uranium reduction.

In order to further demonstrate that U(IV) is not generated as the result of nonenzymatic reduction of U(VI) by sulfide produced from sulfate reduction, researchers performed uranium/sulfide experiments. In these experiments, *D. desulfuricans* were suspended in bicarbonate buffer containing uranium and 1.0 mM sulfide, and incubated at various temperatures. The results of these experiments showed optimum uranium reduction occurred at temperatures consistent with enzymatic rather than nonenzymatic reduction (Lovley and Phillips, 1992). Additionally, in experiments with bicarbonate buffer solutions containing uranium and

sulfide alone (no cells), no reduction of U(VI) was observed (Lovley, et.al., 1991).

D. desulfuricans does not have the ability to utilize acetate as an electron donor, indicating that *D. desulfuricans* may not have a complete TCA cycle, and accumulates acetate as an end product of the oxidation of lactate (Stainer, et.al., 1986). One such pathway is shown in Figure 4. This figure shows part of the fermentation pathway of propionibacteria (Gaudy and Gaudy, 1980). No removal of uranium by *D. desulfuricans* was observed in experiments with no electron donor (Lovley and Phillips, 1991). As a result, lactate has been utilized as the electron donor or carbon source in all of the uranium removal experiments with *D. desulfuricans*.

In addition to uranium and sulfate reduction, *D. desulfuricans* has been found to have one of the most versatile respiratory metabolism known. There is no other organism known that can use the reduction of O_2 , NO_3^- , NO_2^- , SO_4^{2-} , SO_3^{2-} , $S_2O_3^{2-}$ or S^0 for energy conservation (Dilling and Cypionka, 1990). This versatility makes *D. desulfuricans* a likely organism for removal of uranium in wastewaters. It has been suggested that *D. desulfuricans* may be more practical in some applications for uranium removal than *G. metallireducans*. Unlike *G. metallireducans*, it was found that there was no growth of *D. desulfuricans* in solutions where U(VI) was the sole electron acceptor. It is suggested a bioreactor could be established for removing uranium with *D. desulfuricans* if low concentrations of sulfate were included. The sulfate reduction could provide the energy necessary to support biomass growth. The *D. desulfuricans* would not have the iron interferences such as abiotic

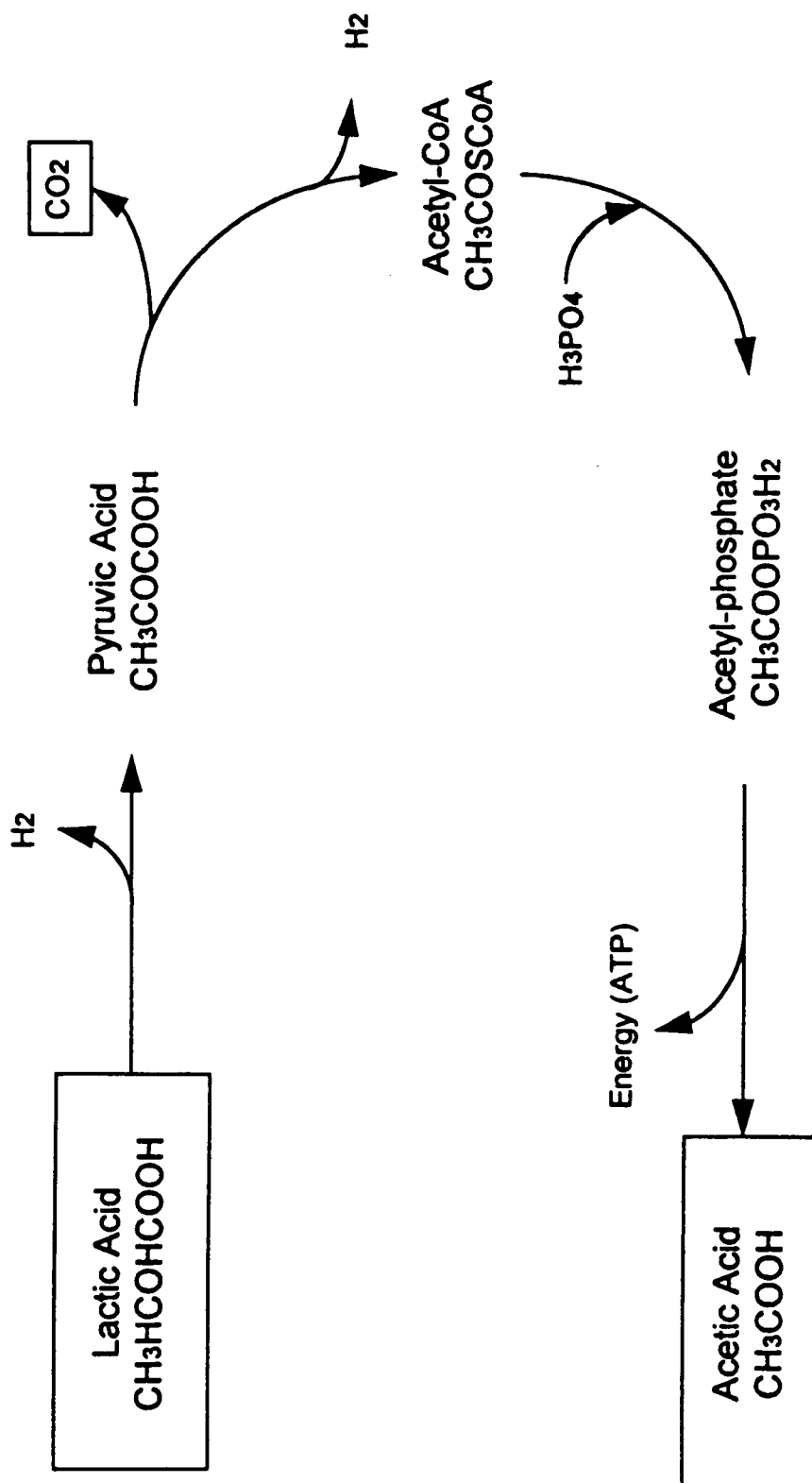


Figure 4. Fermentation of Lactic Acid to Acetic Acid

Adapted from Gaudy and Gaudy, (1980)

uranium oxidation that may result from a Fe(III) based growth media (Lovley and Phillips, 1992).

At this time, there is little information on reduction of uranium which may have direct application to wastewaters. Research to date has focused primarily on natural waters and very dilute solutions. The only information regarding biological uranium removal in the presence of ions other than carbonate comes from one study involving concurrent removal of U(VI) and sulfate. This experiment utilized a sulfate concentration at approximately 2.0 mM or 192 mg/L. Test solutions were prepared containing approximately 2.0 mM sulfate and 1.0 mM uranium. The results indicated sulfate had no significant effect on U(VI) removal. In addition, U(VI) did not influence the rate of sulfate reduction; the two were reduced simultaneously (Lovley and Phillips, 1991).

Generally, wastewaters contain additional anions that have not been found at high concentrations in the uranium reduction test solutions utilized to date. These ions include NO_3^- , SO_4^{2-} , PO_4^{2-} , Cl^- , F^- , etc., and have a potential to form complexes with uranium. Some of these complexes, by their presence, may reduce the solubility of uranium. In addition, they may reduce the bioavailability of the uranium, thus preventing reduction.

The wastewaters at the Y-12 West Tank Farm indicate sulfate levels as high as 146 mM or 14,000 mg/L. In addition, these wastewaters contain nitrate concentrations as high as 1,000 mM or 62,000 mg/L. To determine if biological removal is a viable technology for uranium removal in wastewaters, the microorganisms will need to be evaluated under conditions more representative of actual wastes. A variety of mathematical models are available to determine the influences and potential impact of competing

complexes. The metal speciation model MINTEQA2 is considered to be a versatile state-of-the-art equilibrium solution chemistry program.

MINTEQA2 is a geochemical equilibrium speciation model for dilute aqueous solutions (Allison et.al., 1991). The original model was developed at Battelle Pacific Northwest Laboratory. The fundamental mathematical structure of MINTEQA2 was obtained from the MINEQL model and combined with the U.S. Geological Survey's thermodynamic database WATEQ3. As a result, the model can be utilized to calculate the equilibrium composition of dilute aqueous solutions. The model also can be used to calculate mass distributions between dissolved, sorbed, and multiple solid phases for a variety of conditions.

To predict equilibrium composition, chemical analysis providing the total dissolved concentration of the components in the sample to be modeled is required. Any other relevant uniform measurements such as pH, pe, and partial pressures of gasses may be specified as equilibrium values. Solids can be specified as potentially present at equilibrium or definitely present and not subject to total dissolution.

CHAPTER IV

MATERIALS AND METHODS

The organism used in this study was *Desulfovibrio desulfuricans* ATCCC 29577, obtained from the American Type Culture Collection, Rockville, Md. Anaerobic techniques in this study for growing the *D. desulfuricans* cultures and inoculation of the test solutions are based upon methods developed by Lovley and Phillips (1992).

Growth Solution

The cells were cultured from a stock solution in a growth media as listed in Table II. The growth media was flushed with H₂:CO₂ (80:20) for

Table II. *D. desulfuricans* Growth Media

Component	Concentration
NaHCO ₃	2.5 g/L
NH ₄ Cl	1.5 g/L
NaH ₂ PO ₄	0.6 g/L
KCl	0.1 g/L
MgSO ₄	1.5 g/L
Na ₂ SO ₄	1.5 g/L
60% Lactic Acid syrup	20 mL/L
yeast extract	2 g/L
peptone	5 g/L
mineral mix	10 mL/L
vitamin mix	10 mL/L

10 minutes followed by subsequent flushing with CO₂:N₂:H₂ (5:80:15) for 5 minutes. The purges were performed to remove dissolved oxygen from the media. The flush gasses were passed through a column of reduced hot

copper filings prior to being bubbled into the media at a moderate rate. This was done to insure there was no trace O_2 in the gas stream. Forty ml of the media was transferred into 100 ml serum bottles. The head space of each bottle was flushed with the $CO_2:N_2:H_2$ gas mixture for 1 minute. After bottles containing the media had been sterilized using an autoclave, 5 ml of 2 g/L *L*-cysteine-HCl was added for a final concentration of 0.01 mg/L. *L*-cystene-HCl was added to help maintain reduced conditions in the growth media and expedite microbe growth. The *L*-cystene-HCl was added after sterilizing to prevent it from precipitating out of solution at elevated temperatures.

Growth and Harvesting

Each serum bottle of growth solution was then inoculated with a 5 mL solution containing approximately 10^5 cells/ml in order to obtain an initial concentration of 10^3 cells/ml. The inoculated bottles were then placed in a 35°C incubator for 48 hours, ensuring that the cells were in the log-growth phase (Figure 5) at the time the cells were to be harvested (Ganesh 1993).

Cells were harvested by transferring the two day old inoculated media into centrifuge tubes and spinning them in a Beckman Model J2-21 Centrifuge at 11,000 rpm for 25 min. at 10°C. The growth media was decanted, leaving a pellet of cells in the bottom of the centrifuge tubes. The cells were washed and resuspended two times with an anaerobic bicarbonate buffer solution which contained: $NaHCO_3$, 2.5 g/L and 60% lactic acid, 1.5 ml/L. The bicarbonate buffer solution had also been flushed with the $H_2:CO_2$ and $CO_2:N_2:H_2$ gasses in the same manner as previously explained for the growth solution. The bicarbonate buffer was then sterilized using

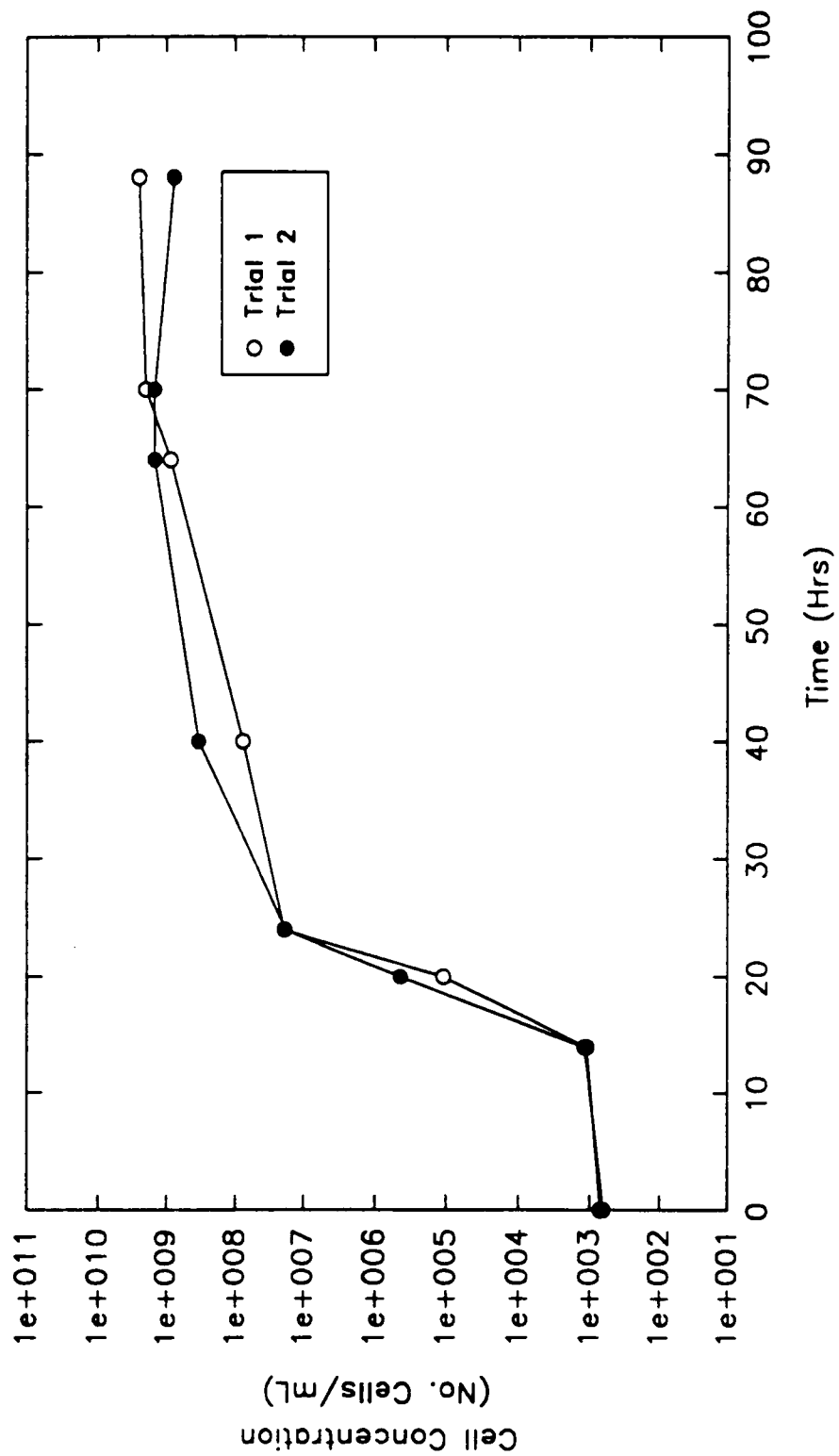


Figure 5. *D. desulfuricans* Growth Curve

an autoclave and cooled to approximately 4°C. The bicarbonate buffer was cooled to minimize biological activity during the harvesting process. A centrifuge tube containing buffer solution and cells was placed in a boiling water bath for approximately 20 min to provide heat killed cell controls.

Uranium Test Solutions

The uranium removal test solutions were comprised of bicarbonate buffer, uranyl acetate, NaNO_3 , and/or Na_2SO_4 . Analytical data and reports have shown waste at the Y-12 West End facility contains as much as 30,000 mg/L nitrate (as NO_3^-). The analytical data also indicates the waste contains 8,000 mg/L sulfate (as SO_4^{2-}). Nitrate concentrations up to 50,000 mg/L were selected in this study to insure that the full range of nitrate-containing waste received at the Y-12 West Tank Farm would be covered. Similarly, 10,000 mg/L of sulfate was selected as the upper boundary for study.

The solutions containing bicarbonate buffer and NaNO_3 and/or Na_2SO_4 were flushed with the $\text{H}_2:\text{CO}_2$ and $\text{CO}_2:\text{N}_2:\text{H}_2$ gas mixtures in the same manner as the growth solution. Nine mL of the nitrate and/or sulfate solutions were placed into 25 mL pressure tubes (reactors), and the head spaces were flushed with the $\text{CO}_2:\text{N}_2:\text{H}_2$ gas mixture for 1 minute. After sterilizing, 0.5 ml of 2 g/L uranyl acetate from an anaerobic stock solution was added to each tube to prepare a solution containing 100 mg/L uranium. The low concentration uranium reactors were prepared by diluting a 2 g/L stock solution at 1:10 with anaerobic bicarbonate buffer; 0.5 ml of diluted uranium solution was added to the test solutions resulting in a final uranium concentration of 10 mg/L. The low uranium containing matrix was

examined to determine if reduced uranium concentrations impact the extent and rate of uranium removal by the microorganism.

Uranium Removal Batch Reactors

A total of eight and/or ten pressure tubes were used to make up a set of batch reactors for each test parameter. The test parameters were specified by the concentrations of sulfate and/or nitrate in a set of reactors. Six of the reactors were inoculated with 0.5 ml of harvested live cells for a concentration of 10^8 cells/ml. These reactors were used to evaluate uranium removal at days 0, 1, 2, 3, 4, and 5. Two of the reactors were inoculated with heat killed cells for a concentration of 10^8 cells/ml as a control. If ten reactors were prepared, the remaining two tubes would be inoculated with anaerobic buffer solution for a no-cell control.

Sampling and Analysis

At day 0, one reactor each of the live cells, heat killed cells, and no-cells was sampled immediately after inoculation to obtain "Time = 0" data points. Approximately 5 to 7 ml of solution was drawn through a 0.2 micron filter (Gelman Acrodisc) for each sample. A 0.2 micron filter was used to insure the microorganisms would be separated from the filtrate. The filtrate was placed in a 4°C cooler to insure biological activity was terminated in the sample before analysis. The sampled reactors were subsequently discarded. The remaining reactors were placed in a 30°C incubator. One of the live cell reactors was sampled and discarded every 24 hours over a five day period to obtain the day 1, 2, 3, 4, and 5 data points. At day 5, the remaining control(s) were also sampled.

The samples were analyzed for uranium, nitrate, and/or sulfate as required. Uranium concentrations in the samples were analyzed on a Chemchek KPA-11 uranium analyzer. Sulfates were analyzed with a Perkin Elmer Series 410 HPLC with an Interaction Chromatography Inc., ION-120 ion exchange column. The column eluent was 4.0 mM salicylate buffer (pH = 7.8) at a flowrate of 1.3 ml/min. A UV detector was used at a wavelength of 254 nM. Nitrates were analyzed with a Perkin Elmer Series 410 HPLC with an Millipore Corp., Waters Chromatography Division, IC-Pak A Column 0.46 x 5.0 cm ion exchange column. The column eluent was 5.0 mM lithium gluconate at pH = 8.5 and a flowrate of 1.2 ml/min. A UV detector was used at a wavelength of 214 nM.

CHAPTER IV

RESULTS AND DISCUSSION

The evaluation of soluble uranium removal by *D. desulfuricans* was performed in buffer solutions containing sodium bicarbonate and lactate. Experiments were conducted in batch reactors containing degassed buffer solutions amended with sodium sulfate, sodium nitrate, and a combination of sodium nitrate/sulfate to produce solutions of varying concentrations. The matrix developed during the course of this study is shown in Table 3. The concentrations of the sulfate solutions were varied from 0 to 10,000 mg/L. The concentrations of the nitrate solutions were varied from 100 to 50,000 mg/L. Concentrations of the combined nitrate/sulfate solutions were varied to include the four end most and two internal possibilities. These solutions were chosen to determine the potential for nitrate and/or sulfate to inhibit or stop uranium removal by the microbes. The initial uranium concentration was maintained at approximately 100 mg/L in all of the solutions.

An additional set of experiments was performed in a similar fashion as above, utilizing lower initial concentrations of uranium. In these experiments the initial uranium concentrations were approximately 10 mg/L. The sulfate concentrations were varied between 1,000 and 10,000 mg/L, while the nitrate concentrations were varied between 5,000 and 50,000 mg/L.

Table III. *D. desulfuricans* Uranium Removal Test Matrix

Test Solution	Uranium (mg/L)	Sulfate (mg/L)	Nitrate (mg/L)	Duration (Days)
1	100	0	0	5
2	100	100	0	5
3	100	500	0	5
4	100	1,000	0	5
5	100	5,000	0	5
6	100	10,000	0	5
7	100	0	100	5
8	100	0	500	5
9	100	0	1,000	5
10	100	0	5,000	5
11	100	0	10,000	5
12	100	0	50,000	5
13	100	100	100	5
14	100	100	1,000	5
15	100	100	50,000	10
16	100	5,000	1,000	5
17	100	10,000	100	5
18	100	10,000	50,000	10
19	10	1,000	0	5
20	10	5,000	0	5
21	10	10,000	0	5
22	10	0	5,000	5
23	10	0	10,000	5
24	10	0	50,000	5

Sulfate Reactors

The results for the sulfate matrix (test solutions 1 - 6) are presented in Figure 6. Figure 6 contains representative control data from the 1,000 mg/L sulfate heat killed cells reactors. These control data are consistent with most of the sulfate control results. The 10,000 mg/L sulfate no cell control showed a slight drop in the uranium concentration. A graph showing the entire sulfate reactors control data set is provided in Appendix A. Figure 6 indicates uranium removal occurred in all biologically active sulfate solutions. A dark, nearly black, pellet formed in the bottom of the biologically active tubes after one day. These pellets were very fragile: they would break up if the tube was shaken or the water surface passed over them. This pellet is believed to be U(IV) as UO_2 which was found in the experiments conducted by Lovley Phillips (1992).

