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Title: Uranium and Technetium Bio-immobilization in Intermediate-Scale Permeable Reactive Barriers

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Groundwater at Oak Ridge National Laboratory's Field Research Center (FRC) is contaminated with U(VI) and Tc(VII), has pH values as low as 3.3, and nitrate concentrations as high as 120 mM. The objective of this research was to determine if in-situ bio-immobilization is a viable treatment alternative for this water.

A laboratory column packed with crushed limestone and bicarbonate was used to model in-situ pH adjustment. Denitrification and metal reduction were modeled in columns packed with FRC sediment with ethanol as the electron donor. Two intermediate-scale physical models deployed in the field were packed with limestone and sediment and were stimulated with ethanol to support denitrification, U(VI) reduction, and Tc(VII) reduction of FRC groundwater.

The limestone/bicarbonate column maintained a pH of above 5 for nearly one hundred pore volumes without significant loss in hydraulic conductivity. The high-nitrate (~ 120 mM) column study provided rates of denitrification (~ 15.25 mM/day), ethanol utilization (~ 13 mM/day), and technetium reduction (~ 120 pM/day) by sediment microorganisms, but no uranium reduction was detected. Results of the low nitrate (3 mM) column study indicate that once the pH of FRC water is adjusted to pH ~ 7 and nitrate is removed, uranium (~ 3 μM) and technetium (~ 500 pM) reduction occurred with ethanol as the electron donor at rates of ~ 0.5 μM/day and 57 pM/day.

Similar results were obtained in two intermediate-scale (~ 3 m long) physical models. Data from the high-nitrate, low-pH model indicate that the pH was increased

and nitrate and technetium reduction were occurring. Decreased U(VI) concentrations were measured in the presence of high nitrate concentrations. Thus, U(VI) precipitates may form or sorption of U(VI) may occur near the inlet in the pH adjustment region. The maximum pseudo-first order rates of reduction measured during the seventh week of model operation were: nitrate at 0.76 day^{-1}, Tc(VII) at 0.28 day^{-1}, and U(VI) at 0.12 day^{1-}. Ethanol concentrations were reduced from ~ 180 mM to zero in ~ 10 days during the seventh week of model operation. No Fe(II) production was measured.

Concentration data collected from the low nitrate, neutral pH model indicate that nitrate, uranium, and technetium reduction were occurring, though the model had been operational for only ~ 6 weeks. No Fe(II) production was detected but sulfate reduction was occurring.

The results of the laboratory experiments and the performance of the intermediate-scale physical models suggest that bio-immobilization is a viable treatment alternative for the contaminated groundwater at the FRC.

Uranium and Technetium Bio-immobilization in Intermediate-Scale Permeable Reactive Scale Barriers

by

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1.0 INTRODUCTION

1.1 Background

The United States Department of Energy (DOE) maintained a nuclear weapons research and production program during the arms race of the Cold War. This program consisted of a network of 113 facilities across the country dedicated to nuclear testing and weapons development (1, 3). These facilities generated large quantities of hazardous waste while processing weapons materials. Some facilities remained operational for more than 50 years.

Hazardous waste was often discharged to surface lagoons or buried in landfills. Many of these storage facilities failed over time, which resulted in contaminated subsurface zones. While some fraction of the contaminants in these zones may be sorbed to soil particles, a significant portion is often dissolved in the groundwater. The groundwater then transports the dissolved contaminants downgradient to potential receptors (water wells, springs, and streams).

Radionuclides are some of the most prevalent compounds present as groundwater contaminants at these facilities (Figure 1). Some radionuclides have extremely long half-lives and can be quite mobile. Therefore treatment systems must be designed and implemented to remediate these sites in a timely and cost-effective fashion.

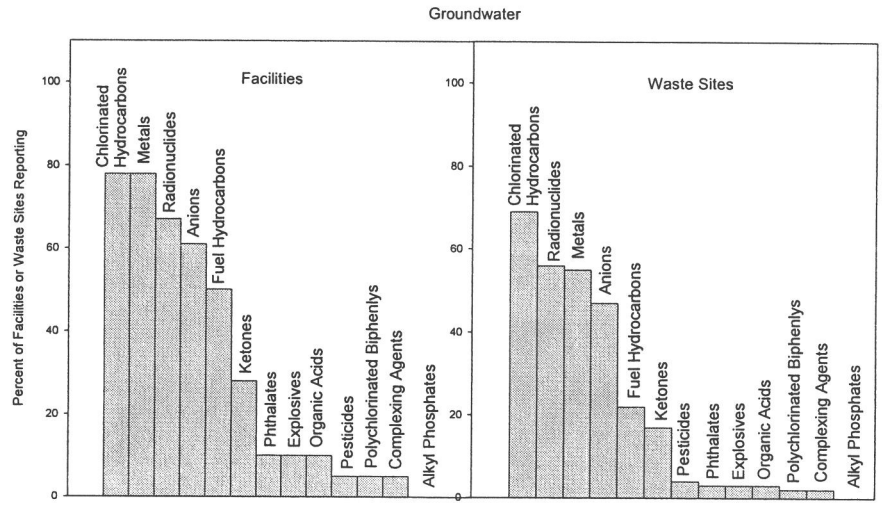


Figure 1. Distribution of contaminants by compound classes in groundwater at 18 DOE facilities and 91 waste sites (2).

Presently more than 7,000 individual sites within the DOE weapons complex have been found to contain subsurface contamination (3). Restoring the environmental quality at such a large number of sites presents a challenge to the DOE that is astounding in both magnitude and complexity. Therefore, although the Cold War is long over, the legacy of nuclear weapons production lives on in the form of widespread groundwater contamination.

1.2 Aquatic Chemistry of Uranium

Uranium is a common groundwater contaminant at DOE sites. It is one of the heaviest elements in the periodic table with a molar mass of 238.03 g in its most stable configuration. It is classified as a radionuclide because subatomic particles can be spontaneously transferred from its nucleus. There are three naturally occurring uranium isotopes: ^{238}U, ^{235}U, and ^{234}U (Table 1).

Table 1. Properties of naturally occurring uranium isotopes (4)

Isotope	Half-life (years)	Fraction of Total Uranium (%)
^{238}U	4.51×10^{9}	97.7
^{235}U	7.0×10^{8}	0.3
^{234}U	2.48×10^{5}	<0.01

^{238}U is the most abundant form of uranium in the environment, accounting for nearly 98 percent of all uranium present in the earth's crust. The ^{238}U isotope decays by releasing two neutrons and protons (an alpha particle) from its nucleus. ^{234}U is formed as a product of the ^{238}U decay series (4). Alpha particle emission releases little energy (5). ^{235}U however may receive a neutron to form a very unstable atom, which can instantaneously decay and release a tremendous amount of energy in a process called fission. For comparative purposes, ^{235}U theoretically contains, by weight, 4 million times more energetic potential than coal and nearly 10 million times more than TNT (4, 6)!

To harvest its energetic potential for use in nuclear weapons and reactors, uranium ore is mined from the earth's crust. As previously noted, the majority of uranium is present as ^{238}U which has little value. Thus, the ^{235}U isotope must be extracted from the ore and concentrated. This process is typically performed by

washing uranium containing ore in nitric acid. Naturally the waste generated during this processes contains large concentrations of nitrate, uranium isotopes, other radionuclides, and acid soluble metals (2). Years of improper disposal of such waste has generated groundwater contamination that is highly persistent and toxic.