The removal of uranium by *D. desulfuricans* in the sulfate reactors indicated a rapid reduction and subsequent removal of uranium from solution (Figure 6). The *D. desulfuricans* in test solutions containing 0, 100, 500, 1,000 and 5,000 mg/L sulfate removed over 90% of the initial uranium in one day. After approximately three days, the concentration of uranium in each of these solutions fell below the detection limit of approximately 0.1 mg/L. Solutions containing no sulfate (uranium is the sole electron acceptor) and 100 mg/L sulfate confirm the results obtained by Lovley (Lovley and Phillips, 1992). In these experiments rapid uranium removal was observed in solutions containing uranium as the sole electron acceptor, and in the concurrent reduction experiments. The experiments

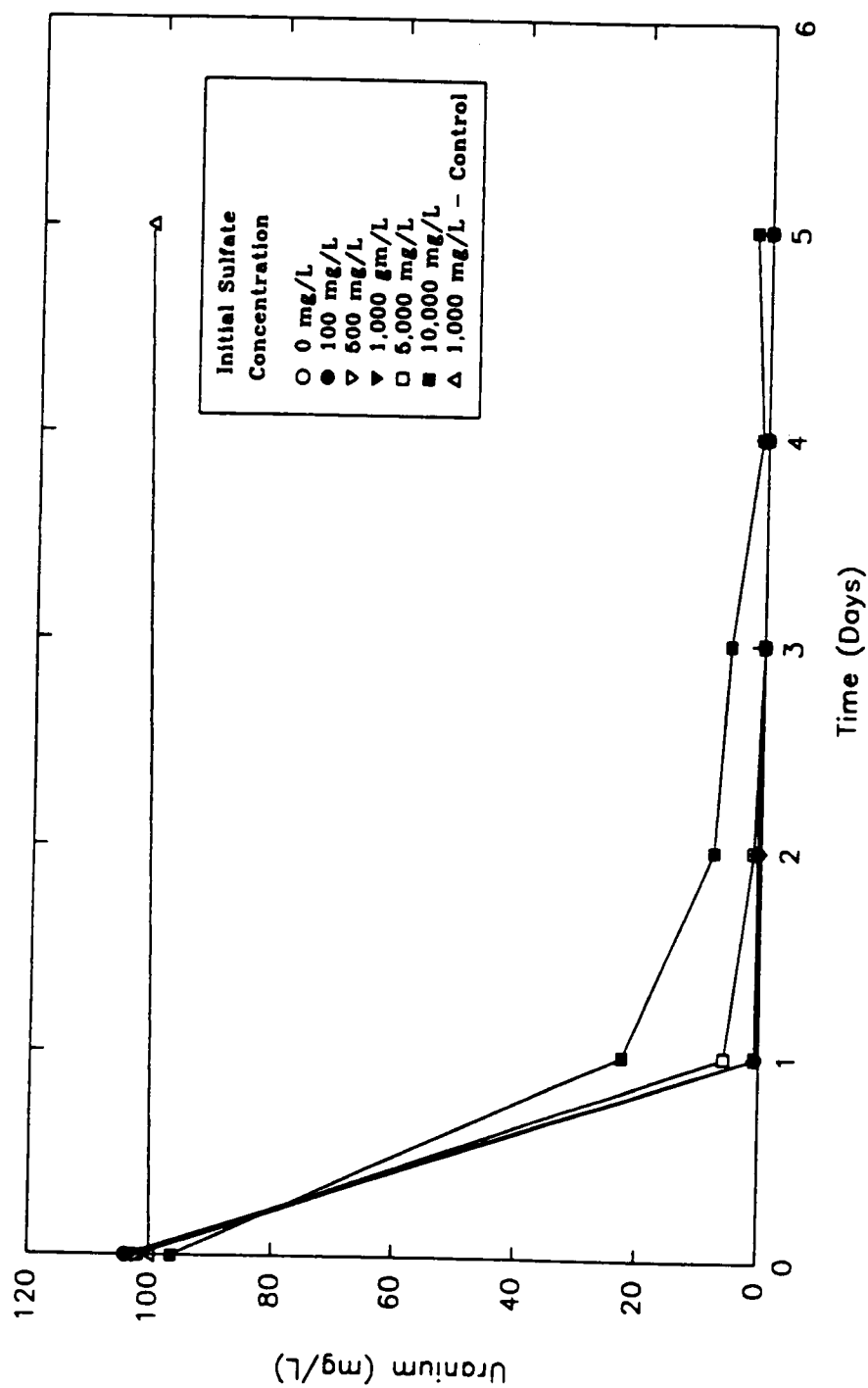


Figure 6. Uranium Removal in Sulfate Solutions

with the solutions containing 500, 1,000, and 5,000 mg/L sulfate showed no significant deviation from the results found in the literature.

Removal in the solutions where uranium is the sole electron acceptor strongly indicates enzymatic influences. Results in the control experiments containing no cells and heat killed cells (Figure 7) indicate no uranium removal in the 0 mg/L sulfate reactors after five days. As a result, it may be concluded that uranium removal in the live cell reactors may be a result of biological activity. In these reactors, biological activity occurs due to the fermentation of lactate to acetate, coupled to uranium removal.

In the test solution containing 10,000 mg/L sulfate, a slight drop in the rate and extent of uranium removal was observed. Approximately 76% of the initial uranium was removed from solution after one day, and the concentration of uranium in the test solution did not drop below the 0.1 mg/L detection limit after five days. The lowest uranium concentration measured during the experiment was approximately 1 mg/L which was observed on day four. There are four possible causes for the lower rate of uranium removal in the 10,000 mg/L sulfate solution:

- 1) changes in the uranium complexes,
- 2) competition between the uranium and sulfate ions as electron acceptors,
- 3) sodium inhibition, and
- 4) ionic strength inhibition.

The potential for changes in the uranium complexes effecting biological removal has been evaluated through computer modeling. The

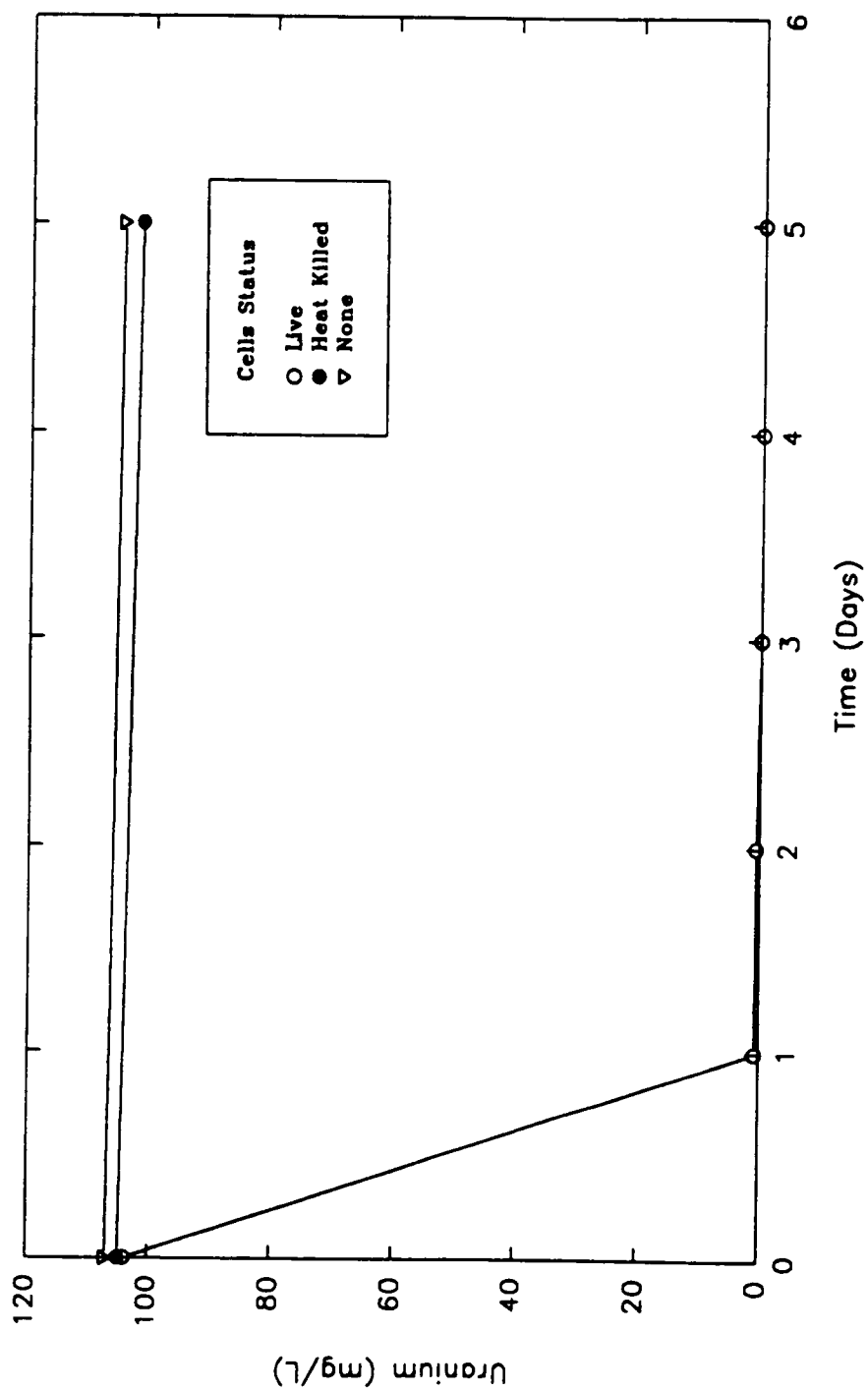
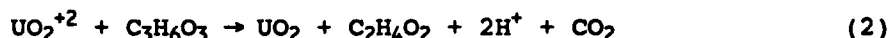


Figure 7. 0 mg/L Sulfate Reactors

results of this modeling indicate no significant changes in the uranyl complexes for the different test solutions. As a result, changes in uranium species should not account for the decline in the rate of removal. Uranium complexes are addressed in greater detail in Chapter V, Computer Simulation. The potential for uranium and sulfate competition as electron acceptors can be evaluated in two ways. First through stoichiometric evaluation, then followed by an examination of the biological pathways. Possible overall reactions for the removal of sulfate and uranium with the fermentation of lactate to acetate is shown in the following equations:



The Gibbs free energy for the removal of sulfate to sulfite through reaction 1 is approximately -236 KJ/mole, while the free energy release for the removal of the uranyl ion to uraninite through reaction 2 is approximately -335 KJ/mole. Based on thermodynamics alone, it appears uranium removal would be the preferred pathway, but the literature indicates removal of sulfate to sulfite occurs through a very complex pathway as shown in Figures 2 and 3. These pathways allow the microorganism to donate a total of eight electrons to the sulfur atom. The uranyl ion is only able to accept two electrons to complete removal to uraninite. As a result, the full removal pathway may not be utilized, and the additional free energy released by the uranium reaction could be wasted. Therefore, even though the uranium reaction is the more thermodynamically favorable reaction, the sulfate removal pathway may be more efficient at utilizing the free energy. Evidence for this may be

found in the studies by Gorby and Lovley (1992) where there was no observed growth of *D. desulfuricans* in solutions containing uranium as the sole electron acceptor. Based on this evidence, uranyl/sulfate competition should not be ruled out.

The removal of uranium by *D. desulfuricans* in sulfate solutions containing low concentrations (approximately 10 mg/L) of uranium is shown in Figure 8. Figure 8 contains representative control data from the 1,000 mg/L sulfate heat killed cells reactors. These control data are consistent with most of the low concentration uranium sulfate control results. A slight drop (< 2 mg/L) in the uranium concentration was observed in the 1,000 mg/L no cell and 5,000 mg/L heat killed cells reactors controls. A graph showing the entire low concentration uranium sulfate reactors control data set is provided in Appendix A. Data in Figure 8 indicates a significantly lower rate of removal than for comparable solutions with higher uranium concentrations (100 mg/L). It is observed that, as the sulfate concentration in the test solution increased, the ultimate amount of uranium removed decreased. This decrease in uranium removal is illustrated in Table IV. The decrease in the amount of uranium removed may

Table IV. Initial Sulfate Concentration versus Ultimate Uranium Removal

Sulfate Concentration (mg/L)	Uranium Reduced (percent)
1,000	97
5,000	90
10,000	71

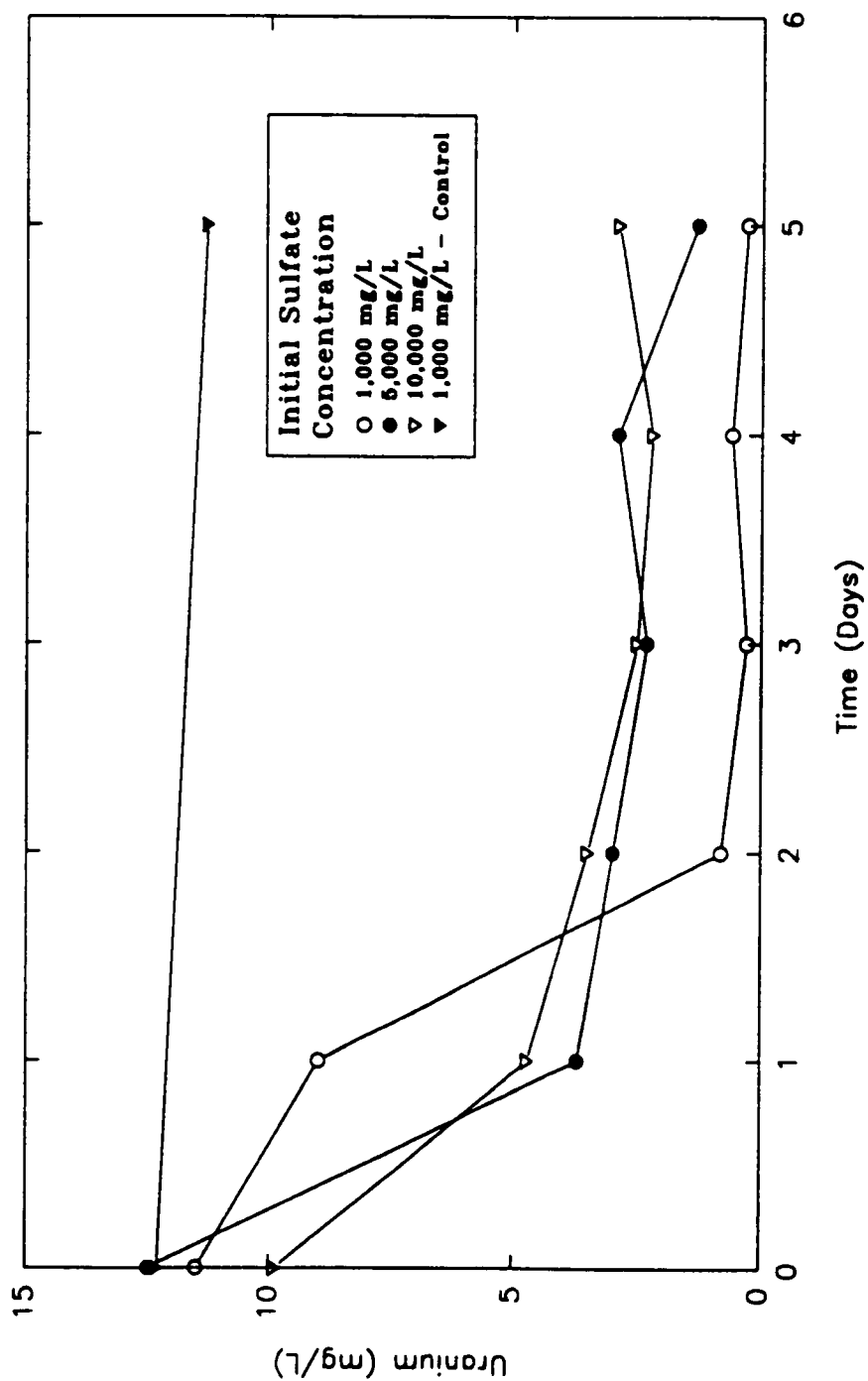


Figure 8. Low Level Uranium Removal in Sulfate Solutions

be an additional indication of competition between the sulfate and uranyl ions. In these solutions, sulfate ions may have a greater bioavailability than the uranium; and therefore, the sulfate may be preferentially reduced.

When the sulfate reactors were sampled and the filtrate transferred to vials for quantitative analysis, there was the distinct odor of hydrogen sulfide (rotten eggs) present, indicating the *D. desulfuricans* was reducing sulfate (SO_4^{-2}) to sulfite (S^{-2}). This odor was noticed after the second day and immediately upon making the transfer. The odor tended to be the strongest in the tubes containing 1,000 and 5,000 mg/L sulfate.

The changes in the sulfate concentrations due to biological removal by *D. desulfuricans* in the batch reactors containing 100, 500, and 1,000 mg/L sulfate are shown in Figure 9. Data from the 5,000 and 10,000 mg/L sulfate reactors did not indicate sulfate removal. Some sulfate removal is believed to have taken place in the reactors because a distinct sulfide odor was observed during sample collection. It is assumed that the equipment used to analyze sulfate is not accurate enough to distinguish the amount depleted. Figure 9 shows that virtually all of the sulfate in the 100 mg/L reactors was removed within the first day. The concentration of sulfate during days 1 through 4 was in the range of 2 to 3 mg/L. The day 5 sample indicated an increase in the sulfate concentration to approximately 20 mg/L, most likely due to error in the analytical equipment. The most probable cause of the anomaly would be carry-over of sulfate from a previous sample not sufficiently flushed out of the HPLC column.

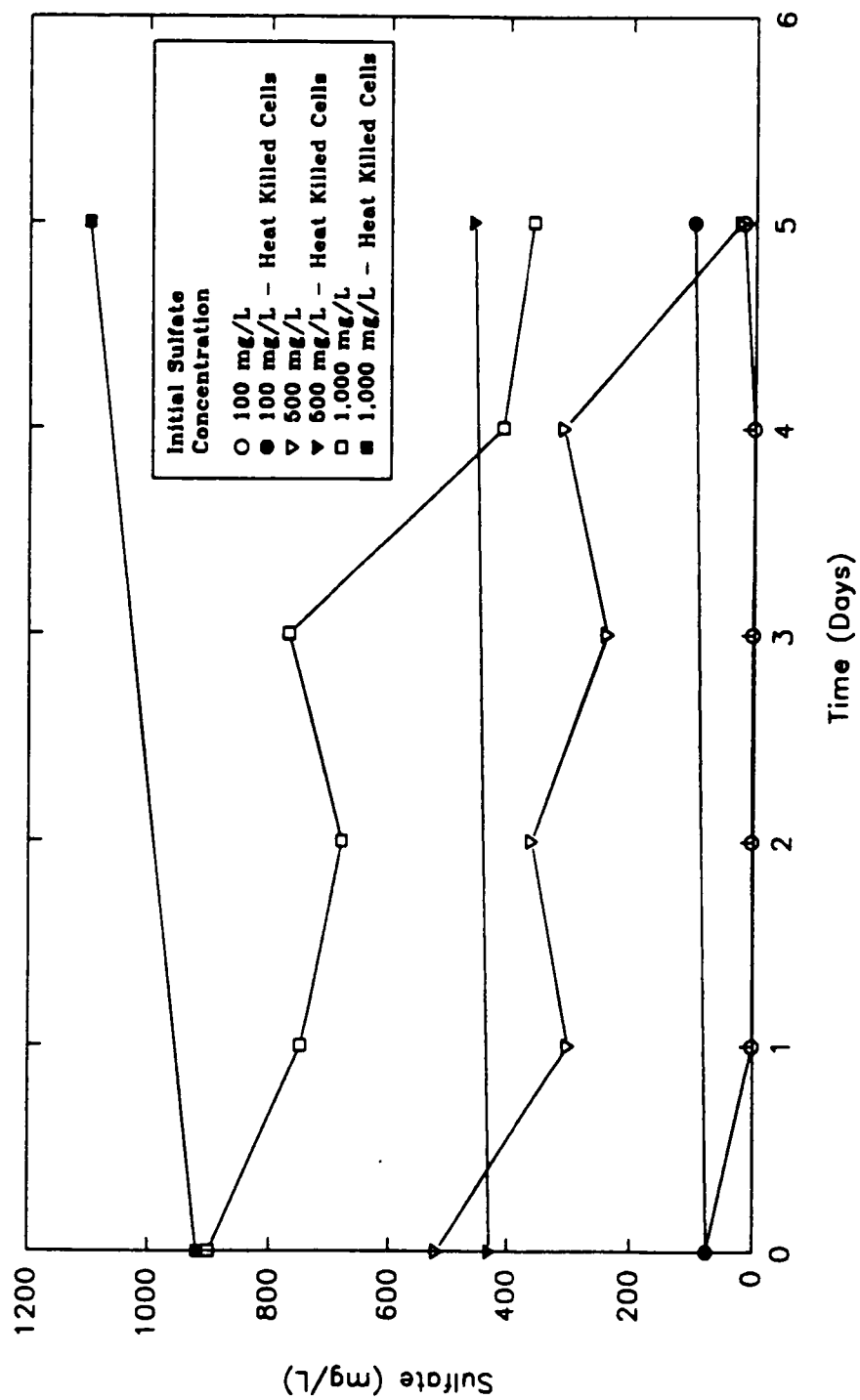


Figure 9. Sulfate Removal in *D. desulfuricans* Reactors

D. desulfuricans in the reactors containing nominally 500 mg/L of sulfate showed a significant amount of removal over the five days as concentrations decrease from approximately 520 at Day 0 to a final concentration of 25 mg/L on Day 5. Removal of sulfate by *D. desulfuricans* in the 1,000 mg/L reactors was similar to that observed in the 500 mg/L reactor, namely, significant removal from approximately 900 mg/L at Day 0 to 360 mg/L by Day 5. Both these removal experiments indicate little to no acclimation period for sulfate in these reactors, consistent with information in the literature regarding concurrent removal (Gorby and Lovley, 1992).

In summary, the removal of soluble uranium in sulfate reactors occurred rapidly (within one day) in solutions containing approximately 100 mg/L U. Varying the concentration of sulfate in these solution up to approximately 5,000 mg/L had little or no effect on the rate of uranium removal. A slight decrease in the rate of removal of uranium was observed in the 10,000 mg/L sulfate reactors. Conversely, lowering the concentration in the reactors to approximately 10 mg/L significantly reduced the rate of uranium removal. The near immediate removal of uranium in these reactors indicates a very short to no acclimation period. Potential causes of the lower rates of uranium removal in the sulfate solutions include: electron acceptor competition, sodium inhibition, and ionic strength inhibition. Each of these factors needs to be evaluated more fully to determine the significance of their impact on uranium removal.

Nitrate Reactors

The results for the nitrate matrix (test solutions 7 - 12) are presented in Figure 10. Figure 10 contains representative control data from the 5,000 mg/L nitrate heat killed cells reactors. These control data are consistent with all of the nitrate control results. A graph showing the entire nitrate reactors control data set is provided in Appendix A. Figure 10 indicates uranium removal occurred in all of the biologically active nitrate solutions. The tubes with the lowest concentrations of nitrates (100, 500, 1000, and 5000 mg/L) formed very distinct black pellets at the bottom. The formation of these pellets was also consistent with the findings by Lovley and Phillips, (1992). The pellets tended to break up easily if the tubes were shaken or if the surface of the water passed over it too quickly. The pellets formed over a longer period of time in the higher nitrate concentration reactors. This was especially true for the tubes containing the highest (50,000 mg/L) nitrate concentration.

The *D. desulfuricans* in test solutions containing 100, 500, 1,000 and 5,000 mg/L nitrate removed over 95% of the initial uranium in one day. After three to four days, the concentration of uranium in each of these solutions went below the detection limit of approximately 0.1 mg/L, consistent with the results seen in the sulfate solutions and with results obtained by previous researchers (Lovley and Phillips, 1992). It is noted that even though these researchers did not utilize nitrates in their solutions, evidence to support removal may be predicted from the research showing *D. desulfuricans* as capable of utilizing nitrate as an electron acceptor (Dilling and Cypionka, 1990). No odor was detected when the

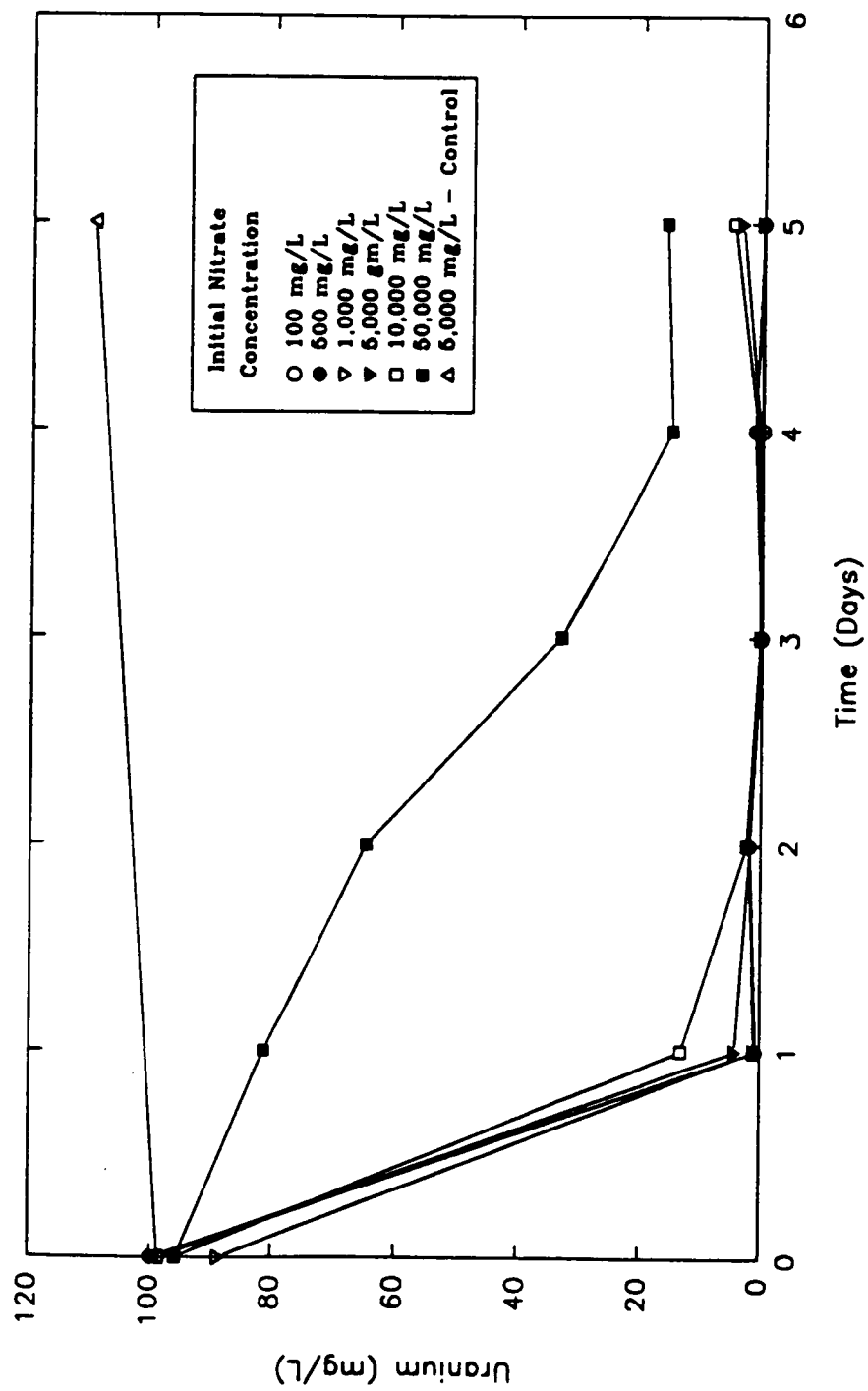
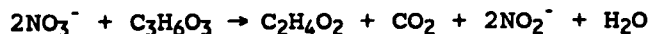


Figure 10. Uranium Removal in Nitrate Solutions

reactors were opened so the NO_3^- may have been converted to nitrite (NO_2^-) rather than NH_4 . This indicates removal of NO_3^- in the test reactors may have occurred in accordance with the equation:



In the test solution containing 10,000 mg/L nitrate, a slight drop in the rate of uranium removal was observed. Approximately 86% of the uranium was removed from solution after one day. In addition, the concentration of uranium in the test solution did not drop below the 0.1 mg/L detection limit. The lowest uranium concentration seen during the experiment was approximately 1 mg/L, which was observed on day four. A more dramatic drop in the rate of uranium removal is observed in the reactors containing 50,000 mg/L nitrate. In these reactors approximately 15% of the soluble uranium was removed during the first day. The concentration of uranium in solution did not decrease as rapidly, nor did the concentration drop to the levels of the other sulfate or nitrate experiments. The lowest uranium concentration was approximately 15 mg/L, on day 4. This corresponds to a removal of approximately 84% over the five days.

As with the sulfate containing reactors, there are four potential causes for these declines in the rate of uranium removal:

- 1) changes in the uranium complexes,
- 2) competition between the uranium and sulfate ions as electron acceptors,
- 3) sodium inhibition, and
- 4) ionic strength inhibition.

The potential for changes in the uranium complexes effecting biological removal has been evaluated through computer modeling. This modeling indicates no significant changes in the uranyl complex for the different test solutions. As a result, changes in uranium species should not account for the decline in the rate of removal. Uranium complexes are addressed in greater detail in Chapter V, Computer Simulation.

Competition of electron acceptors is evaluated through examination of the changes in nitrate concentration over the course of the experiment. Figure 11 shows the change in nitrate concentrations for the 100, 500, and 1,000 mg/L nitrate solutions. The higher nitrate test data (5,000, 10,000, and 50,000 mg/L) does not display evidence of nitrate removal. As with the high sulfate analysis above, the analytical equipment may not be accurate enough to distinguish a decrease due to biological nitrate removal.

The data in Figure 11 shows a delay of approximately two days prior to the start of nitrate removal. Once nitrate removal appears to begin, it proceeded rapidly on days 3 and 4. These results indicate an acclimation period prior to initiation of nitrate removal. This is consistent with experiments conducted using the uranium reducing microorganism *G. metallireducans* (Gorby and Lovley, 1991). In these experiments, *G. metallireducans* cultivated in a ferric citrate based media removed twice as much Fe(III) as in a sodium nitrate based solution. These results are used as evidence for multiple enzyme systems in *G. metallireducans*. In the same way, the acclimation period observed in the *D. desulfuricans* experiment suggests different enzyme pathways for uranium and nitrate removal. Based on this information, it may be suggested that, during the first two days, uranium was the sole electron acceptor in the test

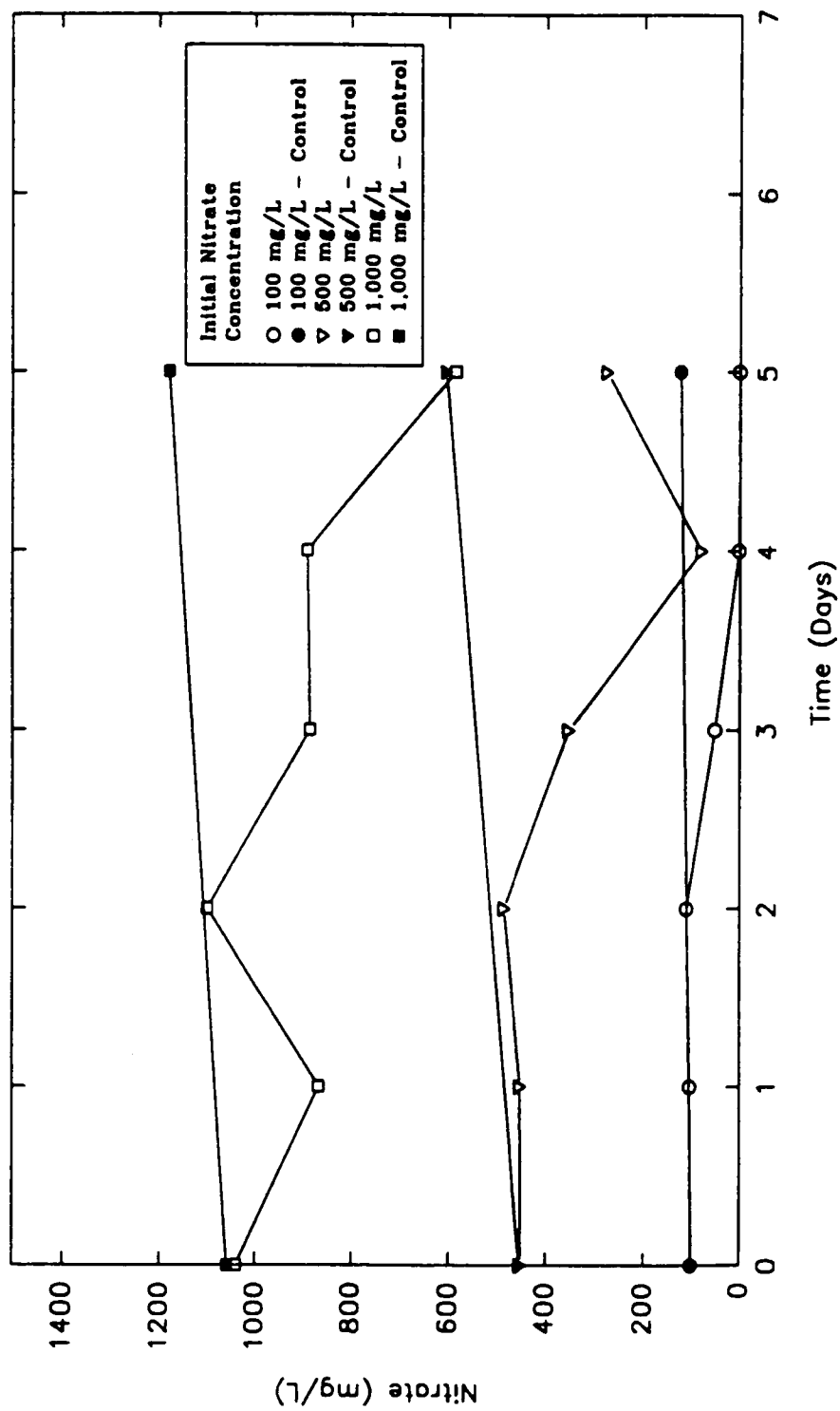


Figure 11. Nitrate Removal in *D. desulfuricans* Reactors

solutions. No competition between nitrate and uranium should have occurred, and all of the test reactors should have behaved in a manner similar to the 0 mg/L sulfate test solution above. Continuing with this evidence, it may be suggested that electron acceptor competition may not account for the decline in the rate of uranium removal seen in the nitrate experiments. Additional studies on sodium and ionic strength inhibition on *D. desulfuricans* should be performed to determine the cause(s) of decline in the rate of uranium removal.

The removal of uranium by *D. desulfuricans* in nitrate solutions containing low concentrations (approximately 10 mg/L) of uranium are shown in Figure 12. Figure 12 contains representative control data from the 10,000 mg/L low concentration uranium nitrate heat killed cells reactors. These control data are consistent with most of the low concentration uranium nitrate control results. A slight increase in the uranium concentration (2 mg/L) was observed in the 50,000 mg/L no cell control reactor. A graph showing the entire low concentration uranium nitrate reactors control data set is provided in Appendix A. Unlike the sulfate reactors above, Figure 11 shows the *D. desulfuricans* in these reactors removed virtually all the uranium within four days. The more rapid removal of uranium in the nitrate reactors may be due to electron acceptor availability. In the experiments containing higher concentrations of uranium, there was evidence that the *D. desulfuricans* needed to acclimate to nitrates for approximately two days. During this time, the only available electron acceptor would be the uranyl ion, thus making it more likely to be reduced.

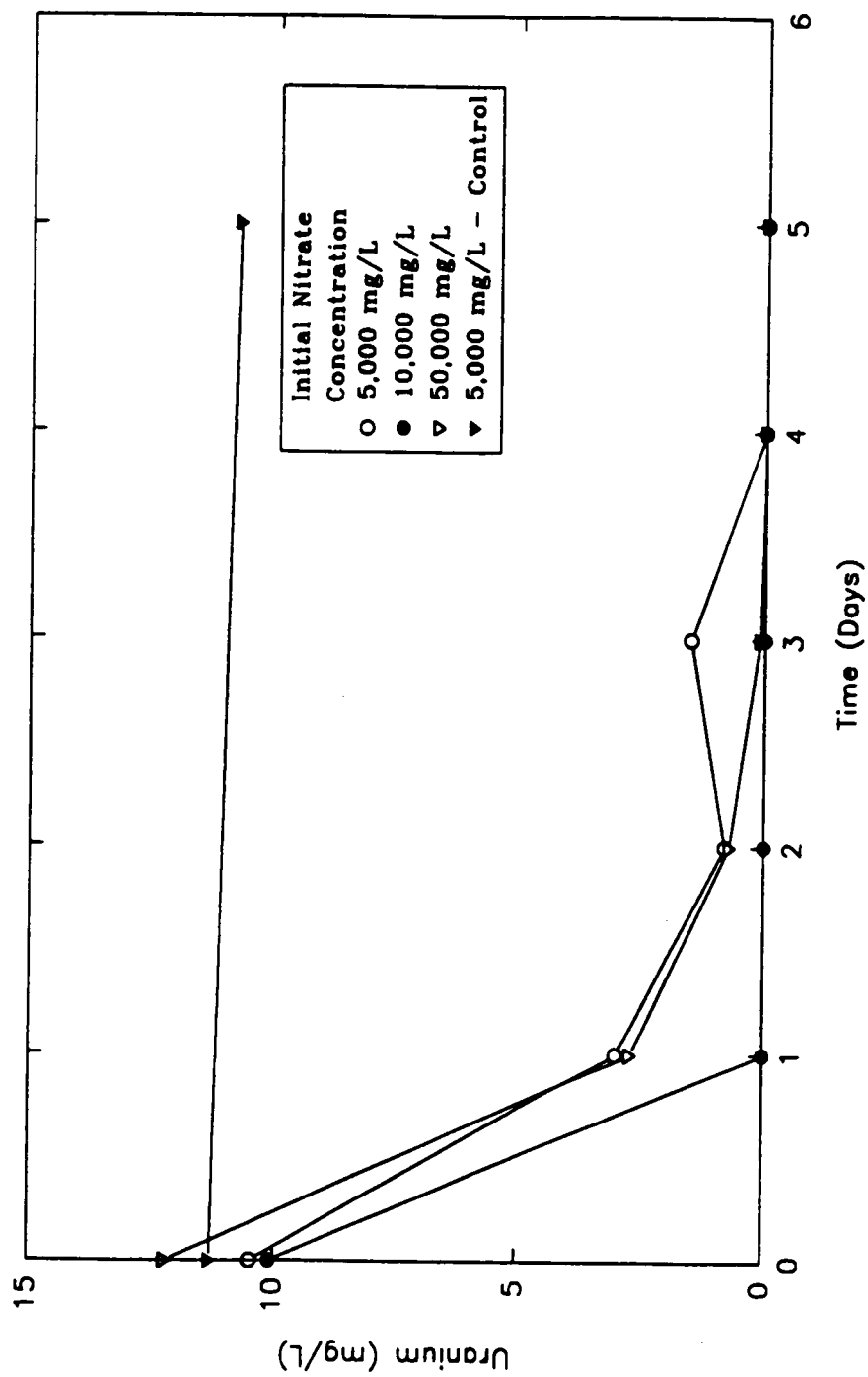


Figure 12. Low Level Uranium Removal in Nitrate Solutions

Combined Nitrate/Sulfate Reactors

A final set of uranium removal experiments was performed with solutions containing sulfates and nitrates. This set of combined sulfate/nitrate reactors was run with the intent to simulate more closely an actual wastewater stream. Results of the combined nitrate/sulfate matrix (test solutions 13-18) are presented in Figure 13. Figure 13 contains representative control data from the 50,000 mg/L nitrate, 100 mg/L sulfate heat killed cells reactors. These control data are consistent with many of the combined sulfate/nitrate control results. A slight decrease (approximately 5 mg/L) was observed in the following:

- 50,000 mg/L nitrate/10,000 mg/L sulfate no cell,
- 1,000 mg/L nitrate/5,000 mg/L sulfate heat killed cells,
- 1,000 mg/L nitrate/100 mg/L sulfate heat killed cells, and
- 100 mg/L nitrate/100 mg/L sulfate heat killed cells controls.