The uranium isotopes exhibits two main oxidations states in groundwater - U(VI) and U(IV). Under oxic conditions, uranium forms the uranyl ion (UO_2^{2+}) in which it exhibits a (VI) oxidation state (12). Uranyl combines with a variety of anions in groundwater such as chloride, carbonate, and nitrate (8). The uranyl-hydroxide and uranyl-carbonate-hydroxide species dominate at circum-neutral pH values and low carbonate concentrations. The neutral charge of the uranyl-hydroxide complex results in a high affinity for sorption (8).

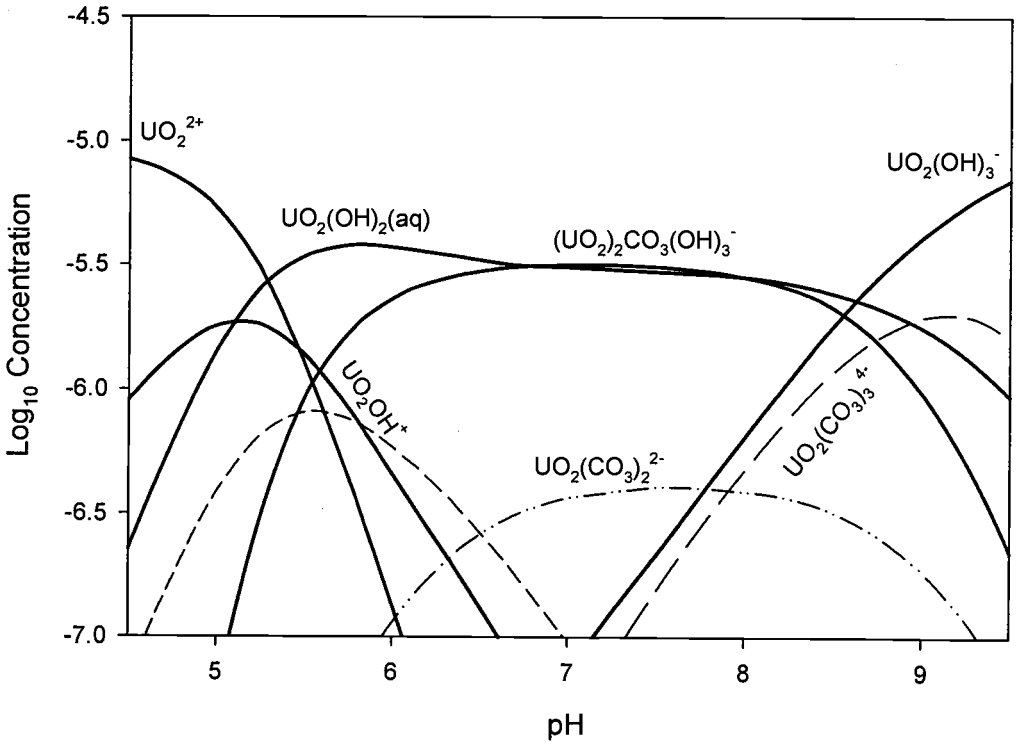


Figure 2. Uranyl speciation as function of pH calculated using thermodynamic data in Grenthe et al. (8). $[CO_3^{2-}]_T=1.5x10^{-4}M$ and $[UO_2^{2+}]_T=1x10^{-5}M$, where the subscript "T" means total concentration (Mineql v.4.06, Environmental Research Software, 1998).

Uranyl-carbonate complexes control uranium speciation in most waters with moderate carbonate concentrations. These complexes typically possess a net negative

charge (Figure 3) and are therefore less susceptible to sorption. Thus one way to prevent sorption of U(VI) is to add bicarbonate to solution (9).

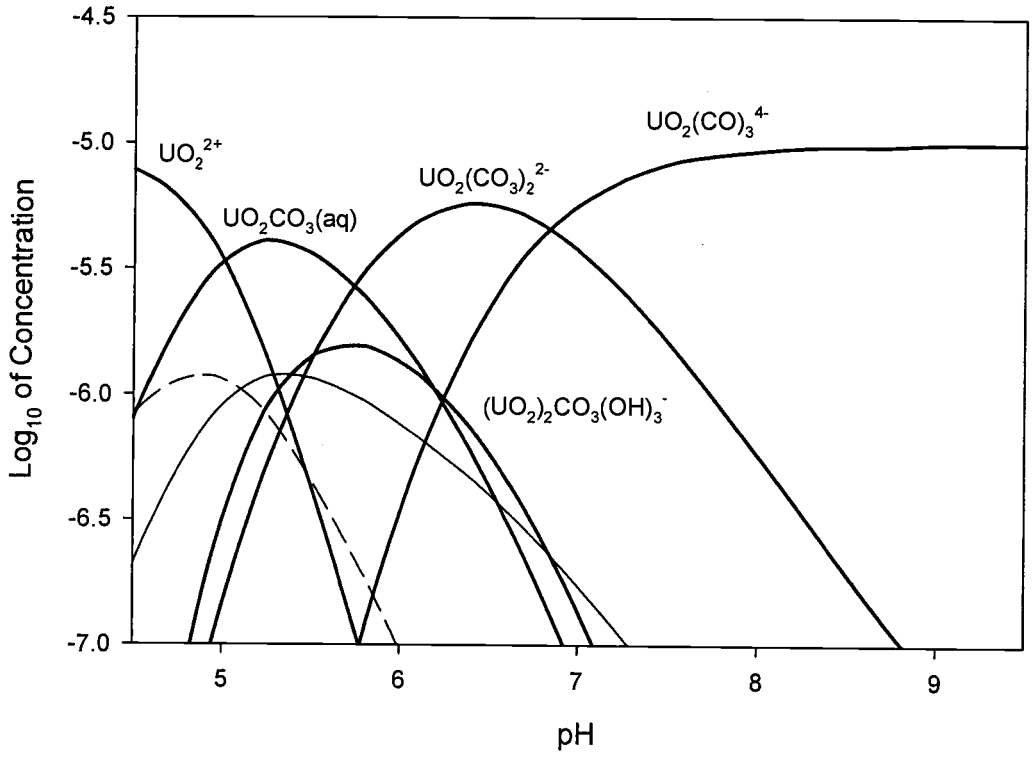


Figure 3. Uranyl speciation as function of pH calculated using thermodynamic data in Grenthe et al. (8). $[CO_3^{2-}]_T=2x10^{-3}M$ and $[UO_2^{2+}]_T=1x10^{-5}M$, where the subscript "T" means total concentration (Mineql v.4.06, Environmental Research Software, 1998).

Uranium (VI) may be reduced to U(IV) directly via microbial respiration or indirectly by fortuitous electron transfer under anaerobic conditions (11). Uranium (IV) has low solubility, and typically precipitates out of solution as uraninite (UO_2). Though uraninite is stable in a reducing environment, it can potentially be reoxidized by oxygen, nitrate, or other soluble species to soluble U(VI).

1.3 Aquatic Chemistry of Technetium

Technetium, atomic number 43, is one of the most abundant products of ^{235}U fission (13). Technetium is also formed by the decay of ^{99}Mo (molybdenum) via beta particle emission, which is equivalent to an electron. There are three long lived

isotopes of this element (Table 2), with ^{99}Tc being the chief fission product of ^{235}U fission reactors.

Table 2. Properties of technetium isotopes (13)

Isotope	Half-life (years)	Source
^{97}Tc	2.6×10^6	^{99}Mo decay
^{98}Tc	1.5×10^6	^{99}Mo decay
^{99}Tc	2.1×10^5	^{99}Mo decay, ^{235}U fission

Technetium-99 is a common groundwater contaminant at DOE sites. Under oxic conditions, ^{99}Tc exists primarily as Tc(VII) in the pertechnetate ion (TcO_4^-) (12). Under slightly reducing conditions, ^{99}Tc exists primarily as Tc(IV) in technetium oxide ($TcO_2 \cdot H_2O$). Technetium oxide has a very low solubility, and precipitates from solution at low concentrations (12).