A graph showing the entire sulfate/nitrate reactors control data set is provided in Appendix A. The tubes containing the lower levels of nitrate in the combined matrix responded similar to the tubes containing the sulfate concentrations from the matrix described previously. A dark pellet formed after the first day, and these tubes tended to be clearer than the tubes containing the 50,000 mg/L nitrate. The high nitrate-containing tubes also formed a film on the inside walls. Samples from these tubes also had the distinct hydrogen sulfide odor that was readily detected after approximately two days. The tubes containing 50,000 mg/L nitrate did not form a pellet after the first day. Based on this observation and the data showing incomplete uranium removal in the 50,000 mg/L nitrate sample, a decision was made to sample the tubes every other

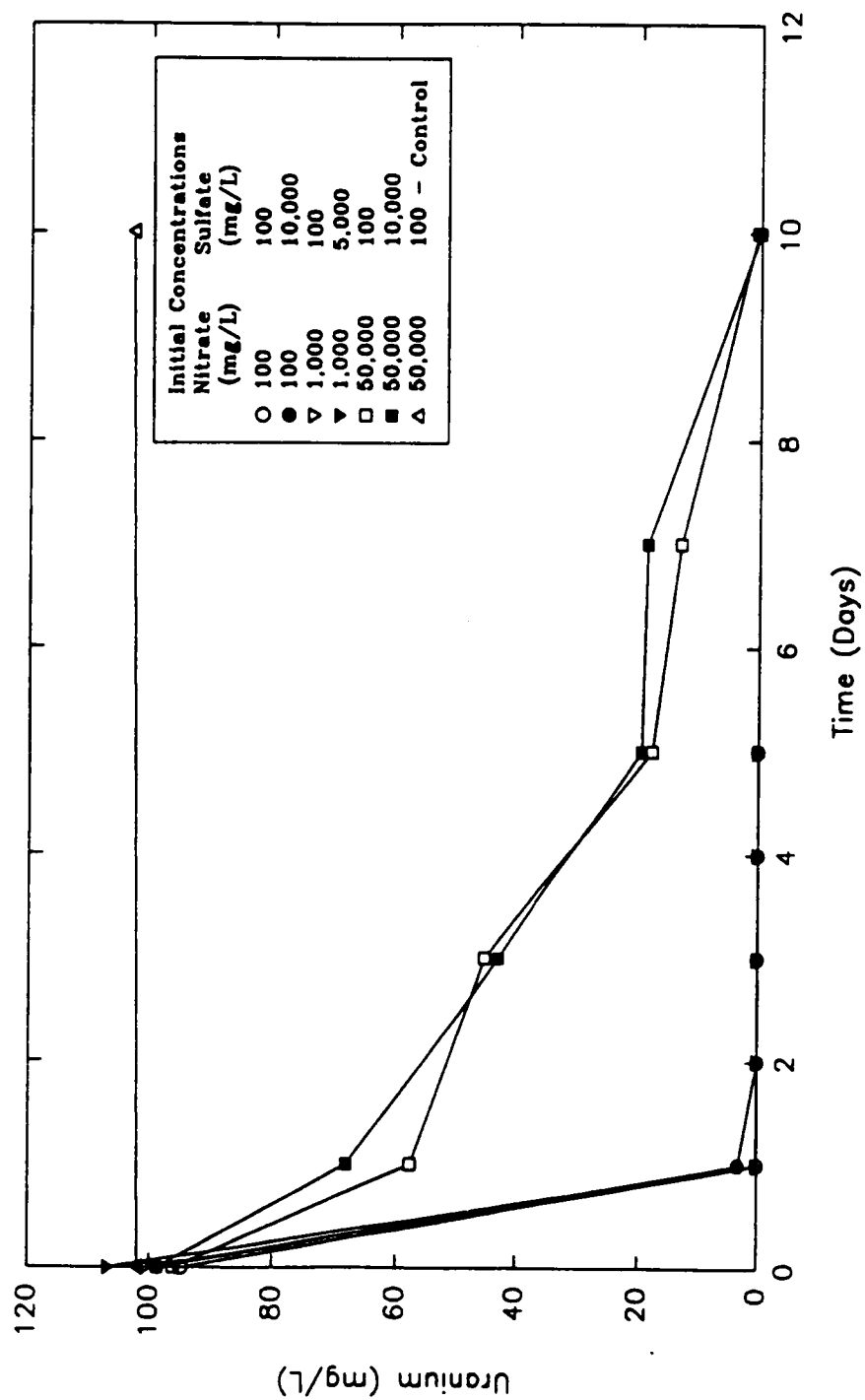


Figure 13. Uranium Removal in Combined Sulfate/Nitrate Solutions

day in order to extend the duration of the test. After five to seven days the dark spot accompanied by whitish film was noticeable in the bottom of the tubes.

In these reactors containing combined nitrates and sulfates, the removal of uranium occurred in a manner consistent with those for the individual anions. Rapid removal of uranium occurred in all of the reactors containing less than 50,000 mg/L nitrate.

In the two sets of reactors containing 50,000 mg/L nitrate, uranium removal appeared to be significantly slower. These reactors were run for 10 days, based on the results from the experiments containing nitrate alone. In the nitrate-only experiments, *D. desulfuricans* did not fully reduce the concentration of uranium in solution below the 0.1 mg/L detection limit in reactors containing 50,000 mg/L nitrate within the five days. This experiment was extended to determine whether or not time was a limiting factor. Figure 13 shows uranium removals at a rate similar to the 50,000 mg/L nitrate reactor in Figure 10. In addition, near complete uranium removal in these reactors was observed at day 10 where the final concentration was removed to > 1 mg/L. From this information, it may be assumed that, if the nitrate-alone experiment was conducted over a longer period of time, additional uranium removal may have been observed.

The decline in the rate of uranium removal appears to be independent of the sulfate concentration. This is demonstrated in Figure 14, which shows removal of uranium in the 50,000 mg/L nitrate solutions containing 0, 100, and 10,000 mg/L sulfate. Based on the discussions for the nitrate-containing reactors above, two potential explanations for the decrease in the rate of uranium removals include sodium inhibition and ionic strength

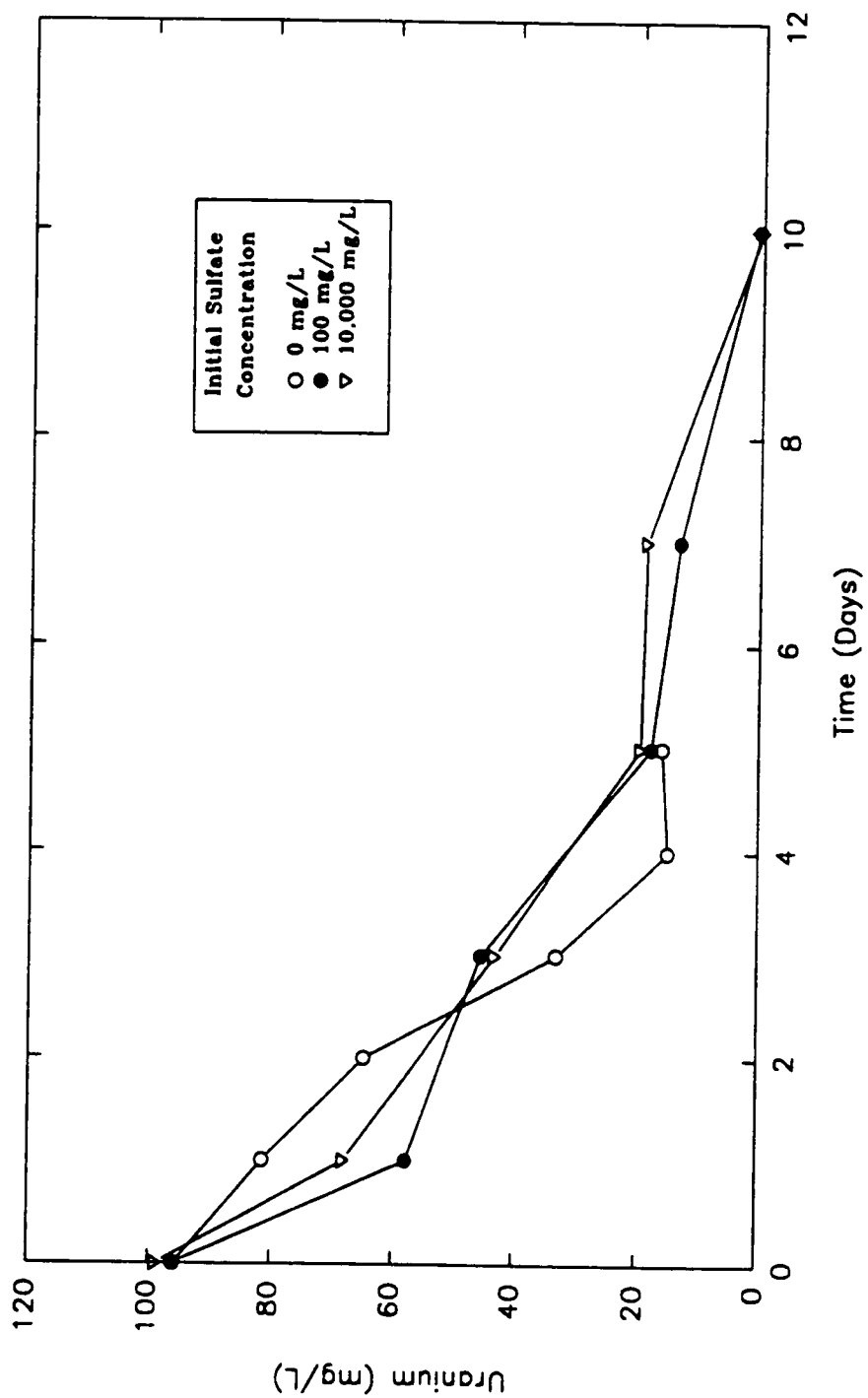


Figure 14. Uranium Removal in 50,000 mg/L Nitrate Solutions

interference. Both of these factors need to be examined to determine their role(s) in the decline in the rate of uranium removal.

CHAPTER V

COMPUTER SIMULATION

Utilization of a geochemical equilibrium model such as MINTEQA2 to evaluate an environmental problem involves following a four step process:

1. Formulate precise chemical questions that can be answered if the chemical composition of the solution is known. The questions must take into account limitations in the data due to uncertainties, such as incomplete sampling and/or analysis.
2. Develop a data set under which the model will operate to produce potential solutions to the questions formulated in step 1.
3. Run the computer program (i.e., MINTEQA2) to solve the mathematical problem.
4. Interpret the output data set(s) to answer the questions posed in step 1.

This process has been followed to assess the applicability of *D. desulfuricans* to actual uranium-containing waste streams. Step one of this process requires the user to define and develop the questions to be solved with the assistance of the model. The questions to be answered in this study are:

- 1) What are the uranium complexes in the nitrate and sulfate test solutions?

2) Will changes in the nitrate and sulfate concentration affect the uranyl complexes?

3) Are these complexes similar to what is predicted in actual wastes?

The answers to these questions should provide insights to bioavailability as a function of uranium speciation. This information should supply a degree of confidence for the application of the technology. If the computer model predicts complexes in the actual waste that are similar to those in the test solutions, there should be a high level of confidence that the microorganism will reduce the uranium. If, however, the uranium complexes in the waste are not similar to those in the test solutions additional research may be required.

Development of the data sets to solve the above questions was accomplished through the use of the "PRODEFA2" program. PRODEFA2 contains a set of questions designed to prompt the user for chemical composition data and constraints regarding the solution(s) to be modeled. When the user completes data entry, PRODEFA2 generates the input data file for MINTEQA2 to run.

To determine all the expected uranium complexes, the data input files were set up to calculate equilibrium conditions for a pH range of 6 to 8. In addition, the model was allowed to precipitate over-saturated solids.

Test Solutions

Twelve data files were used to assess the composition of the sulfate and nitrate test solutions. The compositions of these solutions are described in Table 3. Results from the model on the laboratory test

solutions are presented as uranium distribution diagrams in Figures 15, 16, 17, and 18. Figure 15 shows the uranium distribution diagram for the test solution which contained uranium as the sole electron acceptor. Figures 16 and 17 show the uranium distributions for the 10,000 mg/L sulfate and 50,000 mg/L nitrate test solutions, respectively. Figure 18 shows the uranium distribution diagram for the 10,000 mg/L sulfate; 50,000 mg/L nitrate solution.

A comparison of the distribution diagrams for the test solutions indicate relatively small differences in the uranium complexes expected to be formed. All of the test solutions indicate the primary complexes expected near $\text{pH} = 7$ include $\text{UO}_2(\text{CO}_3)_2^{-2}$ and $\text{UO}_2(\text{CO}_3)_3^{-4}$, consistent with distribution diagrams found in the literature and Figure 1 (Langmuir, 1978). The uranium removal experiments were conducted at $\text{pH} = 7$, so the variations in the rate of removal for the high nitrate solutions may not be due to variations in the uranium complexes. The model indicates the only differences in uranium complexes for the test solutions may occur at a $\text{pH} < 6.2$. At these levels, the uranyl hydroxide complexes $((\text{UO}_2)_3(\text{OH})_5^+$ and $\text{UO}_2(\text{OH})^+$) seem to compete with uranyl sulfate $(\text{UO}_2(\text{SO}_4)_2^{-2})$ complexes. This is seen in the MINTEQ output file for the system containing 10,000 mg/L sulfates. These data are not apparent in Figure 16 because the concentration of these complexes are not high enough to be visible. Since this uranium complex competition is observed to occur at pH below the range of the test solutions, these effects are not considered to be significant. This competition is not noted in the test solutions containing nitrates. As a result, changes in uranyl complexes do not seem to be a source of the decline in the uranium removal rate.

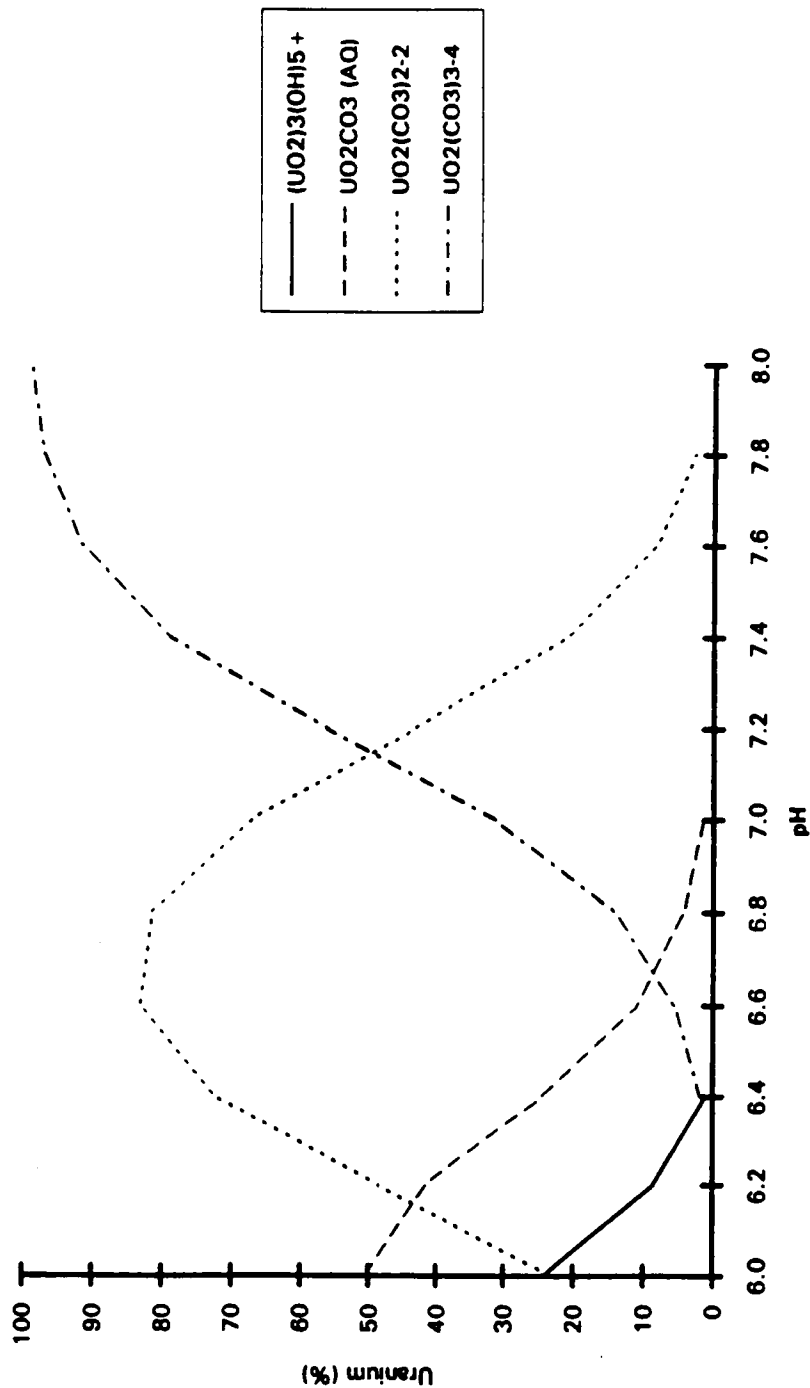


Figure 15. Uranium Distribution Diagram; 0 mg/L Sulfate, 0 mg/L Nitrate Solution

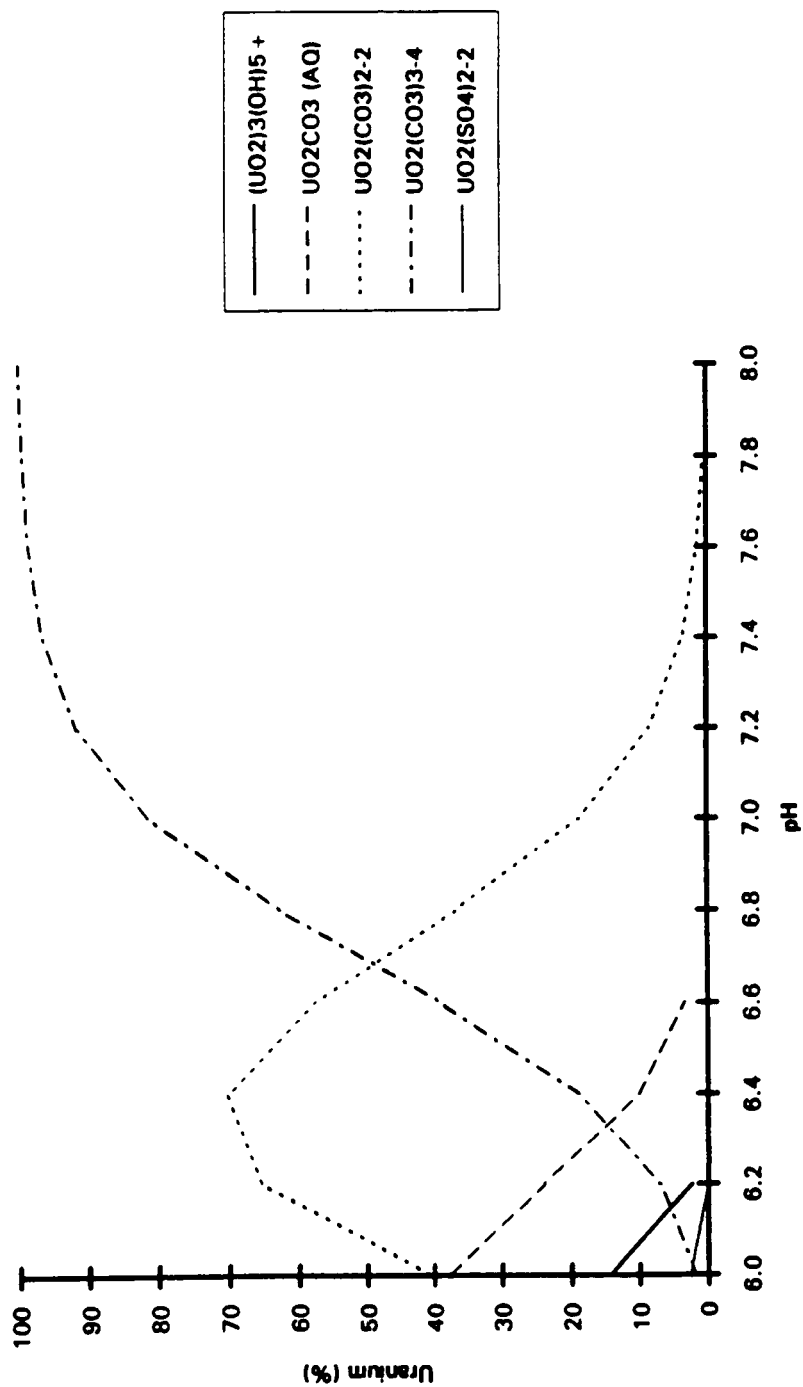


Figure 16. Uranium Distribution Diagram; 10,000 mg/L Sulfate Solution

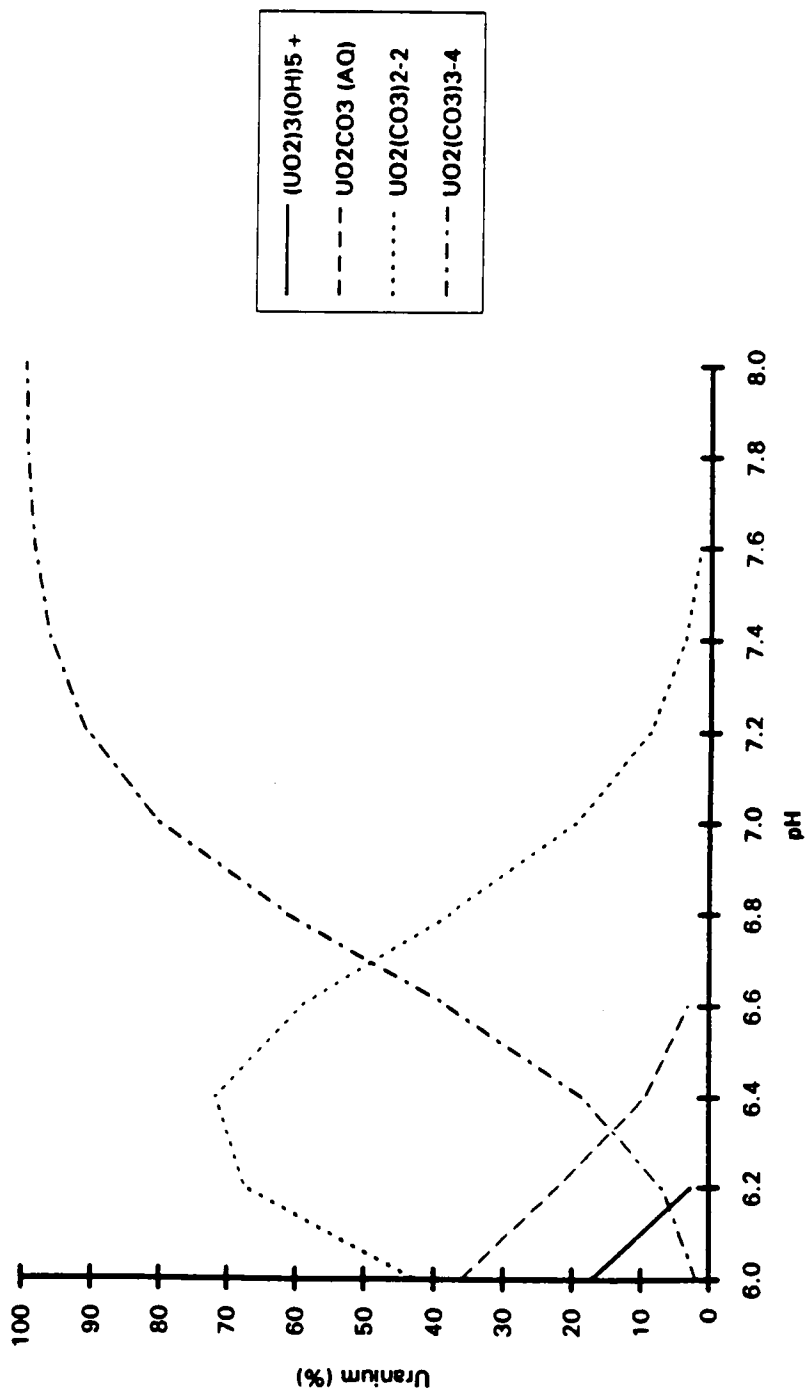


Figure 17. Uranium Distribution Diagram; 50,000 mg/L Nitrate Solution

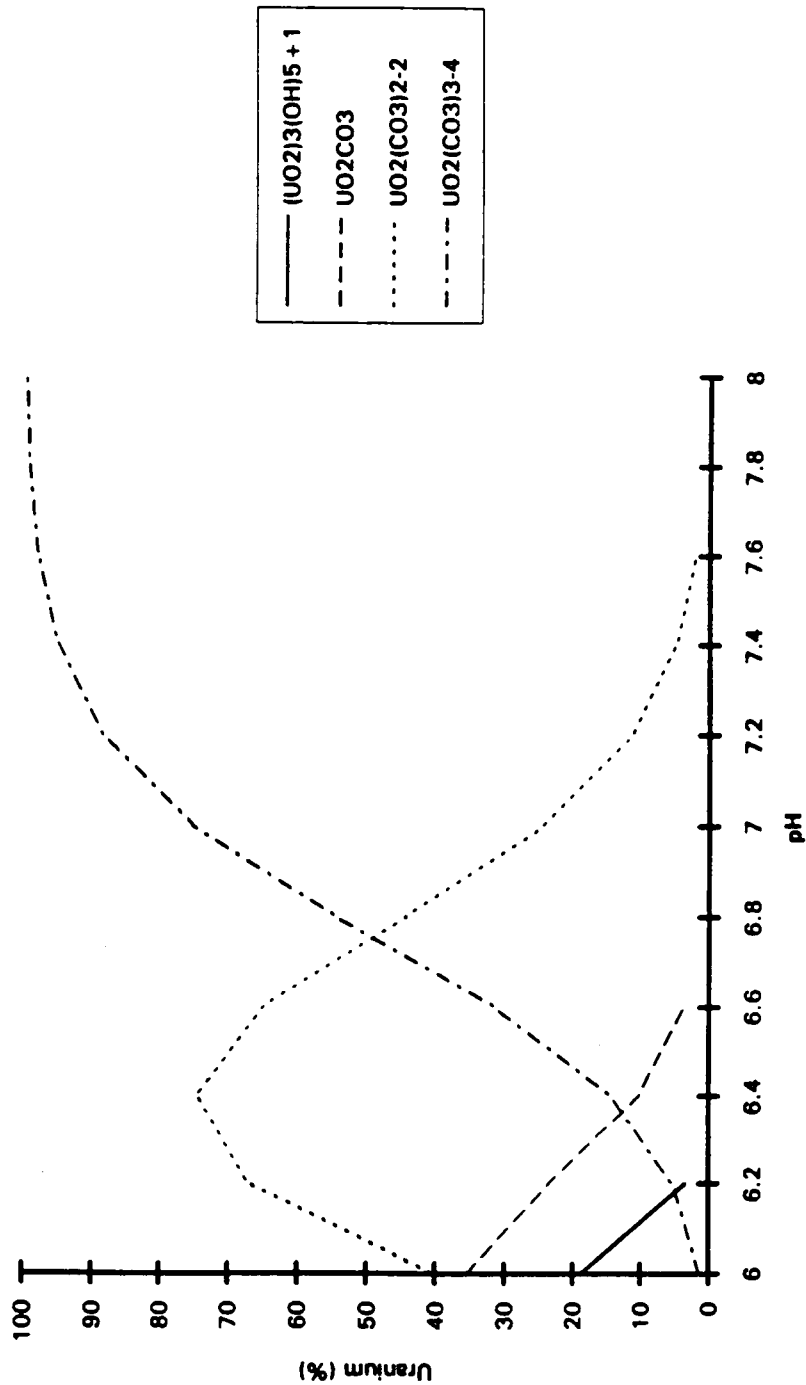
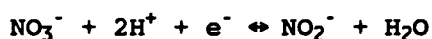
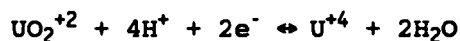


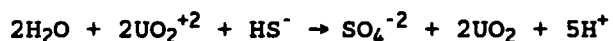
Figure 18. Uranium Distribution Diagram; 10,000 mg/L Sulfate, 50,000 mg/L Nitrate Solution

The MINTEQA2 model was also utilized in an attempt to assess the thermodynamics of the sulfate and nitrate test solutions. This was performed by activating "redox couples" options in the data input files. Redox couple equations from the model are shown below:



Modeling of the sulfate test solution was performed by setting the initial hydrogen sulfide (HS^-) concentration to 0.0 mM and allowing the model to calculate an interim solution. The program then began a set of sweeps where the HS^- concentration was set to 0.01 mM. After solving, the concentration of HS^- was increased incrementally by 0.02 mM for successive sweeps.

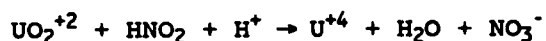
The results of these sweeps showed that, as the concentration of HS^- input into the system increased, a corresponding drop in the concentration of UO_2^{+2} was observed. This drop in UO_2^{+2} corresponded to an increase in the amount of precipitated uranium (as UO_2) and SO_4^{-2} formed, and was consistent with a stoichiometry of:



The calculated free energy released from this reaction is approximately -439 KJ/Mole, indicating the products are thermodynamically stable, and suggesting uranium removal in the sulfate containing reactors may not be totally the result of direct biological activity on the part of *D. desulfuricans*. These data suggest that the sulfide as HS^- may contribute to the formation of uraninite.

It should be noted, however, that MINTEQA2 is a thermodynamic equilibrium model and does not account for reaction rates. If the reaction above is assumed to take place rapidly, it could account for the removal observed in the reactors containing 100 mg/L uranium. As the concentration of sulfate in the test reactors increase the rate of uranium removal from solution should also increase. This was not observed in the test reactors. When the concentration of sulfate was increased to 10,000 mg/L, a decline in the rate of uranium removal was observed. In addition, all of the test solutions containing 10 mg/L uranium showed a significantly lower removal rate. Based on sulfate removal data in the 100 mg/L uranium reactors, it can be assumed there was a significant amount of HS^- present. This HS^- should have been available for rapid chemical removal of the uranium if the above reaction rate is high. The data in Figure 8, showing slower uranium removal at low concentrations than at high concentrations (Figure 6), indicates chemical removal may not have played a significant role in the reactors. These findings are consistent with studies in which no uranium removal was observed in solutions containing only U(VI) and HS^- (Lovley and Phillips, 1992).

Initial modeling of the nitrate-containing solutions showed no removal of uranium with an increased concentration of nitrite (NO_2^-), consistent with thermodynamics. Based on the nitrate and uranium half-cells above, the redox equation might be written as:



However, when the electrode potential for this coupled reaction is calculated, $E^0 = -0.56$ volts (Dean, 1992). With the result that the free energy of this reaction is positive and it will not proceed as written.

The model was next run for conditions of decreasing pe. These results indicated a removal of uranium from UO_2^+ to UO_2 beginning near $\text{pe} = 1.4$. A close inspection of the output file for this condition reveals no proportionate concurrent oxidation of any ionic species, as is stated by Langmuir (1978). It may be assumed the MINTEQA2 model is calculating the most thermodynamically favorable equilibrium conditions utilizing the $\text{UO}_2^{+2}/\text{U}^{+4}$ half-cell alone. Since half-cell reactions cannot occur on their own, these results may be considered suspect (Soneyink, 1980). There is little evidence to support physical chemical reactions in nitrate solutions for removal of uranium. The most likely agent of uranium removal may be assumed to be through direct biological activity.

Y-12 West End Waste

Four data files were generated for the MINTEQ model, based on analytical data for samples of waste treated at the DOE Oak Ridge Y-12 West End Treatment Facility. This data was provided by Y-12 Waste Treatment Operations personnel, and is shown in Table V. Data samples 1, 2, and 3 were provided prior to the start of the batch reactors and were utilized to determine test parameters. Based on a visual inspection of the sample reports, the major (and assumed to be controlling) anions in the waste were determined to be nitrate and sulfate. The uranium concentration of 100 mg/L was selected based on a conservative range for the actual wastes. The four Y-12 waste data files could be modeled under two conditions. First, the alkalinity may be entered as a fixed amount of carbonate (CO_3^{-2}), analogous to a closed system. Second, the model may be run with fixed alkalinity as carbonate which is analogous to a system containing a solution in the presence of CO_2 gas at equilibrium. Under

Table V. Y-12 Waste Analysis

Analyte	Concentration (mg/L)			
	Sample 1	Sample 2	Sample 3	Sample 4
Al ⁺³	4.8	1.9	.7	23
Ca ⁺²	62	7900	8100	106
Cu ⁺²	.6	9.1	28	17.2
F ⁻¹	43	5.3	7.9	5
Fe ⁺³	1.8	6.1	1.2	179
Li ⁺¹	6.1	6.5	6.5	15.7
Mg ⁺²	400	300	420	136
Ni ⁺²	2	.009	2.7	14.8
NO ₃ ⁻¹	645	21800	16280	62000
K ⁺¹	550	430	250	420
SO ₄ ⁻²	3350	860	790	14000
Na ⁺¹	5100	4300	3300	308
Zn ⁺²	.14	.0009	.0009	5.13
UO ₂ ⁺²	70	137	60	340
PO ₄ ⁻³	80	61	52	3700
CO ₃ ⁻²	455	600	600	1786
Mn ⁺³	N/A	N/A	N/A	71.6

these conditions, the carbonate precipitating out of solution is made up by CO_2 dissolving from the gas phase. In this way the total alkalinity of the solution remains constant. The Y-12 waste waters were assumed to be closed systems, so fixed carbonate was chosen for the data sets.

The uranium distribution diagrams for the Y-12 West End wastewaters are shown in Figures 19, 20, 21, and 22.

The results from the sample 1 data indicate only uranyl phosphate complexes form. The model suggests only soluble uranyl phosphate ($\text{UO}_2(\text{HPO}_4)_2$) will be present from pH 6.0 to 6.7. At pH = 6.8, sodium autunite ($\text{Na}_2(\text{UO}_2)_2(\text{PO}_4)_2$) begins to precipitate, reaching near completion at pH = 8.0. A sensitivity analysis indicates calcium plays a major role in the formation of sodium autunite in this waste water. As the concentration of calcium is increased, the sodium autunite is predicted to begin precipitation at lower pH. Doubling the amount of calcium (120 mg/L) predicted formation of sodium autunite at pH = 6.6.

Distribution diagrams predicted by MINTEQ for samples 2 and 3 are nearly identical. Only a small amount of uranium is predicted to be soluble. The most prevalent soluble complex is $\text{UO}_2(\text{CO}_3)_2^{-2}$, which reaches a maximum uranium concentration of approximately 7 mg/L near pH = 6.6. Most of the uranium in each sample is predicted to be precipitated. Below pH = 6.8, the precipitate is sodium autunite. Near pH = 6.8, the uranium precipitate changes to schoepite ($\text{UO}_2(\text{OH})_2$). A gross sensitivity analysis on the samples indicates the change in uranium precipitate is due to the very high concentration of calcium. At these levels, the calcium precipitates more readily than uranium so it should have more anions available to form salts. As the calcium salts fall out of solution, they