The aquatic chemistry of technetium is less complex than that of uranium. Pertechnetate may form complexes with sugars and organic compounds in solution (14). However, these substances are typically present at very low concentrations in contaminated aquifers and are expected to play an insignificant role in technetium speciation.

Pertechnetate reacts little with other aqueous species in groundwater, but may sorb to iron and aluminum hydroxides in sediments (12). Addition of excess bicarbonate (~ 100 mM) to solution has been shown to remove some sorbed technetium, although technetium does not react with carbonate directly. It is thought that the (-2) charge of the carbonate species may out compete the single (-) charge on the pertechnetate ion for sorption sites (12), thereby releasing sorbed technetium into solution.

1.4 Environmental Health Considerations

Radionuclides are pervasive groundwater contaminants at DOE sites throughout the country (Figure 1). Some radionuclides are extremely persistent, some with half-lives of millions of years. And as the groundwater at these sites is often oxygenated, some of these radionuclides exist in soluble and therefore mobile forms.

Thus, groundwater transport of soluble radionuclide species will continue to increase the extent of contamination unless remedial action is taken.

While alpha and beta particles are less penetrating and therefore potentially less damaging than gamma radiation (short wavelength, high energy rays), their toxicity increases greatly if the radionuclides are ingested (4, 5, 53). Therefore the Environmental Protection Agency has designated Maximum Contaminant Levels (MCLs) for radionuclides in drinking water (Table 3). The MCL for uranium (an alpha emitter) is targeted specifically to the maximum concentration of uranium in water. The MCL for technetium falls under the more general beta emitter category. Millirem units represent the radiation dose absorbed by the body multiplied by a biological efficiency factor, which scales the dose relative to the sensitivity of the body part irradiated (53).

Table 3. Drinking water standards for uranium and technetium (53)

Contaminant	MCL	Potential Health Risk
Uranium	30 ug/L	Increased cancer risk, kidney toxicity
Technetium (Beta/ Photon Emitters)	$4 \frac{millirems}{yr}$	Increased cancer risk

Gamma radiation on the other hand, which is not emitted by either ^{238}U or technetium, possesses enough energy to remove electrons from atoms and molecules in their path. The removal of electrons forms free radicals, which are very reactive in nature. The free radicals can then proceed to attack DNA, RNA, and other molecules that are essential for proper cellular function (4). Receptors of radiation may suffer terminal diseases such as cancer. If a receptor's DNA is damaged however, the effects of radiation may also disrupt life functions in offspring as well.

In summary, radionuclides are especially toxic to organisms in that they disrupt cellular function of receptors for potentially generations after exposure. Therefore grossly contaminated sites should be treated to remove radionuclides as effectively as possible. The following section of this paper addresses advantages and disadvantages of key approaches to treating radionuclide contaminated groundwater.

1.5 Treatment of Radionuclide Contaminated Groundwater

Several alternatives are available for treating radionuclide contaminated groundwater. Some examples include chemical and physical methods, bio-sorption, and bio-immobilization. While several methods may be effective under specific conditions, each has advantages and disadvantages. This section highlights both ex-situ and in-situ groundwater treatment alternatives for uranium and technetium, and the potential benefits of in-situ bio-immobilization.

Pump and treat is a classic example of ex-situ groundwater treatment. This alternative may not be well suited for uranium and technetium removal however, as they are often trace contaminants. Thus, large volumes of water must be extracted to remove small quantities of radionuclides. Significant fractions of uranium and technetium may be sorbed to sediments, which can further decrease treatment efficiency.

Once contaminated groundwater is removed from the subsurface, uranium and technetium may be physically removed by sorption to ion resins. However, this method has proven both costly and ineffective for uranium removal at low concentrations (11). Ion resins have proven ineffective for treatment of technetium as well because pertechnetate (TcO_4^-), the most common form of technetium present in oxic groundwaters, competes only weakly for sorption sites (17). Further, treating large volumes of groundwater with such systems would likely require long-term operation and specialized maintenance, which may be expensive.

Bio-sorption is an additional ex-situ alternative for removal of uranium and technetium from groundwater. In this process contaminated water is contacted with microorganisms (often dead) and provided sufficient time for sorption of radionuclides to the organisms (11). Though bio-sorption systems may remove most contaminants of interest, the waste generated is concentrated in radionuclides and organics (bio-mass), which requires further treatment and special, costly disposal.

Recently in-situ chemical treatment with zero-valent iron (Fe(0)) has been applied to immobilize radionuclides in groundwater (47). Electrons are transferred from Fe(0) to reduce uranium, technetium, and other oxidized co-contaminants such as

nitrate and sulfate. The reduced radionuclides precipitate on the surface of the Fe(0), potentially rendering the reducing surface inaccessible (47). This coating effect could drastically reduce the reductive potential of the iron during its design life. Further, with the iron reducing capacity decreased, the precipitated radionuclides may then be re-oxidized and re-solublized, thereby reversing the treatment process. That said, permeable reactive barriers containing Fe(0) are being investigated for possible use in remediation of radionuclide-contaminated groundwater. Research is still under way concerning the viability of such systems for treatment of radionuclides at the Oak Ridge National Laboratory (47).

A promising new treatment alternative is bio-immobilization. The objective of this approach is to control the solubility, and thus the mobility, of redox-sensitive metals or radionuclides by using indigenous microorganisms to change the oxidation state of the contaminant. As mentioned previously, groundwater contaminated with nuclear processing waste often has low pH and high nitrate concentrations. These oxidizing conditions cause radionuclides such as uranium and technetium to remain in soluble forms. Indigenous microorganisms may be stimulated to reduce uranium and technetium in-situ, thus immobilizing the radionuclides and preventing further contamination downgradient.

Groundwater contaminant plumes typically have excess concentrations of potential electron acceptors, such as oxygen, nitrate, sulfate, oxidized metals, and oxidized radionuclides, but may have small concentrations of potential electron donors. The opposite may also be true. Plumes may have abundant reduced carbon, but lack electron acceptors required for microbial respiration. When the limiting substrate is added to a contaminated aquifer, be it the electron acceptor or donor, microbes can grow and flourish (12). Typically nitrate, sulfate, and ferric iron serve as the natural electron acceptors in the absence of oxygen (41). When the conditions are iron-reducing or sulfate-reducing, U(VI) and Tc(VII) may serve as alternative electron acceptors for microbial respiration.

Bio-immobilization is an attractive treatment alternative because it occurs in-situ and could potentially be more easily implemented than other remediation technologies. It may be that only an organic electron donor (ethanol, acetate), must be

supplied to stimulate a 'bio-barrier' of indigenous metal reducing organisms. (Trace nutrients such as phosphorous may be required in some cases.) While seemingly simple at the macroscopic scale, many interactions occurring at the microscopic scale add a great deal of complexity to this approach. Bio-immobilization processes must therefore be understood at all scales so that optimal field-scale treatment systems may be designed and implemented.

1.6 Biologically Mediated Reduction of Technetium

Reductive precipitation of technetium can proceed via two primary mechanisms in reduced sediments: direct electron transfer (abiotic), or enzymatic reduction. Several strains of anaerobically cultured bacteria have coupled enzymatic reduction of Tc(VII) to oxidation of organic carbon or dihydrogen (Table 4). All studies show that enzymatically reduced technetium forms a black, electron dense precipitate (TcO_2) at the cell's periphery. Laboratory studies indicate that dihydrogen and formate result in the highest rates of Tc(VII) reduction. This is likely due to compatibility of these substrates with the formate dehydrogenase enzyme system (28, 16). Lloyd et al. showed that the rates of technetium reduction by E. *coli* and D. *sulfuricans* with formate and dihydrogen as electron donors are large enough that flow-through treatment systems for technetium contaminated groundwater may be feasible (25, 26).