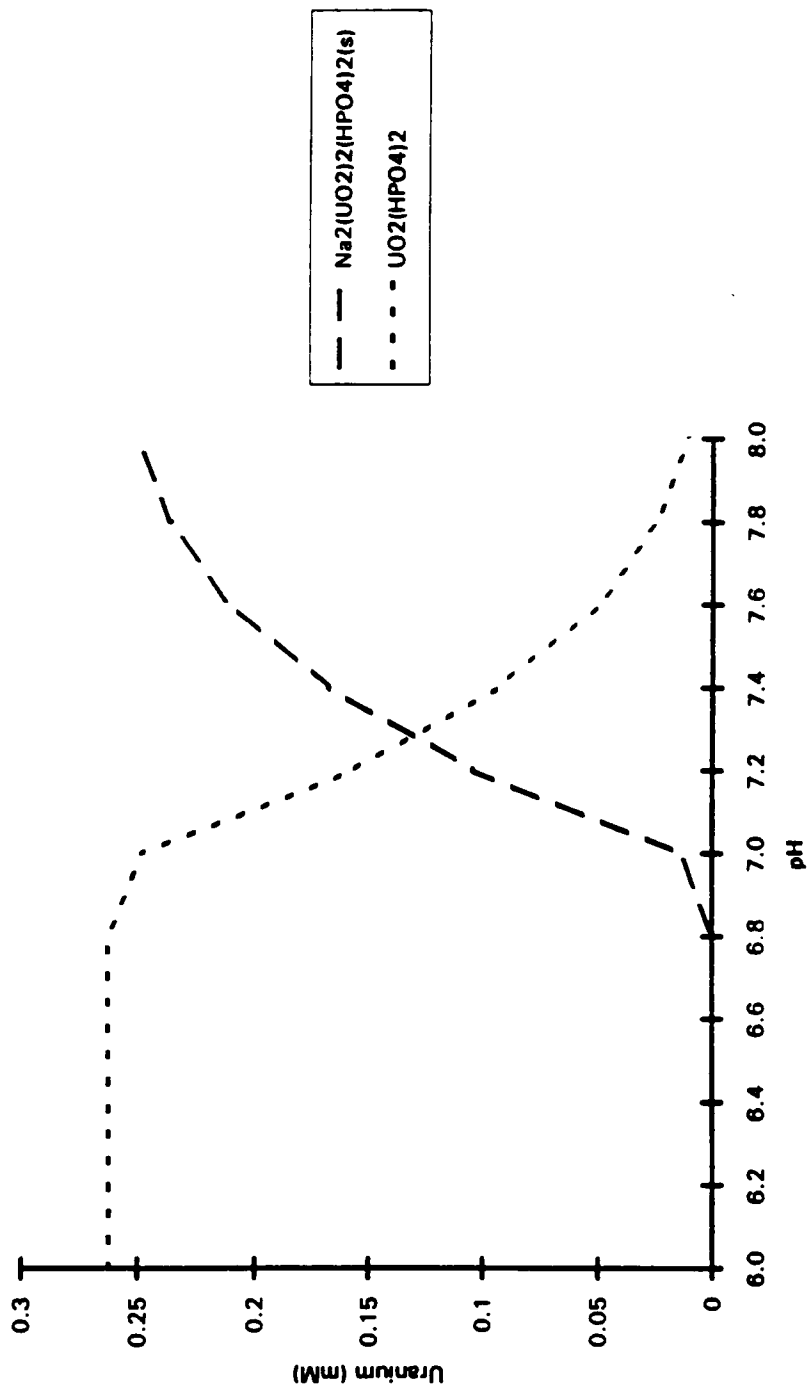


Figure 19. Uranium Distribution Diagram for Y-12 Waste Sample 1

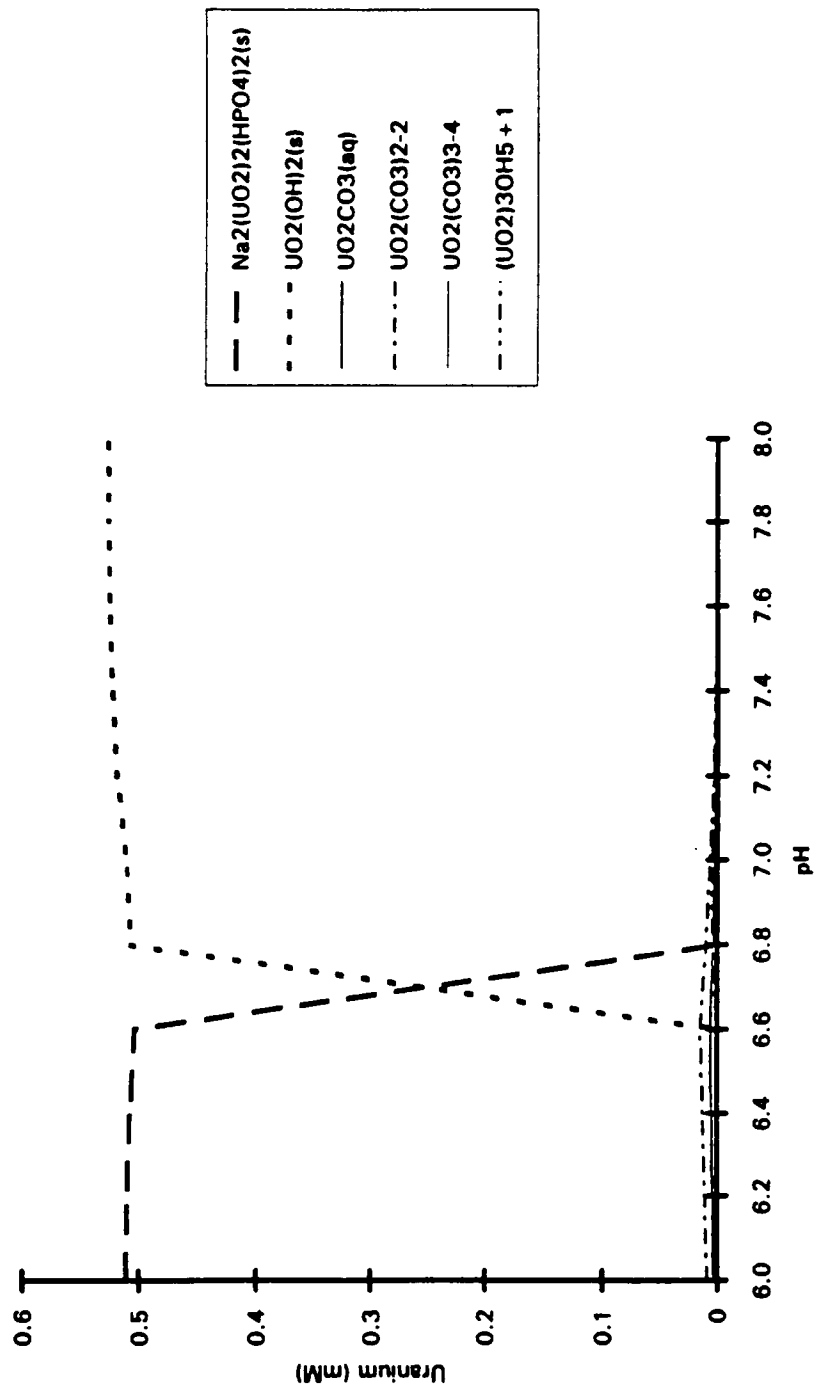


Figure 20. Uranium Distribution Diagram for Y-12 Waste Sample 2

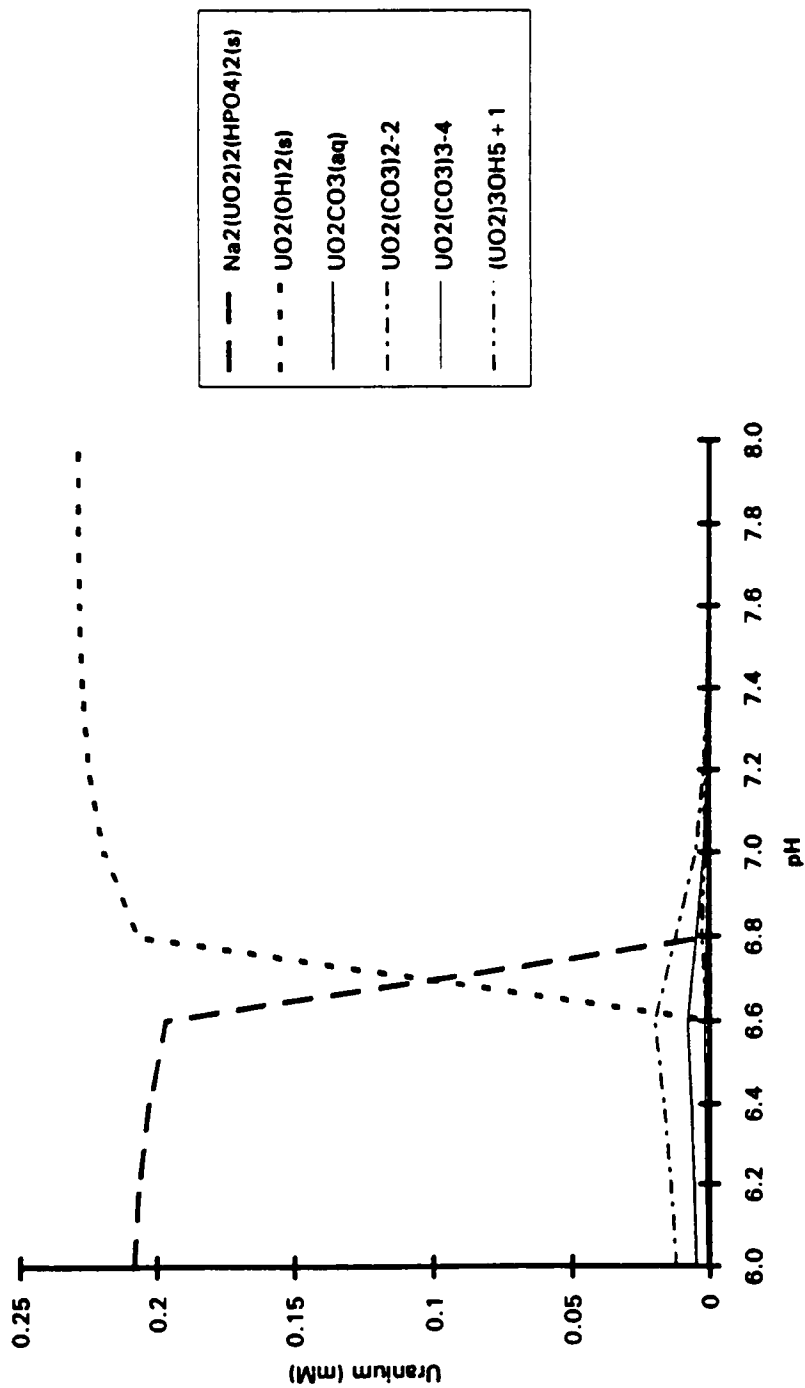


Figure 21. Uranium Distribution Diagram for Y-12 Waste Sample 3

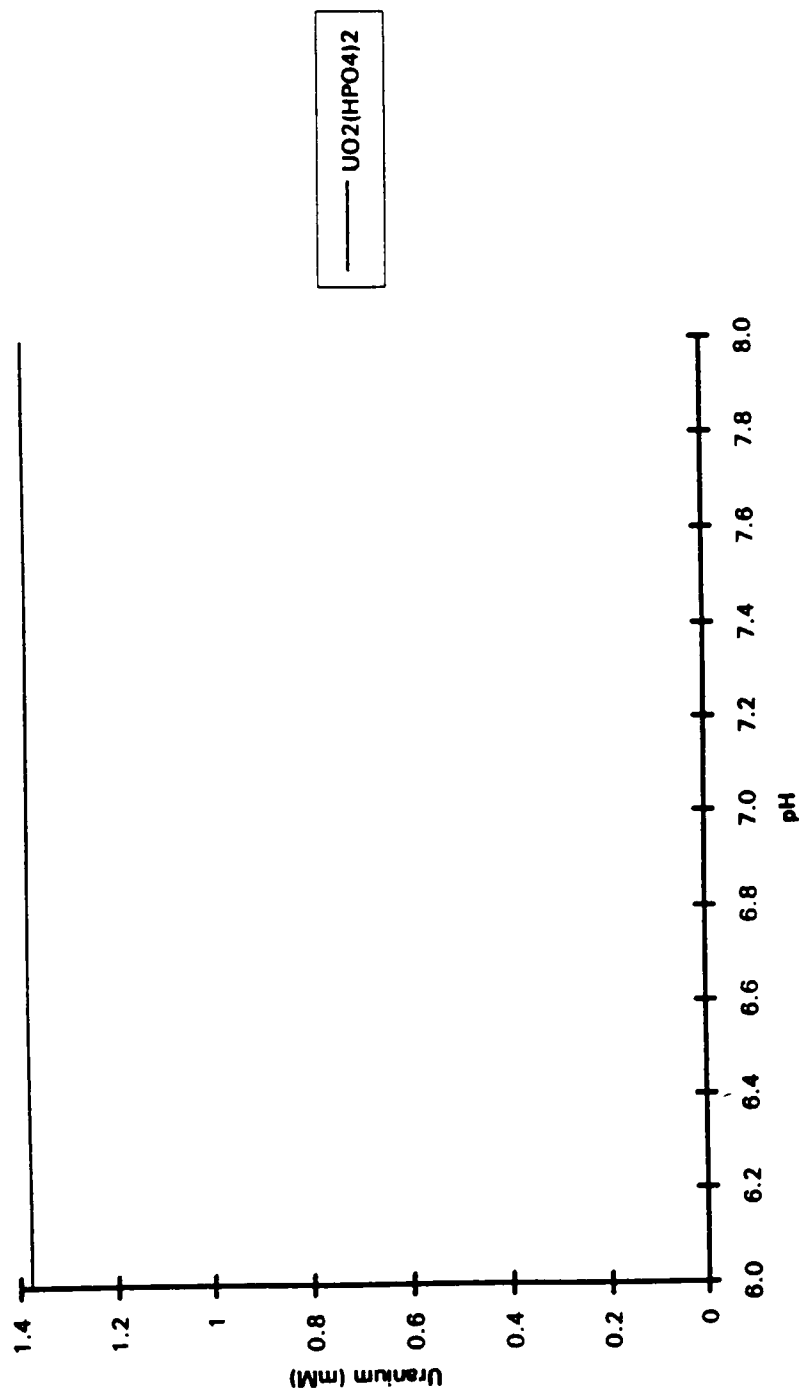


Figure 22. Uranium Distribution Diagram for Y-12 Waste Sample 4

first pull out fluoride, then the carbonates and phosphates. The calcium salts falling out of solution deplete the phosphate and carbonate, making it unavailable to the uranium. At pH = 6.8, the only anion that will complex with the uranium at these levels appears to be hydroxide. As these complexes form, the uranium precipitates as schoepite. At pH = 7.6, the model predicts virtually all of the uranium will be precipitated as schoepite.

The sample 4 distribution diagram indicates the only form of uranium in solution will be uranyl phosphate, and no uranium precipitates will be formed as a result of solubility effects. Sensitivity analysis of the waste water indicates no uranyl phosphate precipitates form due to the low concentrations of both sodium and calcium. At approximately 2,000 mg/L sodium and 3,000 mg/L calcium, sodium autunite begins to precipitate at pH 6.4. At these levels, the model suggests that not all of the uranium precipitates. The maximum precipitation (66%) seems to occur near pH = 6.6. As the pH increases to 7.2, all of the uranium will have resolubilized. In addition, the uranium complexes developed at 7.2 will be in the carbonate forms.

The results of these equilibrium modeling studies indicate phosphates have a major impact on uranium complexes expected to be formed in the Y-12 West End wastewaters. Three of the wastewaters modeled indicate that, in normal pH range (approximately 7), little to no uranium will be found in solution. The nature of the waste suggests most of the uranium will not be stable in solution under these conditions.

It was noted that the model predicts a significant amount of calcium, iron, magnesium, aluminum, and copper salts will fall out of

solution in addition to the uranium. Most of the calcium precipitates result from complexing with carbonate. Since carbonate complexes are needed to keep uranium soluble, the model was run without calcium in the data input file. This would keep the carbonate in solution and predict the impact it would have on the uranyl phosphate complexes.

The results from these calculations are represented in Figure 23. Figure 23 shows the uranium complexes for Sample 2 without calcium. This figure was consistent with results obtained for samples 1, 3, and 4. A comparison of Figure 23 with Figure 20 indicates no significant difference in the uranium complexes. These Figures indicate phosphate in the solution seems to have a significant effect on the uranium complexes. The model indicates uranium has a higher affinity for phosphate than for the carbonate. The model indicates that, even with as much as 590 mg/L of the carbonate in solution, uranium favors the phosphate complexes, which in turn has a significant effect on the uranium solubility.

Because the phosphate seems to have a significant impact on the uranium solubility, the model was run without it in the data input files. The results from these calculations are represented in Figure 24. Figure 24 was consistent with results from samples 3 and 4 which indicate if there is no phosphate in the solutions, Uranyl hydroxide (UO_2OH_2) begins to form as a major precipitate near $\text{pH} = 6.2$. This is due to the large amount of carbonate being complexed and precipitated with the calcium. When the carbonate is removed by the calcium, the only anion available to complex with the uranium is hydroxide (OH^-). Sample 1 was not consistent with these results. Figure 25 shows the uranium complexes for sample 1 without phosphates.

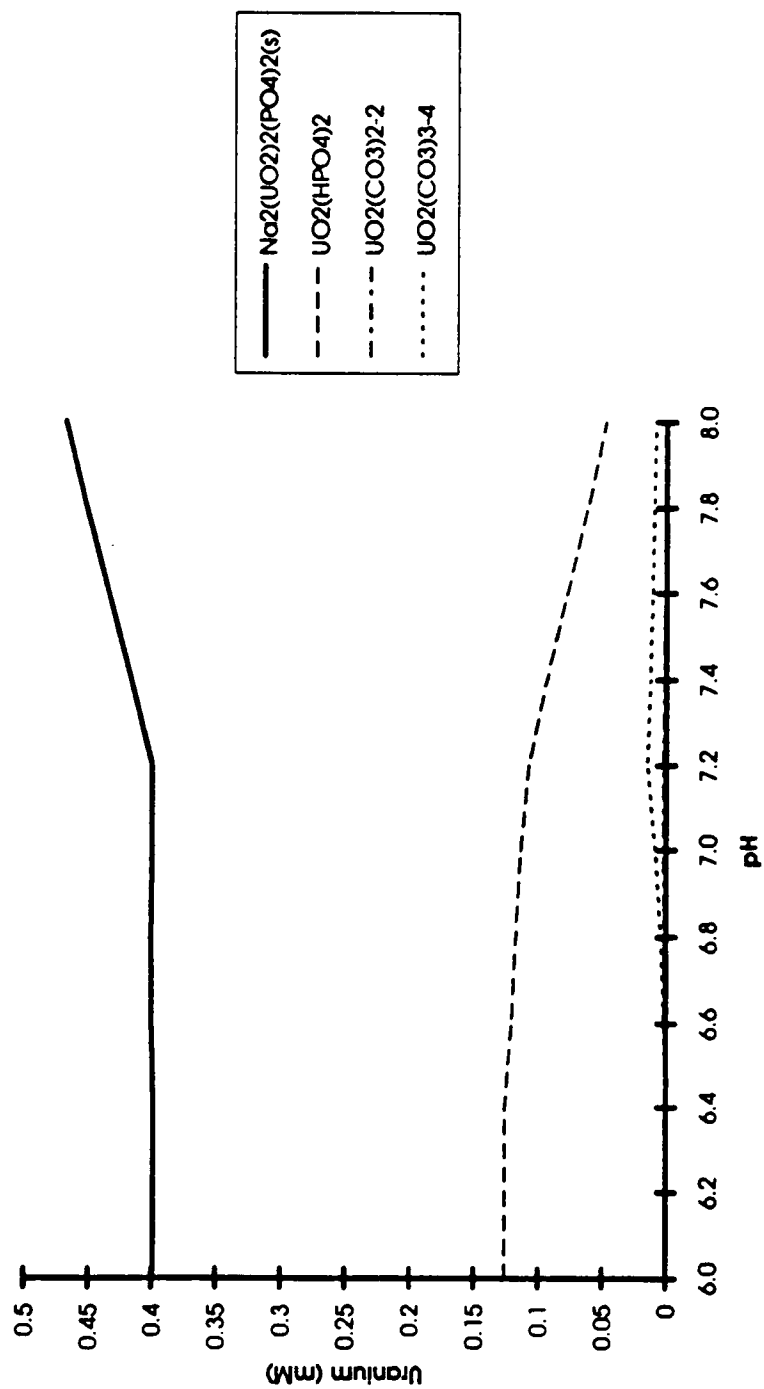


Figure 23. Uranium Distribution Diagram for Y-12 Waste Sample 2 Without Calcium

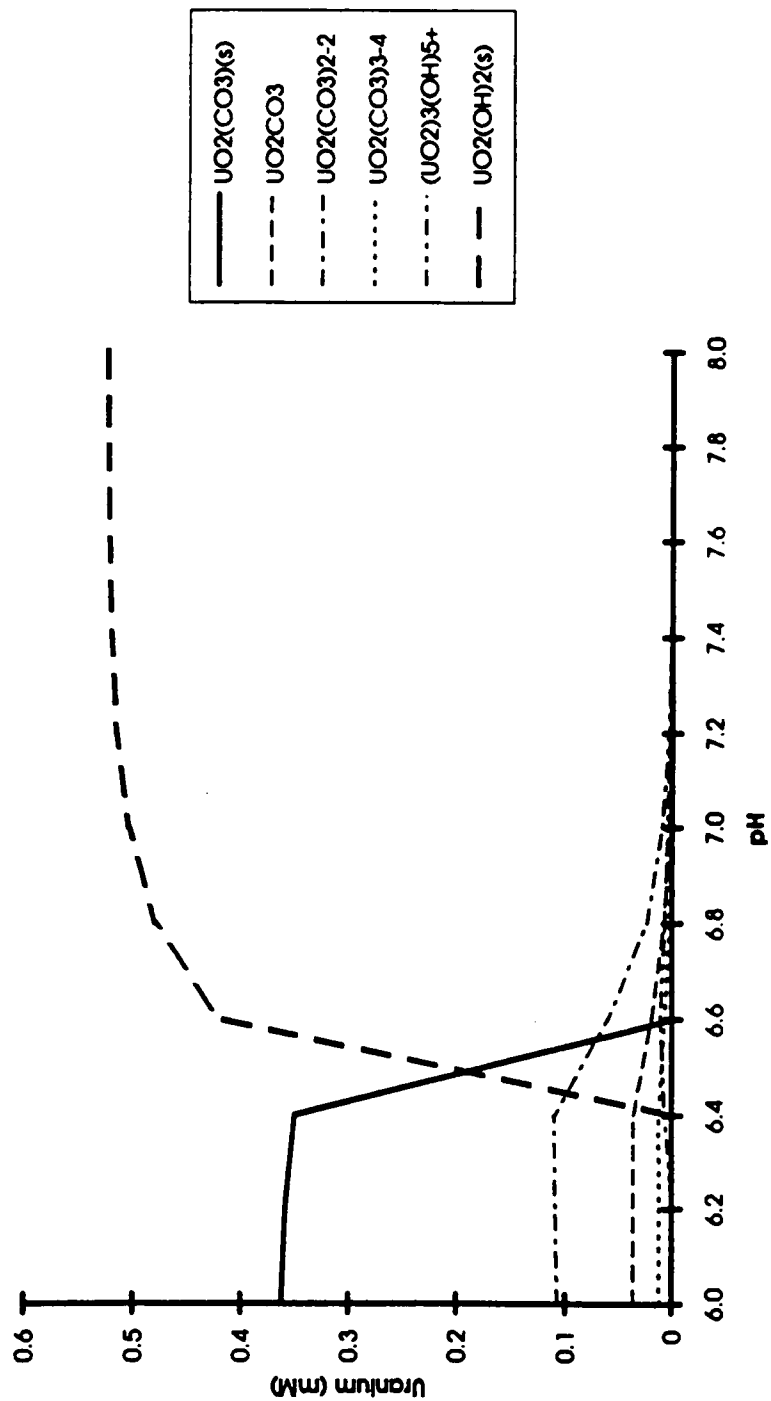


Figure 24. Uranium Distribution Diagram for Y-12 Waste Sample 2 Without Phosphate

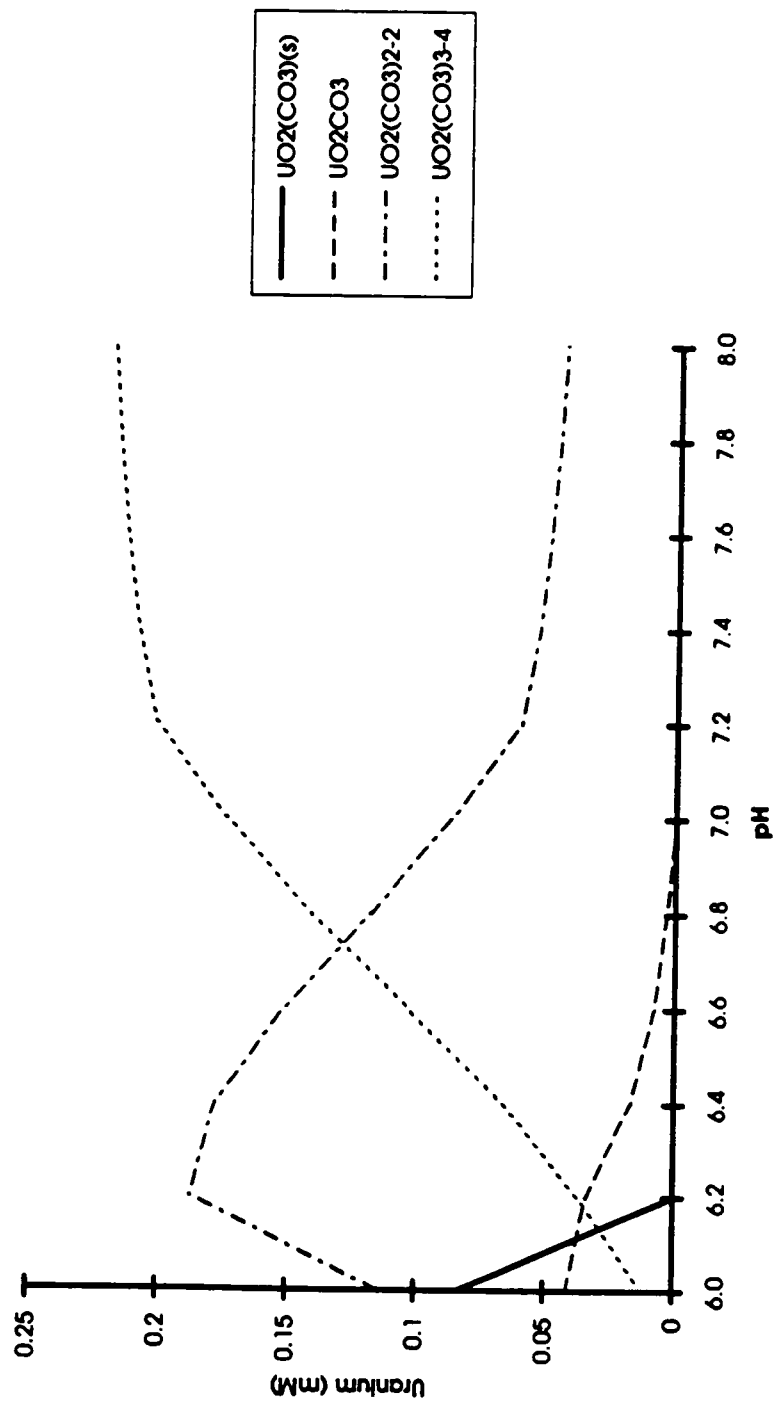


Figure 25. Uranium Distribution Diagram for Y-12 Waste Sample 1 Without Phosphate

This figure indicates a significant amount of uranium should be soluble in the carbonate form. These results are due to the relatively low calcium concentration (62 mg/L) in the wastewater. This lower calcium concentration allows more carbonate to be in solution, which in turn makes the carbonate available for complexing with uranium.

Based on these results from sample 1 without phosphate, the model was run without calcium nor phosphate in the input data files. The results from these calculations are represented in Figure 26. Figure 26 is consistent with results from samples 1, 3, and 4 which indicate if there is no calcium nor phosphate in the wastewater, the uranium should be soluble in the carbonate forms.

These results suggest if the wastewaters received at the Y-12 West End Facility do not contain phosphates nor significant quantities of calcium there is a potential to segregate the uranium. If these wastes could be neutralized with sodium hydroxide rather than lime (CaO), the uranium may stay in solution as uranyl carbonate. These conditions may allow the uranium to be biologically available for selective removal by *D. desulfuricans*.

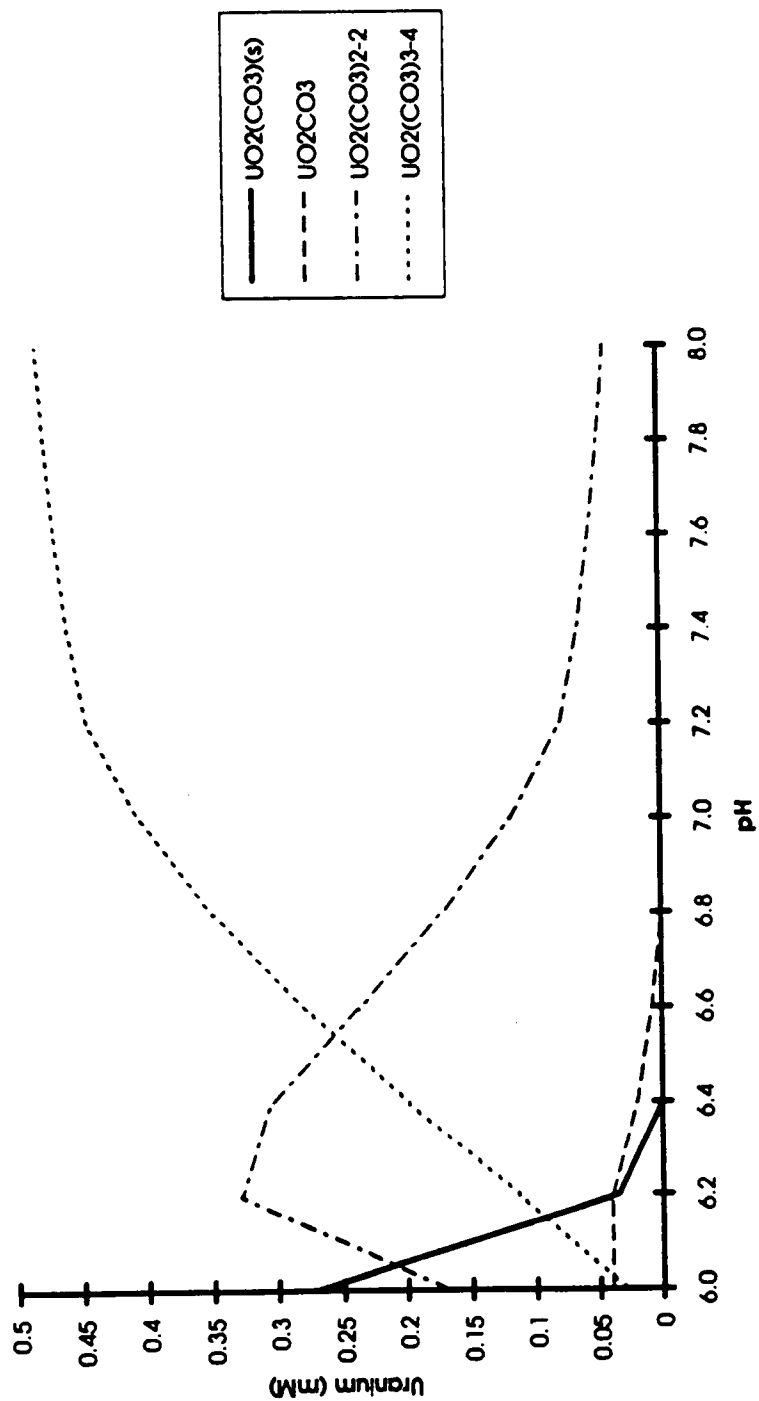


Figure 26. Uranium Distribution Diagram for Y-12 Waste Sample 2 Without Phosphate nor Calcium

CHAPTER VI

CONCLUSIONS

Biological removal of heavy metals such as uranium is a potentially feasible wastewater treatment technology. Microorganisms have been identified which, under anaerobic conditions, will enzymatically reduce U(VI) to U(IV). In solution, this changes the uranyl complex (UO_2^{+2}) to uraninite (UO_2). The uraninite crystallizes and settles out of solution.

The use of *D. desulfuricans* to biologically reduce soluble uranium in waters containing high concentrations of nitrates and sulfates may be possible. In test reactors containing 0 to 5,000 mg/L sulfate, soluble uranium concentration was reduced from nearly 100 to <0.1 mg/L in approximately one day. Results from the 10,000 mg/L sulfate solution demonstrated a slight drop in the rate of uranium removal. A more significant decline in the rate of uranium removal was observed in reactors containing lower initial concentration of uranium. A possible explanation for this decline in rate of uranium removal is competitive interference between the uranyl (UO_2^{+2}) and sulfate (SO_4^{+2}) ions.

In the reactors containing the lowest concentrations of sulfate (100 to 1000 mg/L), sulfate removal was observed. Sulfate removal at higher concentrations may not have been observed due to lack of instrument accuracy. The production of sulfide in the test reactors is significant with respect to the set of solutions initially containing 10 mg/L uranium. In these reactors, complete removal of uranium was not observed as would have been expected by thermodynamics. These results support the concept of direct biological removal of uranium through the metabolism of lactate.

In test reactors containing nitrates ranging from 100 to 10,000 mg/L, the soluble uranium was removed from approximately 100 to below 0.1 mg/L within one day. At 50,000 mg/L nitrate, uranium removal occurred much more slowly. After five days, the uranium concentration in solution was reduced from approximately 100 to 15 mg/L. There are two potential causes of this decline in the rate of uranium removal. These include sodium and ionic strength inhibition.

In the reactors containing lower concentrations of nitrate (100 to 1,000 mg/L), some nitrate removal was observed. In all of the cases where nitrate removal was observed, there was an indication of a two day acclimation period. This suggests *D. desulfuricans* may be capable of utilizing nitrates. This also indicates uranyl/nitrate competition may not be a likely cause of the decline in the rate of uranium removal observed in the 50,000 mg/L nitrate solution. If competition was the controlling factor, then during the two day acclimation period the uranium should have been the sole electron acceptor and thus removed in a manner consistent with the solution containing no sulfate and no nitrate.

In the combined nitrate/sulfate solution tests, near complete uranium removal was observed in all of the reactors. The *D. desulfuricans* removed the uranium from 100 to below 0.1 mg/L within approximately one day in the reactors containing 100, 1,000, and 5,000 mg/L nitrate, independent of the sulfate concentrations in the solutions. In the reactors containing 50,000 mg/L nitrate, uranium removal from 100 to below 1 mg/L occurred over 10 days. The increased time necessary to reduce the uranium was observed for both the high and low concentrations (100 and 10,000 mg/L) of sulfates. As with the nitrate-only test solutions, the

cause of this decrease in the uranium removal rate may be due to sodium and/or ionic strength inhibition.

Computer modeling of the sulfate and nitrate test solutions indicated that variations of the uranyl complexes were not significant in the experimental conditions. As a result, changes in uranyl complexes should not account for the decline in the rate of uranium observed in the highest sulfate and/or nitrate solutions. Thermodynamics predicts that removal of uranium through oxidation of nitrite to nitrate is not favorable, suggesting the uranium in the nitrate test reactors was removed through direct biological activity.

Computer modeling of Y-12 West End high nitrate waste indicates this stream may not be suitable for biological uranium removal under current conditions. The model predicted three of the waste streams would have very little soluble uranium, if any. The phosphate concentration in the solutions was relatively small; however, it seems to have sufficient affinity to complex with uranium and precipitate. In waste waters containing high concentrations of calcium, the uranium precipitate changed to an uranyl hydroxide.

If the wastewaters do not contain phosphates nor significant quantities of calcium, it may be possible keep the uranium from precipitating with other metals. By keeping the uranium in solution, it may then be possible to selectively remove it biologically using *D. desulfuricans*.

In addition, the computer model suggests the uranium may be more soluble at pH <7. Additional studies should be performed to evaluate the

potential for removal of uranium by *D. desulfuricans* in solutions at lower pH range.

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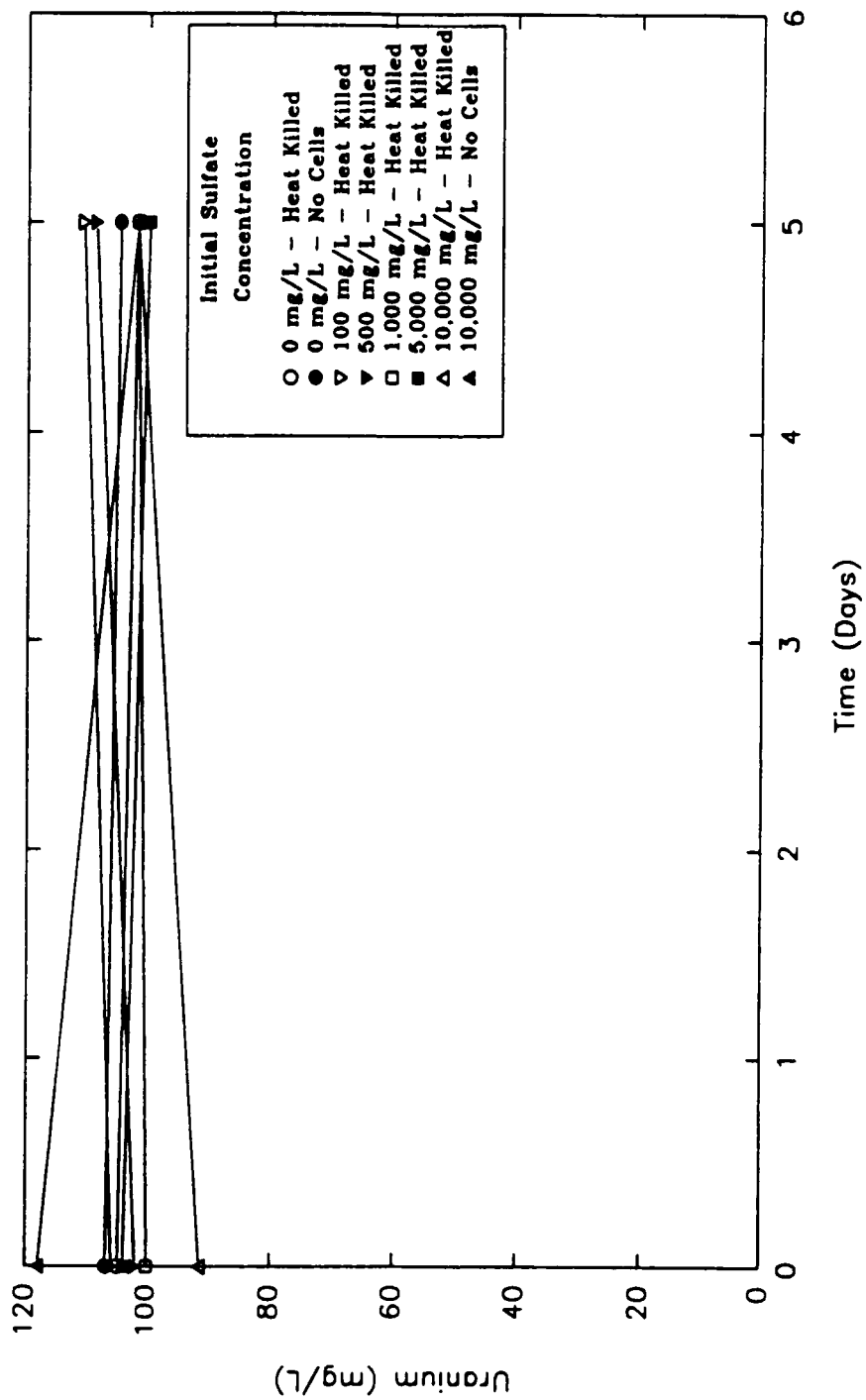
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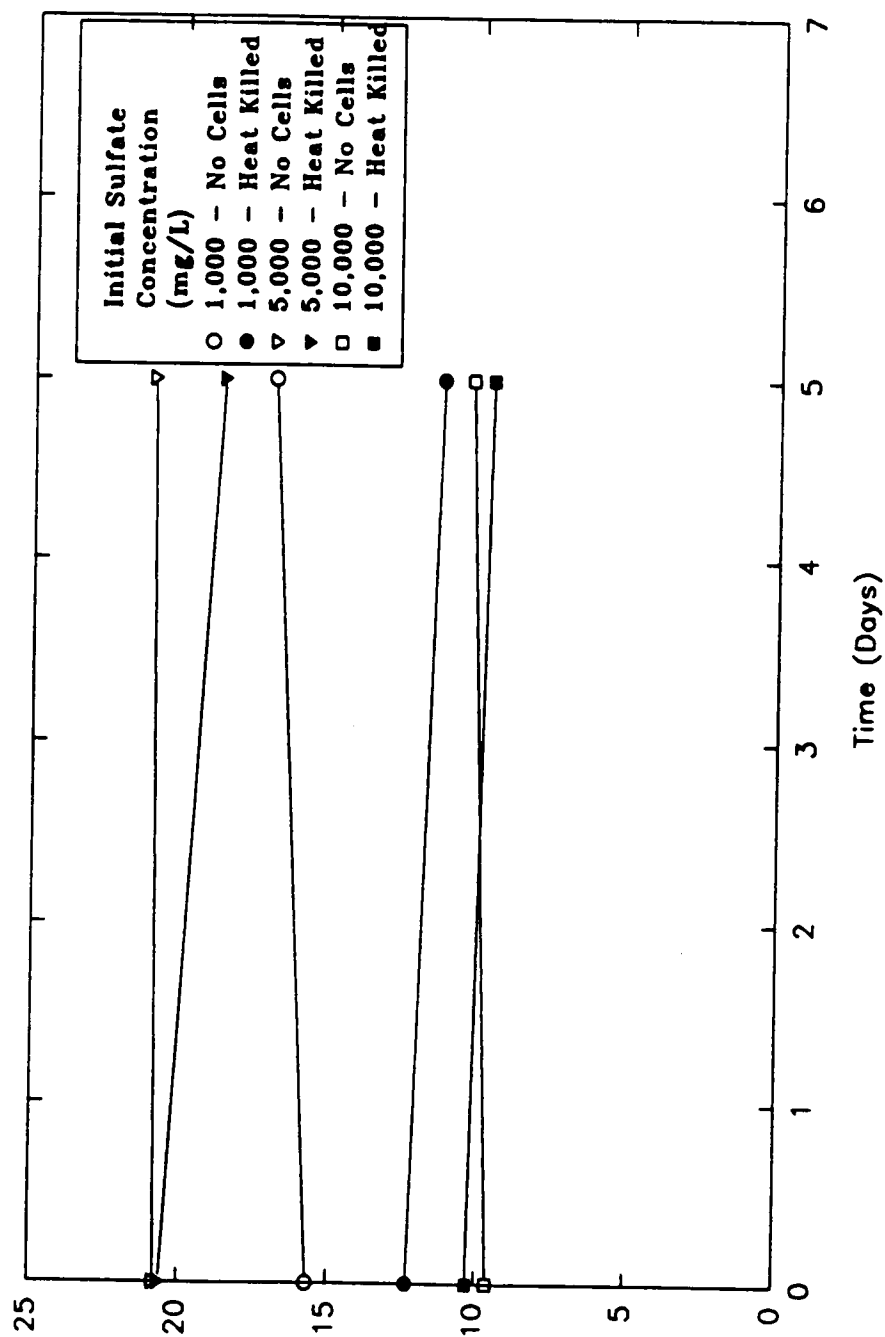
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APPENDIX

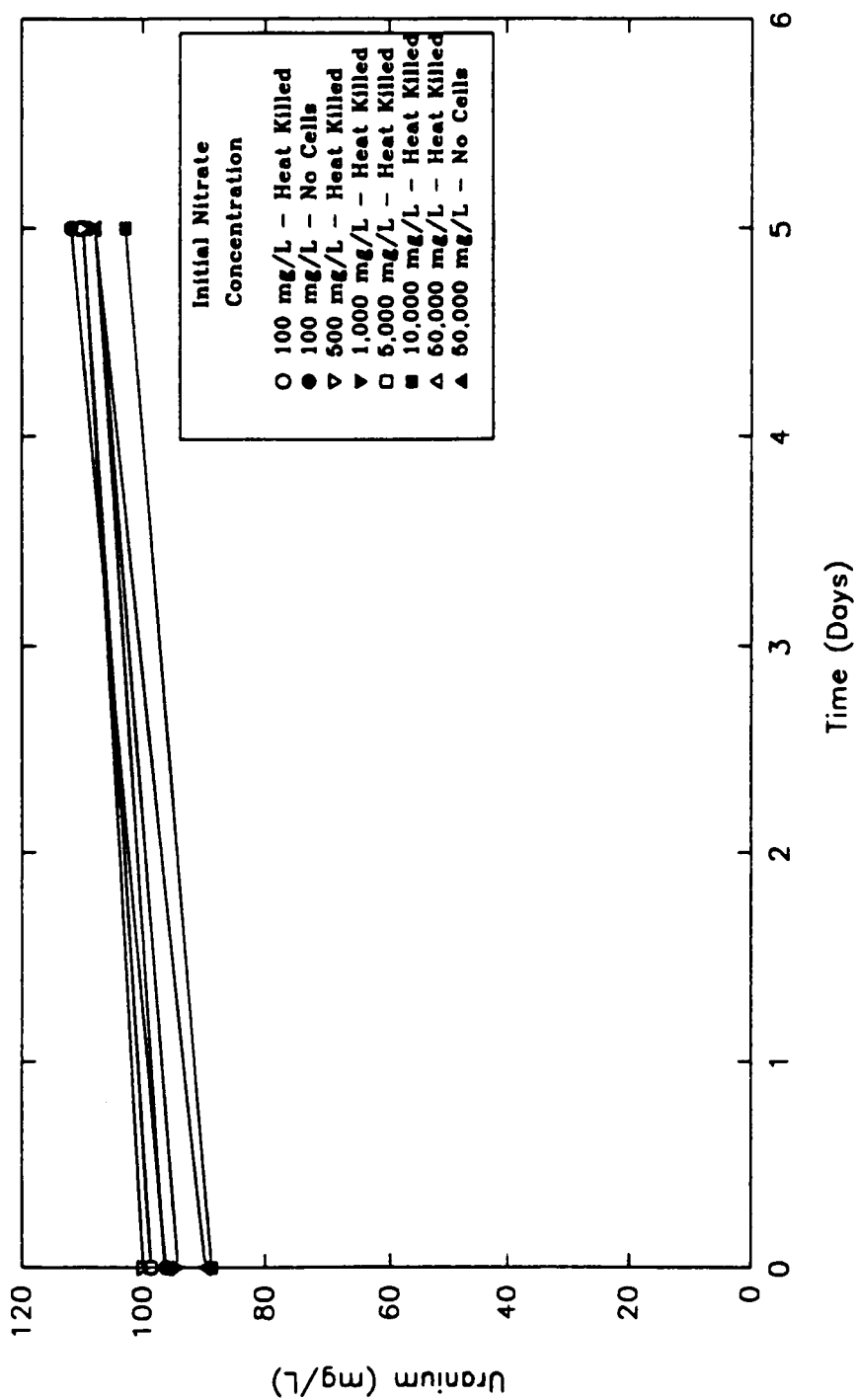
APPENDIX A. Uranium Reduction Reactor Control Graphs