Abiotic reduction of technetium can occur in solution as catalyzed by Fe(II). Cui and Eriksen (21) concluded that while pertechnetate reduction via ferrous iron is thermodynamically favorable, the kinetics are slow. They postulated that abiotic reduction of technetium is likely dominated by electron transfer from Fe(II) bearing minerals, or precipitates- i.e. that the electron exchange is surface catalyzed (46). This finding was corroborated by Lloyd et al. (16, 17) in studies that demonstrated that technetium is not effectively reduced by Fe(II) in solution, but rather is reduced by Fe(II) containing oxides. The rate of technetium reduction by ferrous iron containing solids was further increased by adding humics to serve as soluble electron shuttles between aqueous TcO_4^- and solid phase or sorbed Fe(II) (17).

Table 4. Microbial strains capable of enzymatic Tc(VII) reduction.

Microorganism	Substrate	Notes	Reference
Shewanella *putrefaciens* (soil culture)	H_2, Lactate	Cultured from anaerobic sediments in New Mexico.	24
Clostridium *sphenoides* (pure culture)	Citrate		23
Clostridium *sp.* (pure culture)	Glucose		23
E. *coli* (pure culture)	Formate	Ethanol not effective as electron donor for Tc(VII) reduction, inhibited by nitrate	28, 29, 16, 26
Indigenous microbes	Lactate, NO_3^-, SO_4^-	Sandstone from Germany and clayey soil from the Czech Republic	22
Desulfovibrio *desulfuricans* (pure culture)	H_2, Formate	Tc(VII) reduction unaffected by nitrate when H_2 serve as electron acceptor	29, 15, 25
Geobacter *sulfurreducens*	H_2	Catalyzed by Fe(II) containing solids in the presence of humics and U(IV) which serve as electron shuttles	16,17
Desulfovibrio *vulgaris*, D. *gigas*	Dextrose, Lactate	Anaerobic mixed soil cultures and pure cultures reduced Tc(VII)	27

Lloyd et al. (16) speculated that because the U(VI)/U(IV) and Tc(VII)/Tc(IV) reduction couples occur at redox potentials E^0 = 0.344 and 0.738 V respectively, that U(IV) may serve as a catalyst for the abiotic reduction of Tc(VII). In their experiments, G. *sulfurreducens* was able to reduce U(VI) and Tc(VII) in the presence of 10 mM acetate. Because acetate does not support technetium reduction by G. *sulfurreducens*, they concluded that Tc(VII) was reduced by U(IV)! U(IV) may serve as an electron shuttle for Fe(III) reduction as well (58).

The implications of these findings are quite promising because uranium and technetium are often co-contaminants in groundwater at DOE legacy sites. Tc(VII) may be reduced as a byproduct of U(VI) reduction (17) and by surface catalyzed reactions with Fe(II) containing solids (16, 17, 21). These two processes imply than in-situ bio-immobilization of uranium and technetium could be more easily achieved

than previously thought. Further, if uranium reduction is stimulated in-situ via substrate delivery, the activity of Fe(III) reducing microorganisms can produce the ferrous iron containing mineral magnetite. Studies have shown that biogenically formed magnetite is the most effective form of Fe(II) for reducing Tc(VII) (55).

1.7 Biologically Mediated Reduction of Uranium

Though U(VI) may be reduced by Fe(0) (47), is not abiotically reduced by aqueous, sorbed, or mineral Fe(II), even in the presence of soluble electron shuttles such as anthraquinon-2,6-disulfonate (AQDS) (44, 45). Reductive precipitation of uranium is an enzymatically catalyzed reaction in reduced sediments. Several anaerobic bacteria have been found to couple the oxidation of reduced carbon sources and dihydrogen to the reduction of U(VI) (Table 5). In all studies U(VI) was reduced to U(IV), and precipitated extracellularly as uraninite (30, 31, 11, 32, 34, 20, 36, 35, 39).

S. *putrefaciens* and G. *metallireducens* can couple growth to reduction of uranium, while D. *sulfurreducens* can not. D. *sulfurreducens* does not conserve energy from U(VI) reduction, but rather does so fortuitously in the presence of sulfate (35). Sulfate additions to mixtures of U(VI) and sulfate reducing organisms were shown to increase the rates of U(VI) reduction by stimulating growth of the sulfate reducers (35, 39).

Lloyd et al. theorized that the enzyme system responsible for the reduction of U(VI) by the iron reducing microorganism G. *sulfurreducens* may be located at the cells periphery because both U(IV) and Fe(II) are precipitated extracellularly (16). They concluded that U(VI) reduction is not strictly a cell surface mediated reaction by observing that U(VI) reduction was not inhibited by removing surface proteins from the cells, though Fe(III) reduction was inhibited by protein removal.

Table 5. Microbial strains capable of U(VI) reduction.

Microorganism	Substrate	Notes	Reference
Pseudomonas *aeruginosa*, P. *stutzeri*	Ethanol	Denitrifiers cultured from anaerobic sediments in Arizona	30
Shewanella *putrefaciens*	Ethanol	Sulfate and Fe(III) reducer, cultured from Arizona sediment	30, 33, 20, 43
Desulfovibrio *desulfuricans*	Lactate, or H_2	Pure culture, survives in U concentrations of 24 mM. Conserves no energy from U(VI) reduction. Copper may inhibit U(VI) reduction (11).	11, 33, 16, 34, 35, 37
Geobacter *metallireducens*	Acetate	Fe(III) and nitrate reducer	16, 32, 43, 33, 20, 49
Desulfovibrio *gigas*, D. *baculatus*, D. *vulgaris*, D. *desulfuricans*, Pseudomonas *putida*	Lactate	Arsenic and molybdenum inhibited U(VI) reduction at 1 mM	36
Indigenous microbes	Ethanol	Cultures from Arizona, Germany, and New Mexico	39
Indigenous microbes	Acetate	Culture from New Mexico sediment	44
Indigenous microbes	Acetate, Lactate, Formate	Stimulated in landfill leachate contaminated Oklahoma aquifer. Nitrate inhibited U(VI) reduction at 5 mM.	42
Desulfotomaculum	-	Species found to dominate indigenous populations in contaminated New Mexico sediment	40
P. *islandicum*	H_2	Cultured from hydrothermal groundwater	48

The enzyme systems responsible for uranium and technetium reduction are different. Uranyl (UO_2^{2+}) traverses the cell wall, gains electrons, and ultimately is expelled as extra-cellular uraninite (UO_2). Conversely, pertechnetate (TcO_4^-) is taken up by the cell as an analogue of sulfate and is precipitated either in the periplasm, or closely associated with the cell (19). The site of precipitate deposition may contribute to the stability of reduced forms of uranium, technetium, and other metals and

radionuclides in the subsurface. Determining the roles of microorganisms in stabilizing radionuclide precipitates could reveal important information about optimal design parameters for in-situ bio-immobilization treatment systems.

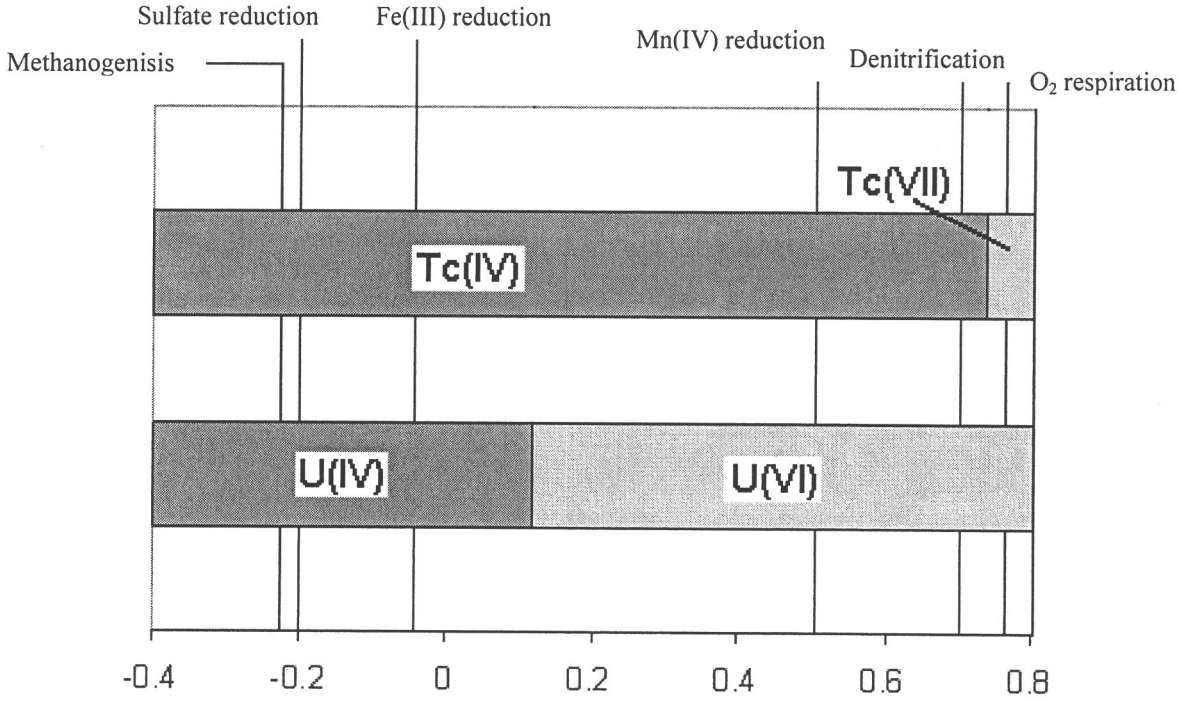


Figure 4. Oxidation of uranium and technetium as a function of standard reduction potentials at pH 7 (52, 16, 21).

Uranium and technetium differ too in their standard redox potentials (Figure 4). While Tc(VII) reduction may proceed concomitant with denitrification, U(VI) reduction may not take place until denitrification is ~ complete. Nitrate concentrations of 7.5 mM were shown to inhibit U(VI) reduction in field push-pull tests (42). In a separate study, 120 mM, but not 3 mM, nitrate concentrations inhibited U(VI) reduction in field push-pull tests (9). Both studies reported that nitrate and denitrification intermediates may reoxidize U(IV) in sediments. Similar outcomes were observed in laboratory studies when incubated sediments were amended with acetate (44). Both 5 and 7.5 mM nitrate additions to reduced sediments resulted in reoxidation of previously reduced uranium (44). U(VI) and Fe(III) reduction resumed in both studies when denitrification was complete.

Though concerns of uraninite reoxidation certainly warrant skepticism about bio-immobilization in nitrate contaminated aquifers, there is hope! Pyrite (FeS_2) or mackinawite ($FeS_{0.9}$) may protect uraninite from reoxidation by controlling the redox potential in-situ (30). Sulfate reducing conditions must be achieved for these protective solids to form. Thus sufficient electron donor and residence times must be provided in-situ for complete denitrification so that Fe(III), U(VI), and sulfate reducing conditions can be stimulated and maintained.

1.8 Denitrification

As observed by Istok et al. (9), Finneran et al. (44), and Senko et al. (42), nitrate and denitrification intermediates can hinder reductive precipitation of U(VI) in groundwater. Thus, understanding denitrification is critical for designing in-situ treatment zones which support optimal conditions for U(VI) and Tc(VII) reduction.

Denitrification is understood to be the complete reduction of nitrate to dinitrogen. Many bacteria can perform denitrification. Facultative aerobic bacteria, for instance, are quite resilient in that in the absence of their preferred electron acceptor oxygen, they can modify or repress enzymatic expression to accommodate an alternative electron acceptor. These organisms are ubiquitous in soils and aquifers, which is quite advantageous to the development of in-situ remediation schemes.

Assimilative nitrate reduction is performed by plants, fungi, and bacteria. In this process, nitrate is first reduced to ammonia, which may then be used as a nitrogen source for growth. Dissimilative nitrate reduction also involves the reduction of nitrate, and may be further divided into two categories: dissimilatory nitrate reduction to ammonia (DNRA) and denitrification.

Denitrification proceeds through a sequence of four enzymatic reductions. The first reaction is the conversion of nitrate (NO_3^-) to nitrite (NO_2^-). It is catalyzed by the enzyme nitrate reductase. The next conversion is that of nitrite to nitric oxide (NO). This reaction is catalyzed by an enzyme unique to denitrifiers called nitrite reductase, which is located in the cells periplasm. Nitric oxide is reduced to nitrous oxide (N_2O) in the third reaction, catalyzed by the enzyme nitric oxide reductase. The final

reaction is catalyzed by nitrous oxide reductase, which reduces nitrous oxide to dinitrogen (50).

These enzymes are only expressed by denitrifiers under anoxic conditions. Thus, it is critical that anoxic conditions be maintained within the denitrifying region of the subsurface. This constraint should not be problematic as populations of both aerobic microbes and facultative denitrifiers are present in the region of substrate delivery and can quickly consume any oxygen entering the denitrifying zone if sufficient donor is present.

Holmes et al. (51) found that nearly 40 % of the organisms present in acetate stimulated sediments from a uranium contaminated site in Shiprock, N.M. were of the Geobacteraceae family (51). They further concluded that of this 40 %, between 55 and 65 % were Desulfuromonas while the remaining were Geobacter species. While Desulfuromonas are obligate sulfate reducers, Geobacter *metallireducens* may use nitrate as an alternative electron acceptor. Both Pseudomonas (30) and Geobacter (49) species have been shown to reduce nitrate and U(VI) in groundwaters. These findings suggest that microorganisms capable of reducing U(VI) in nitrate contaminated groundwater are ubiquitous in the subsurface.

1.9 Uranium and Technetium Bio-immobilization

Bio-immobilization of U(VI) and Tc(VII) in groundwater is a promising treatment strategy. Laboratory studies with pure cultures and natural mixed communities have demonstrated that indigenous microorganisms are capable of coupling oxidation of exogenous electron donors to the reduction of nitrate, Tc(VII), U(VI), Fe(III), and sulfate. While nitrate is a co-contaminant of concern because of its potential to inhibit the bio-immobilization process, Abdelouas et al. (30) demonstrated that reduced sulfur compounds may provide protection against this reoxidation.

Istok et al. (9) noted in field push pull test and Senko et al. (42) noted in laboratory studies, that dosing reduced sediments containing uraninite with nitrate reoxidized previously immobilized U(IV). Finneran et al. (44) noted similar findings in laboratory studies. This oxidation effect is to be expected as dosing the sediments

with nitrate changes the redox conditions of the sediment both chemically and biologically. This 'dosing' effect as created in lab and small scale field studies would likely not be encountered in large scale stimulated aquifers if electron donor was not limiting.

If nitrate concentrations flowing into the reduced aquifer region were constant, then a denitrification zone of a defined width would develop based on groundwater velocity, denitrification rate, donor delivery, and other factors. Fe(III) and U(VI) reducing conditions could develop downgradient of this zone. If high nitrate groundwater entered the denitrification region, the region might expand, possibly extending down gradient into the previously Fe(III) and U(VI) reducing regions. In this case formerly immobilized uraninite may be reoxidized to mobile U(VI) species, along with Fe(II) and other redox sensitive metals. One could visualize this as a contraction of the reducing zone.