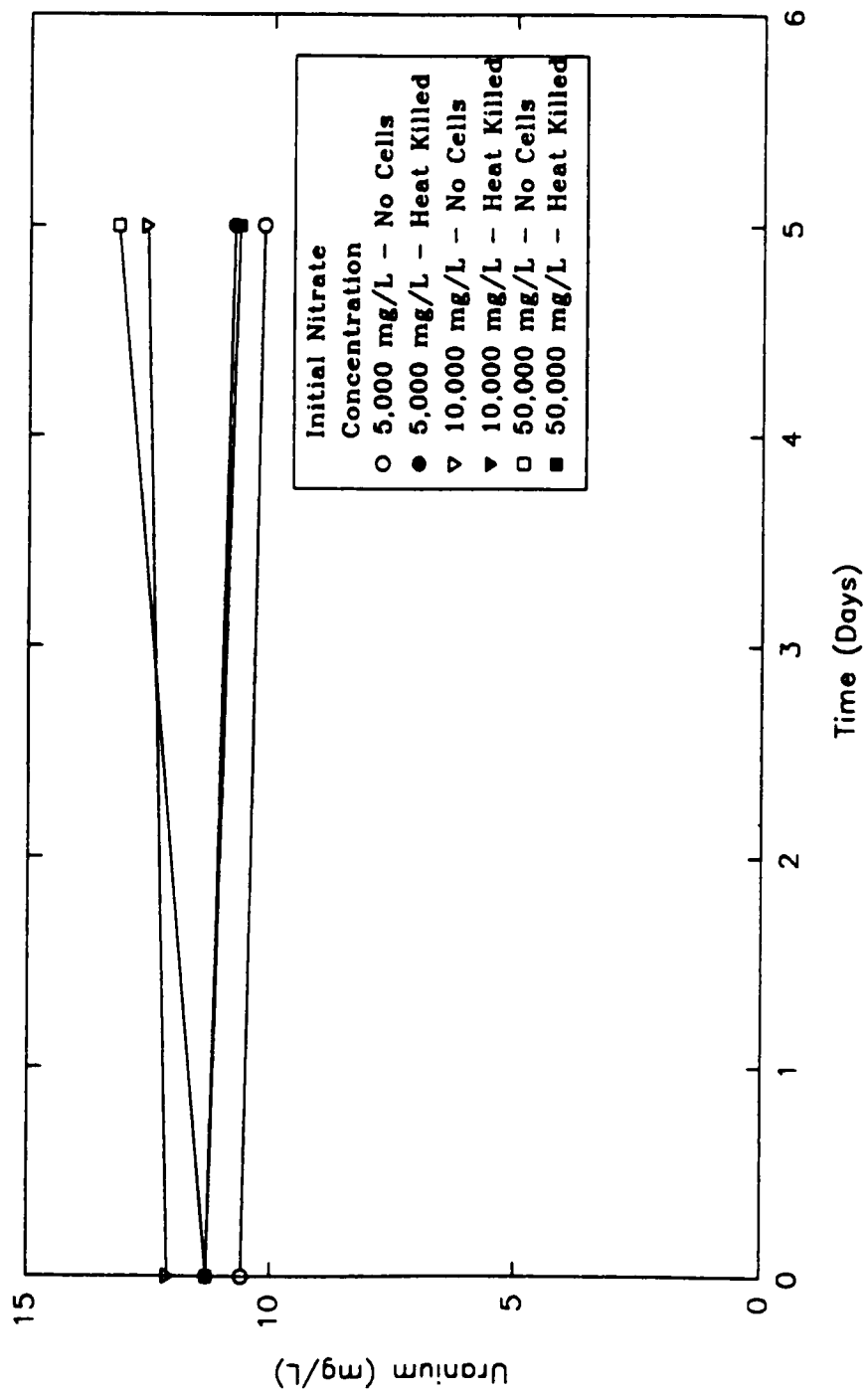
Sulfate Reactor Controls



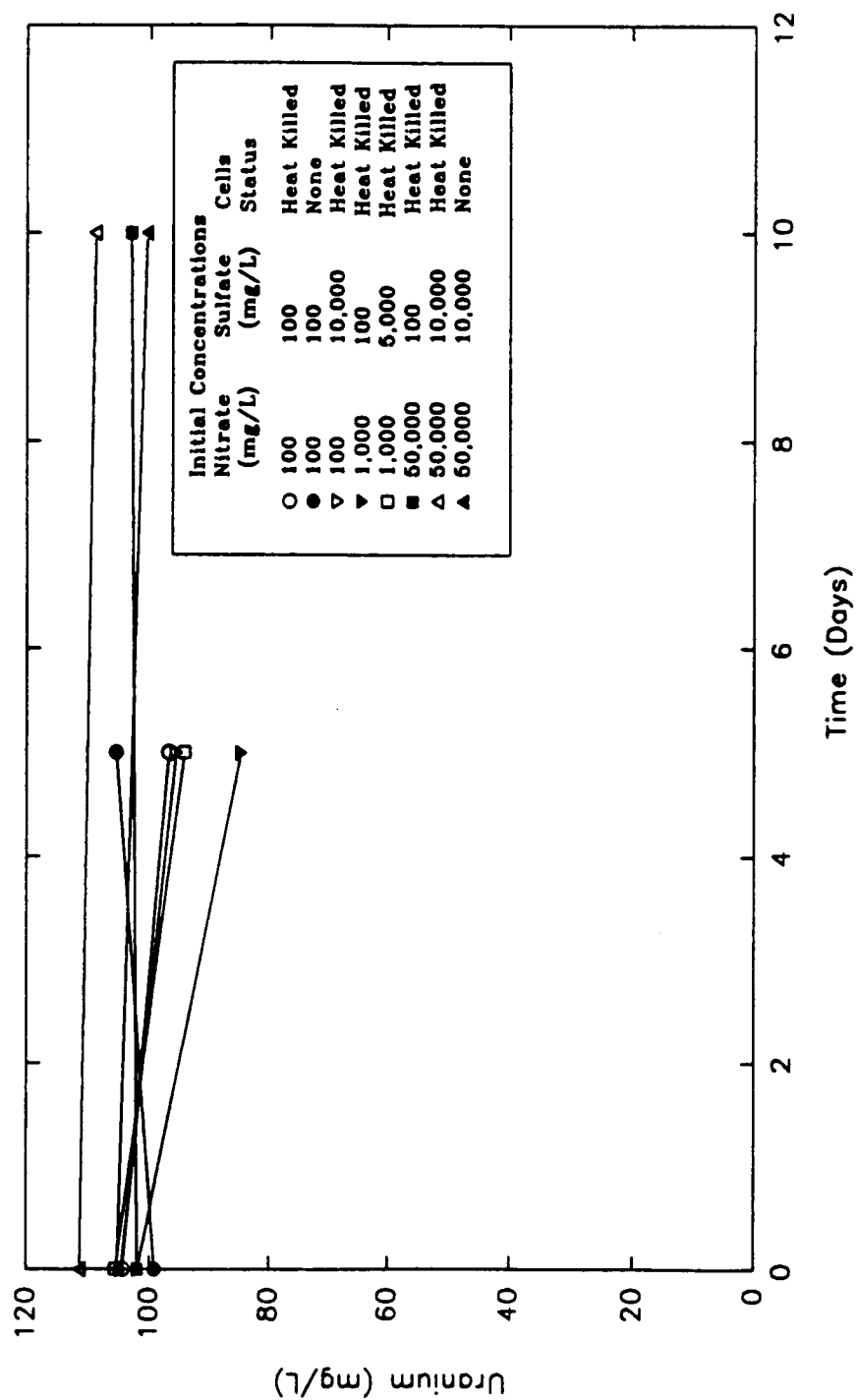
Low-Concentration Uranium Sulfate Reactor Controls



Nitrate Reactor Controls



Low-Concentration Uranium Nitrate Reactor Controls



Combined Sulfate/Nitrate Reactor Controls

APPENDIX B. Analytical Results

Sulfate Matrix Results

Uranium Concentrations (mg/L)						
SO ₄ Concentration (mg/L)	0	Time (Days)				
		1	2	3	4	5
0	104	0.695	0.592	<0.1	<0.1	<0.1
	105					102
	107					105
100	104	0.682	0.725	<0.1	<0.1	<0.1
	106					111
500	102	0.692	<0.1	<0.1	<0.1	<0.1
	102					109
1,000	103	0.704	<0.1	<0.1	<0.1	<0.1
	100					102
5,000	102	5.81	1.31	<0.1	<0.1	<0.1
	104					100
10,000	96.5	22.6	7.92	5.59	0.991	2.48
	91.3					102
	118					102

Sulfate Matrix Results

Sulfate Concentrations (mg/L)						
Nominal SO ₄ Concentration (mg/L)	Time (Days)					Cells Status
	0	1	2	3	4	
0	0	0	1	4	0	0 Live
	0					0 Killed
	0					0 None
100	76	2	3	3	2	20 Live
	76					100 Killed
500	520	300	360	240	310	25 Live
	430					460 Killed
1,000	900	750	680	770	410	360 Live
	920					1100 Killed
5,000	4200	4900	5100	5900	5800	5300 Live
	4900					6200 Killed
10,000	9200	8900	9600	11000	9000	9200 Live
	10000					11000 Killed
	9900					11000 None

Low Uranium Sulfate Matrix Results

Uranium Concentrations (mg/L)						
SO ₄ Concentration (mg/L)	Time (Days)					Cells Status
	0	1	2	3	4	5
1,000	11.5	9.0	0.8	0.3	0.6	0.3 Live
	15.7					17 Killed
	12.3					11.3 None
5,000	12.5	3.7	3.0	2.3	2.9	1.3 Live
	20.6					18.7 Killed
	20.8					21.0 None
10,000	9.9	4.7	3.5	2.5	2.2	2.9 Live
	10.3					9.6 Killed
	9.6					10.3 None

Nitrate Matrix Results

Uranium Concentrations (mg/L)

NO ₃ Concentration (mg/L)	Time (Days)					Cells Status	
	0	1	2	3	4		5
100	98.6	0.86	1.94	0.1	0.07	<0.1	Live
	98.6					110	Killed
	96.2					112	None
500	100	0.782	1.83	0.155	1.4	<0.1	Live
	100					110	Killed
1,000	88.8	1.03	1.8	.223	.234	.162	Live
	94.1					108	Killed
5,000	99.0	4.16	1.84	.361	.554	3.45	Live
	98.7					110	Killed
10,000	95.9	13	2.39	.353	.98	4.88	Live
	88.6					103	Killed
50,000	95.8	81.4	64.8	33	15	16	Live
	89.6					108	Killed
	96.4					108	None

Nitrate Matrix Results

Nitrate Concentrations (mg/L)						
Nominal NO ₃ Concentration (mg/L)	Time (Days)					
	0	1	2	3	4	5
100	100	103	109	50.6	2.4	1.8
	98.7					123
	110					111
500	450	452	486	355	79.6	279
	454					605
1,000	1040	867	1100	886	892	586
	1060					1180
5,000	4480	5130	6070	5920	5920	5760
	5620					6100
10,000	12100	11300	13300	12700	12400	13900
	11500					12800
50,000	80400	79400	90500	35500	40300	37900
	27400					93300
	83300					36500
						None

Low Uranium Nitrate Matrix Results

Uranium Concentrations (mg/L)

NO ₃ Concentration (mg/L)	Time (Days)						Cells Status
	0	1	2	3	4	5	
5,000	10.5	3.0	0.8	1.5	<0.1	<0.1	Live
	11.3					10.8	Killed
	10.6					10.2	None
10,000	10.1	<0.1	<0.1	<0.1	0.1	<0.1	Live
	23.1					21.8	Killed
	12.1					12.6	None
50,000	12.2	2.7	0.7	0.1	<0.1	<0.1	Live
	11.3					10.7	Killed
	11.3					13.2	None

Nitrate/Sulfate Matrix Results

Uranium Concentrations (mg/L)							
NO ₃ /SO ₄ Concentration (mg/L)	Time (Days)					Cells Status	
	0	1	2	3	4		5
100/100	94.8	<0.1	<0.1	<0.1	<0.1	<0.1	Live
	104.4					96.8	Killed
	99.2					105.5	None
100/10,000	98.7	3.3	0.7	<0.1	<0.1	<0.1	Live
	104.7					95.6	Killed
1000/100	100.6	<0.1	<0.1	<0.1	<0.1	<0.1	Live
	102.0					84.8	Killed
1000/5000	106.8	0.2	<0.1	<0.1	<0.1	<0.1	Live
	105.6					94.2	Killed

Nitrate/Sulfate Matrix Results

Uranium Concentrations (mg/L)						
NO ₃ /SO ₄ Concentration (mg/L)	Time (Days)					Cells Status
	0	1	3	5	7	10
50,000/100	96.1	57.8	45.4	17.9	13.2	0.6
	102.1					103.2
50,000/10,000	98.8	68.1	43.1	19.7	18.8	<0.1
	111.4					109
	105.3					100.6
						None

APPENDIX C. MINTEQ Data Input Files

Test Solution with 10,000 ppm SO4

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	2.380E-01	-0.79 y	/Na+1
992	8.400E-04	-3.08 y	/Acetate
732	1.041E-01	-0.98 y	/SO4-2

3 1

330	6.0000	0.0000	/H+1
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Test Solution with 5,000 ppm SO4

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	1.339E-01	-0.79 y	/Na+1
992	5.205E-02	-3.08 y	/Acetate
732	1.041E-01	-0.98 y	/SO4-2

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with 1,000 ppm SO4

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	5.058E-02	-0.79 y	/Na+1
992	1.041E-02	-3.08 y	/Acetate
732	1.041E-01	-0.98 y	/SO4-2

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with 500 ppm SO4

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	4.017E-02	-0.79 y	/Na+1
992	5.205E-03	-3.08 y	/Acetate
732	1.041E-01	-0.98 y	/SO4-2

3 1

330	6.0000	0.0000	/H+1
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Test Solution with 100 ppm SO4

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	3.184E-02	-0.79 y	/Na+1
992	1.040E-03	-3.08 y	/Acetate
732	1.041E-01	-0.98 y	/SO4-2

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with NO Nitrate or Sulfate

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	2.976E-02	-0.79 y	/Na+1
992	1.040E-03	-3.08 y	/Acetate

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution containing 50,000 ppm NO3

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	8.361E-01	-0.79 y	/Na+1
492	8.064E-01	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution Containing 10,000 ppm Nitrate

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	1.910E-01	-0.79 y	/Na+1
492	1.613E-01	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with 5,000 ppm Nitrate

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	1.104E-01	-0.79 y	/Na+1
492	8.064E-02	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with 1,000 ppm Nitrate

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	4.589E-02	-0.79 y	/Na+1
492	1.613E-02	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with 500 ppm Nitrate

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	3.782E-02	-0.79 y	/Na+1
492	8.060E-03	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with 100 ppm Nitrate

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	3.137E-02	-0.79 y	/Na+1
492	1.610E-03	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test solution containing 50,000 ppm NO3 and 10,000 ppm SO4

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	1.044E+00	-0.79 y	/Na+1
492	8.064E-01	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate
732	1.041E-01	-0.98	/SO4-2

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with 50,000 ppm NO3 and 100 ppm SO4

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	8.382E-01	-0.79 y	/Na+1
492	8.064E-01	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate
732	1.040E-03	-0.98	/SO4-2

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with 1000 ppm NO3 and 5000 ppm SO4

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	1.050E-01	-0.79 y	/Na+1
492	1.613E-02	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate
732	5.205E-02	-0.98	/SO4-2

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with 1000 ppm NO3 and 100 ppm SO4

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	4.797E-02	-0.79 y	/Na+1
492	1.613E-02	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate
732	1.040E-03	-0.98	/SO4-2

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with 100 ppm NO3 and 10000 ppm SO4

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	2.396E-01	-0.79 y	/Na+1
492	1.610E-03	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate
732	1.041E-01	-0.98	/SO4-2

3 1

330	6.0000	0.0000	/H+1
-----	--------	--------	------

Test Solution with 100 ppm NO3 and 100 ppm SO4

25.00 MOLAL 0.000 0.00000E-01

0 0 1 0 3 0 0 0 1 1 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 y	/H+1
140	2.976E-02	-1.48 y	/CO3-2
893	4.200E-04	-3.38 y	/UO2+2
500	3.345E-02	-0.79 y	/Na+1
492	1.610E-03	-0.79 y	/NO3-1
992	8.400E-04	-3.08 y	/Acetate
732	1.040E-03	-0.98	/SO4-2

3 1

330	6.0000	0.0000	/H+1
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Characterization of Y-12 West Tank Farm Waste

Sample 1

25.00 PPM 0.000 0.00000E-01

0 0 1 1 2 0 0 0 1 2 1 0 0

ACTIVITY 330 20

0.20

0 0 0

330	0.000E-01	-3.00 y	/H+1
30	4.800E+00	-3.75 y	/Al+3
150	6.200E+01	-2.81 y	/Ca+2
231	6.000E-01	-5.02 y	/Cu+2
270	4.300E+01	-2.65 y	/F-1
281	1.800E+00	-4.49 y	/Fe+3
440	6.100E+00	-3.06 y	/Li+1
460	4.000E+02	-1.78 y	/Mg+2
540	2.000E+00	-4.47 y	/Ni+2
492	6.450E+02	-1.98 y	/NO3-1
410	5.500E+02	-1.85 y	/K+1
732	3.350E+03	-1.46 y	/SO4-2
500	5.100E+03	-0.65 y	/Na+1
950	1.400E-01	-5.67 y	/Zn+2
893	7.000E+01	-3.59 y	/UO2+2
580	8.000E+01	-3.07 y	/PO4-3
140	4.550E+02	-1.90 y	/CO3-2

3 1

330	3.0000	0.0000	/H+1
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6 1

7015002	114.4000	-39.3900	/FCO3APATITE
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Characterization of Y-12 West Tank Farm Waste

Sample set 2

25.00 PPM 0.000 0.00000E-01

0 0 1 1 3 0 0 0 1 2 1 0 0

ACTIVITY 330 20

0.20

0 0 0

330	0.000E-01	-3.00 y	/H+1
30	1.900E-01	-3.75 y	/Al+3
150	7.900E+03	-2.81 y	/Ca+2
231	9.100E+00	-5.02 y	/Cu+2
270	5.300E+00	-2.65 y	/F-1
281	6.100E+00	-4.49 y	/Fe+3
440	6.500E+00	-3.06 y	/Li+1
460	3.000E+02	-1.78 y	/Mg+2
540	9.000E-03	-4.47 y	/Ni+2
492	2.180E+04	-1.98 y	/NO3-1
410	4.300E+02	-1.85 y	/K+1
732	8.600E+02	-1.46 y	/SO4-2
500	4.300E+03	-0.65 y	/Na+1
950	9.000E-04	-5.67 y	/Zn+2
893	1.370E+02	-3.59 y	/UO2+2
580	6.100E+01	-3.07 y	/PO4-3
140	6.000E+02	-1.78 y	/CO3-2

3 1

330 3.0000 0.0000

/H+1

6 3

7015002 114.4000 -39.3900

/FCO3APATITE

2089303 -5.4040 12.0450

/SCHOEPITE

6015001 4.8480 -0.2610

/GYPSUM

Characterization of Y-12 West Tank Farm Waste

Sample set 3

25.00 PPM 0.000 0.00000E-01

0 0 1 1 2 0 0 0 1 2 1 0 0

ACTIVITY 330 20

0.20

0 0 0

30	7.000E-01	-3.75 y	/Al+3
150	8.100E+03	-2.81 y	/Ca+2
231	2.800E+01	-5.02 y	/Cu+2
270	7.900E+00	-2.65 y	/F-1
281	1.200E+00	-4.49 y	/Fe+3
440	6.500E+00	-3.06 y	/Li+1
460	4.200E+02	-1.78 y	/Mg+2
540	2.700E+00	-4.47 y	/Ni+2
492	1.628E+04	-1.98 y	/NO3-1
410	2.500E+02	-1.85 y	/K+1
732	7.900E+02	-1.46 y	/SO4-2
500	3.300E+03	-0.65 y	/Na+1
950	9.000E-04	-5.67 y	/Zn+2
893	6.000E+01	-3.59 y	/UO2+2
580	5.200E+01	-3.07 y	/PO4-3
140	6.000E+02	-2.00 y	/CO3-2
330	0.000E-01	-3.00	/H+1

3 1

330	3.0000	0.0000	/H+1
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6 1

7015002	114.4000	-39.3900	/FCO3APATITE
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Characterization of Y-12 West Tank Farm Waste
Sample set 4

25.00 MG/L 0.000 0.00000E-01
0 0 1 1 3 0 0 0 1 2 1 0 0

ACTIVITY 330 11

0.20

0 0 0

330	0.000E-01	-6.00 N	/H+1
893	3.400E+02	-16.05 N	/UO2+2
270	5.000E+00	-5.59 N	/F-1
492	6.200E+04	-0.09 N	/NO3-1
732	1.400E+04	-1.38 N	/SO4-2
30	2.300E+01	-5.83 N	/Al+3
150	1.060E+02	-3.61 N	/Ca+2
231	1.720E+01	-4.81 N	/Cu+2
281	1.790E+02	-8.90 N	/Fe+3
410	4.200E+02	-2.18 N	/K+1
440	1.570E+01	-2.81 N	/Li+1
460	1.360E+02	-3.26 N	/Mg+2
471	7.160E+01	-4.05 N	/Mn+3
500	9.920E+02	-1.54 N	/Na+1
540	1.480E+01	-4.74 N	/Ni+2
580	3.700E+03	-9.17 N	/PO4-3
950	5.130E+00	-5.46 N	/Zn+2
140	1.786E+03	-6.32 N	/CO3-2

3 1

330 6.0000 0.0000

/H+1

6 1

7015002 114.4000 -39.3900

/FCO3APATITE

VITA

Daniel A. Kucsmas was born in Belleville, Illinois on June 10, 1958. He graduated from Queensbury High School in Glens Falls, New York, "Regents with Honors" in June 1977. The following August he entered Clarkson College in Potsdam, New York, and in May 1981 he received a Bachelors of Science degree in Chemical Engineering. In the fall of 1987 he began attending the University of Tennessee, Knoxville as an evening school student studying toward a Master's degree in Environmental Engineering. This degree was awarded in June, 1994.

The author is currently employed with Martin Marietta Energy Systems in Oak Ridge, Tennessee. He is a process engineer in the Facility Design Services Department of Central Engineering Services Division.