A more favorable response to increased nitrate concentrations might be greater denitrification rates. In this sense, denitrifiers up gradient could potentially serve to protect down gradient U(IV) from reoxidation in aquifers. To date large scale, continuous flowing bio-immobilization experiments in high nitrate groundwater with indigenous microbes have not been conducted. Therefore the extent to which indigenous denitrifiers are capable of accelerating respiration rates based on nitrate availability in a bio-immobilization system is not known.

Denitrification rates are contingent on the electron donor delivery strategy, which is critical to the success of bio-immobilization systems. If insufficient electron donor is supplied downgradient of the nitrate and uranium containing plume, local exhaustion of the substrate, and subsequent passing of contaminants could occur. Or similarly, insufficient residence time might be provided, which could result in passing of nitrate containing water and reoxidation of uraninite downgradient. Also, the optimal type and concentration of donor is not obvious. Optimally functioning bio-immobilization barriers create and maintain conditions which support reduction of oxygen, nitrate, and U(VI) and Tc(VII). Different electron donors may be best suited for different groups of microorganisms as they respire with the different electron acceptors. For instance, a laboratory study showed that acetate is a preferred electron

donor for U(VI) and Fe(III) reduction but acetate does not support Tc(VII) reduction (16). Thus determining the optimal electron donor or donors for each system is important.

Anderson et al. (7) demonstrated that in-situ uranium reduction via aquifer stimulation with acetate additions (~ 1 to 3 mM) is possible. The study was conducted in a shallow, unconfined aquifer that was contaminated with uranium (~ 1 μM) and sulfate (~ 7 mM). No nitrate was present, and the pH of the groundwater was circumneutral. Three rows of acetate injection wells and three rows of downgradient monitoring wells were installed to track changes of concentration over time. Three months of water samples were collected during acetate stimulation, and were analyzed for uranium, Fe(II), sulfate, and acetate. Within the first nine days of acetate injection Fe(II) concentrations increased, and U(VI) decreased. Characterization of the microbial community indicated that Geobacter species accounted for ~ 89 % of those present during that time. By day 50, Fe(II) and sulfate concentrations began to decrease, while U(VI) concentrations increased. Anderson et al. (7) postulated that sulfate reducers near the acetate injection wells were in competition with Geobacter species for Fe(III) as an electron acceptor during the initial phases of aquifer stimulation. Sulfur reducers do not conserve energy for growth when using Fe(III) as their electron acceptor, and thus their populations do not grow. When Fe(III) was depleted near the injection wells however, the sulfate reducers began to flourish while the Geobacter population decreased. Anderson et al. (7) hypothesized that the sulfur reducers consumed the acetate quickly for sulfate reduction, which resulted in increased uranium concentrations downgradient.

This finding is interesting in light of a laboratory study conducted by Lovely et al. (11), which demonstrated that D. *desulfuricans* can reduce U(VI) in the presence of sulfate with lactate as the electron donor. D. *desulfuricans* does not conserve energy using U(VI) as the electron acceptor, but rather reduces it fortuitously in the presence of sulfate. Anderson et al. (7) observed different dynamics with respect to U(VI) reduction by sulfate reducers in-situ. They found that sulfate reducers coupled the oxidation of acetate to the reduction of sulfate, with no U(VI) reduction. In fact, uranium concentrations increased when sulfate reducing conditions were reached.

Two primary differences in experimental conditions could explain these results.

First and most obvious is that different electron donors were used, acetate in the aquifer, and lactate in the microcosms. The two systems clearly are very different environments with different microbial communities. The second difference is more subtle, and potentially more insightful. The ratios of electron acceptor/donor were more proportional in the laboratory experiment than in the field experiment (Table 6). The excess sulfate availability as an electron acceptor relative to uranium could explain the difference in processing by the microorganisms.

Table 6. Contrasts in experimental conditions in two U(VI) reduction studies.

Species	Anderson et al. (2003)	Lovely and Phillips (1992)
Uranium	~ 0.001 mM	~ 0.35 mM
Sulfate	~ 7 mM	2 mM
Donor	Acetate ~ 2 mM	Lactate ~ 10 mM

The ideas presented and research sited in this introduction illustrates that the bio-immobilization of U(VI) and Tc(VII) contaminated groundwater has promise as a treatment alternative. The arguments presented in the previous paragraphs demonstrate that both small and large scale studies are required to better understand and isolate the important biogeochemical dynamics that control the efficacy of bio-immobilization at the field scale- especially with respect to the stability of reduced uranium.

It is important to note too, that studies of microbially stimulated U(VI) and Tc(VII) reduction, with both indigenous and pure cultures, were conducted under circumneutral pH conditions. Though D. *desulfuricans* was shown to reduce uranium in acidic waters (pH ~ 4) (20), few other studies evaluated U(VI) and Tc(VII) reduction capabilities under acidic conditions, or concurrent with pH adjustment or denitrification. Most bacteria grow most rapidly at circumneutral pH - rare conditions for many technetium and uranium contaminated groundwaters. Also, potentially toxic metals (e.g. aluminum, nickel) can be present in low pH groundwater. These could also hinder microbial activity.

Such are the conditions in groundwater near the S-3 ponds located near the Y-12 plant at Oak Ridge National Laboratory (ORNL) in Oak Ridge, Tennessee. Nitric

acid waste containing uranium, technetium, and trace amounts of tetrachloroethylene was disposed to unlined waste ponds from 1951 to 1983 (10). The ponds were neutralized and capped in 1984, but groundwater in the region remains contaminated. It contains uranium and technetium (~ 6 μM, and 13,000 pM respectively), has pH as low as 3.3, and nitrate concentrations as high as 140 mM.

Istok et al. (9) performed a series of field push-pull test in the contaminated aquifer at ORNL. The indigenous microorganisms were stimulated in separate wells using acetate, ethanol, and glucose as the electron donors. pH adjusted groundwater obtained from a well on site was used as source water in the push-pull tests. Control wells were included in the study, which received pH adjusted groundwater with no electron donor. Nitrate, uranium, and technetium concentrations were not reduced in these wells but were in wells that received electron donor (Table 7).

Table 7. Maximum reduction rates of NO_3^-, U(VI), and Tc(VII) in field push-pull tests (9).

Electron Donor	NO_3^- Rate (mM/hr)	Tc(VII) Rate (pM/hr)	U(VI) Rate (μM/hr)
Acetate	0.69	107	0.021
Ethanol	3.1	189	0.024
Glucose	3.2	460	0.041

After the sediments had been repeatedly stimulated with the various electron donors, the source water conditions were varied from well to well. In one well, for instance, source water with no electron donor was added to a previously stimulated region of the aquifer. Nitrate and U(VI) reduction were not detected under these conditions, though Tc(VII) was reduced, but at a much slower rate than in the presence of electron donor.

The first electron donor additions to the sediment resulted in slow rates of nitrate, U(VI), and Tc(VII) reduction. Therefore, three donor additions were administered to achieve sufficiently reducing conditions in the sediment such that rates of reduction could be obtained in a reasonable time interval. Upon successfully stimulating the aquifer sediments, the source water was once again administered to the well and the concentrations of nitrate, U(VI), and Tc(VII) were monitored. The contaminants were reduced as expected until hour 50, at which time U(VI)

concentration began to increase. The U(VI) concentration reached a maximum of 20% higher than the U(VI) concentrations in the injected test solution.

1.10 Research Objectives

Istok et al. (9) speculated that the increase in U(VI) concentration was likely reoxidation of U(IV) to soluble U(VI) due to "a biological process somehow linked to donor oxidation". They concluded this by observing that U(IV) was not reoxidized when high nitrate (~ 120 mM) groundwater with no electron donor was added to previously reduced aquifer sediment. They also observed that U(IV) was not reoxidized when low nitrate (~ 1 mM) groundwater and electron donor were added to the aquifer. Thus, the combination of electron donor and high nitrate concentrations seemed to result in reoxidation of U(IV) in previously reduced sediment.

In a separate variation, acetylene gas was dissolved in high nitrate and ethanol containing source water. Tc(VII) and nitrate reduction proceeded during this test, followed by the accumulation of denitrification intermediates NO_2^- and N_2O. Interestingly, the acetylene seemed to inhibit both U(VI) reduction and the reoxidation of U(IV). This finding demonstrates that additional experiments, both highly controlled laboratory tests and field push-pull tests, are required to determine the causes of U(IV) reoxidation.

The research presented in this thesis was inspired by the work presented in the Istok et al. (9) paper, and by previous studies conducted by Dr. Istok and his collaborators. Specifically, the objective of this research was to determine if in-situ bio-immobilization is a viable treatment alternative for this high nitrate, low pH, and uranium and technetium contaminated groundwater. Three questions were identified as the first step in assessing whether or not an in-situ bio-immobilization treatment system could be appropriate at ORNL. First, can the pH be adjusted in-situ without clogging with metal and other hydroxide solids? pH adjustment will likely be required to support microbial growth in-situ. Second, can nitrate be removed at a sufficient rate such that the ~ 140 mM concentrations can be reduced to near zero? Nitrate removal will likely be required prior to establishing the Fe(III) reducing conditions that favor

U(VI) and Tc(VII) reduction. And finally, can U(IV) and Tc(IV) be precipitated in-situ, after nitrate removal, but concurrent with pH adjustment? These questions were the basis for the research presented in this thesis.

2.0 METHODS

2.1 Field Site

The S-3 ponds in Oak Ridge, Tennessee were constructed in 1951 to store nuclear processing waste from the nearby Y-12 plant (10). The ponds were unlined, with a combined storage capacity of approximately 38 million liters (10). Waste water stored in the ponds contained high uranium and nitrate concentrations, in addition to technetium and trace concentrations of solvents and metals from Y-12 and other nuclear processing sites (10). Disposal to the ponds was discontinued in 1983, and the ponds were partially denitrified in situ (10). The treated water was discharged from the ponds to a nearby stream. In 1984 the remaining sludge was buried under backfill, and the ponds were covered "with a multi-layered Resource Conservation and Recover Act cap, and asphalt surface", which is now used as a parking lot (10).

The shallow, unconfined aquifer down gradient of the S-3 ponds consists of unconsolidated silty-clayey saprolite which overlies Nolichucky shale (54). Researchers are allowed access to the contaminated aquifer though the Field Research Center (FRC), which was established by the U. S. Department of Energy's Natural and Accelerated Bioremediation Research (NABIR) Program (56). All groundwater and microbially stimulated sediment used in the experiments presented in this thesis were obtained from the FRC, though other sediments were used as well (Table 8).

Table 8. Soils/media used in laboratory and field experiments.

Material	Source	Description	Uses
Hanford sand	Background site in the Hanford formation, WA.	Unconsolidated basaltic sediment.	Laboratory studies only.
Limestone	Maynardsville Limestone, Tennessee quarry	Crushed and sieved with ¼" sieve.	Laboratory and intermediate-scale models.
Contaminated FRC sediment	FRC soil cores Area 1	Silty, Clayey sediment	Laboratory and intermediate-scale models.
Background FRC sediment	FRC background pit (uncontaminated)	Silty, clayey sediment	Intermediate-scale models

2.2 Groundwater

The FRC provides access to both the contaminated aquifer, and to a nearby uncontaminated background area. The contaminated section of the FRC is currently divided into three areas, Area 1, 2, and 3. This work was performed only in Areas 1 and 2. Though in close proximity, the two areas have very different groundwater characteristics. Groundwater from one well in each area was used in small-scale laboratory and intermediate-scale physical model studies (Table 9).

Table 9. Groundwater concentrations in two wells at the FRC (9).

Area	Well	pH	NO_3^- (mM)	SO_4^{2-} (mM)	U (uM)	Tc (pM)
1	FW21	3.3	142	0.4	5.8	18000
2	GW835	6.4	1	0.8	4.9	410

FW21 water and GW835 water have different compositions. Though some bicarbonate is added to GW835 to ensure the speciation of uranium in field and laboratory experiments, bicarbonate is added to FW21 in larger quantities to adjust the pH to circumneutral conditions as well. Adjusting the pH of FW21 water causes dissolved metals to precipitate from solution as insoluble metal hydroxides, resulting in changes in solution composition (Figure 5). No losses were observed in GW835 after addition of bicarbonate (data not shown).

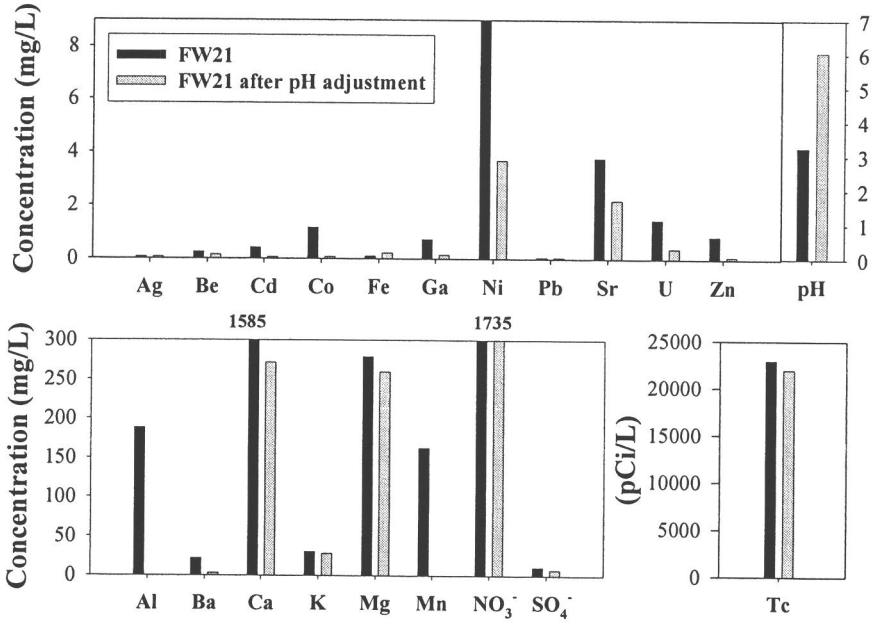


Figure 5. Solution composition change in FW21 groundwater due to pH adjustment with 100 mM bicarbonate.

2.3 pH Adjustment

2.3.1 Density of pH Adjusted FW21 Groundwater

FW21 water was titrated with 0.1 N NaOH and 0.1 N $NaHCO_3$ in 0.5 mL increments. The density of the resulting solution was determined as follows, where density of solution is defined as the sum of mass per volume water and mass of solids formed per volume of solution. Two, 2-L flasks of FW21 water were placed atop a stir plate. Titration with the respective base was performed in each flask, and 20 mL samples were taken incrementally from the flasks. 10 mL of the samples were dispensed into pre-labeled plastic weigh boats, and were dried and weighed to determine the amount of suspended solids in the mixture. The additional 10 mL withdrawn from the mixture were placed into 12 mL pipettes which were sealed at one end to store the samples and allow for settling of the solids.

Visible precipitate formation was detected above a pH of 5 for the NaOH titrated samples, and pH above 4.5 for the $NaHCO_3$ titrated samples. The precipitate formed in both titrations settled to ~ 2.5 mL of solids per 10 mL of settled titrant solution above pH ~ 5.

To determine the mass of precipitate produced during the titration experiment, 0.2 N solutions of NaOH and $NaHCO_3$ were prepared. More concentrated bases were used in this experiment to expedite the titration process. After every 35 mL of $NaHCO_3$, or every 25 mL of NaOH added, 20 mL of titration solution were extracted. 10 mL of the solution were removed and dispensed directly into a pre-weighed plastic weighing boat. The additional 10 mL of the titrant solution were filtered (0.2 μM) into a second plastic weigh boat.

Weigh boats containing filtered and non-filtered titrant solution were dried in an oven; The weight difference between the filtered and non-filtered samples yielded the mass of precipitates formed during titration. The densities of the precipitates were calculated using an average of three values of precipitate mass formed in the circum-neutral pH range for both titrations. Dilution effects as well as the added mass of the base were considered in the calculations.

2.3.2 Precipitate Transport

A column experiment was conducted to determine how much of the precipitate formed during pH adjustment might be retained in the pore space during transport. The experiment was performed using FW21 water which had been treated with sodium bicarbonate to adjust the pH to 6.8. The light colored precipitate that formed in 100 mM bicarbonate FW21 water was suspended in solution via continuous stirring on a magnetic stir plate. The water was then pumped with a peristaltic pump at a flow rate of 1 mL/min through a glass, (2.5 cm inside diameter x 25 cm long) column packed with Hanford sediment. The sediment pack had a porosity of ~ 46 %. Hanford sediment was chosen as pack media for the column because large quantities of this sediment from a single uniform sample were available for laboratory testing.

The effluent of the column was collected on 15 minute intervals. 10 mL samples were dispensed in to weigh boats which were dried and weighed to determine the mass of precipitate passing though the column. The residence time varied somewhat due to clogging in the small diameter (1 mm) tube used to deliver FW21 water to the column via the peristaltic pump.

2.3.3 pH Adjustment Experiments

A glass column (2.5 cm inside diameter x 25 cm long) was packed with a mixture of Hanford sand and $NaHCO_3$ by compacting ~ 10 mL of Hanford sediment followed by ~ 2.5 mL of $NaHCO_3$. This procedure was repeated until the column was fully packed with the layered mixture. The resulting ratio of $NaHCO_3$ to sediment was approximately 11.5 % by weight.

FW21 groundwater was pumped though the pH adjustment column via a peristaltic pump at a flow rate of approximately 1.3 mL/min. Manometers were used to measure head loss during the experiment (Figure 6). Samples were collected on 15 minute intervals. Measurements of volumetric flow rate, head loss, and pH were recorded at the time of each sample collection. Hydraulic conductivity was computed from measured flow rates and head loss using Darcy's Law.

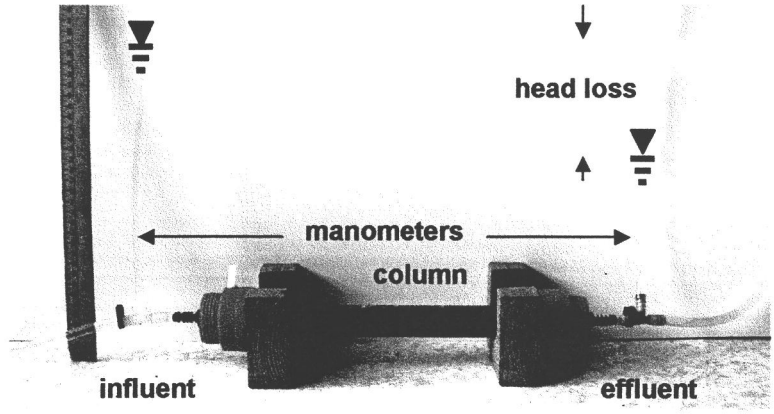


Figure 6. Column used for pH adjustment experiments.

Three variations of this experimental setup were used in an attempt to identify an effective pH adjustment strategy for a flowing system. The first variation reduced the bicarbonate concentration by a factor of two. The second was to use the less soluble base Na_2CO_3 in place of the $NaHCO_3$. The third variation used half the bicarbonate mass used in the original pH adjusting column experiment and crushed Maynardsville Limestone instead of the Hanford sediment.

2.4 Bio-immobilization experiment with pH adjusted FW21 groundwater: "The Laboratory Chamber"

106.4 g of FRC sediment and 52.5 mL of tap water were combined in a beaker. The mixture was stirred continuously until the soil was suspended in solution. Next, 102 mL of tap water were added to 961.23 g of Hanford sediment. This mixture was stirred to ensure moistening of the Hanford sediment surfaces. Finally the two mixtures were combined and stirred until the FRC sediment uniformly coated the Hanford sediment.

The Laboratory Chamber was constructed out of 1 cm thick pieces of glass (25 cm long x 48.3 cm high) (Figure 7). The lower 5.5 cm of the chamber was 'inoculated' with the FRC/Hanford sediment mixture (described above). Moistened Hanford sediment alone was used to pack the remainder of the chamber.

A 'feed' solution was prepared by neutralizing one liter of FW21 to pH ~ 7 by adding ~ 120 mM bicarbonate and adding 100 mM ethanol to serve as an electron donor. This solution was used to stimulate the growth and activity of sediment microorganisms. Each week, approximately three pore volumes of the feed solution were passed though the column using a peristaltic pump at a flow rate of ~ 3 mL/min. Triplicate samples were collected during each weekly feeding beginning on the sixth week of stimulation. Samples were collected from the feed solution, from the first pore volume of effluent, and from the third pore volume of effluent. The pore volume was calculated using the saturated depth, measured flow rate, and an assumed porosity of 40 %.

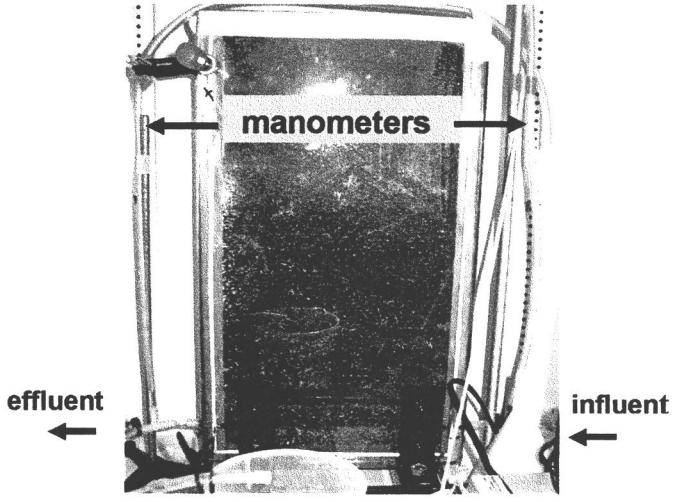


Figure 7. Laboratory chamber used for bio-immobilization experiments with FW21 groundwater.

All samples were analyzed for nitrate, nitrite, sulfate, ethanol, and periodically uranium and technetium concentrations. The change in concentration (influent – effluent both measured in triplicate) and the concentrations in the first pore volume (also measured in triplicate) were determined each week. This change in concentration was then divided by the length of time between feedings (typically 7 days), which yielded the denitrification rate in the chamber. A chamber was used rather than a column because it is open to the atmosphere, which allows nitrogen and other gasses produced by denitrification and donor fermentation/oxidation to escape.

2.5 Bio-immobilization experiment with GW835 groundwater: "The Laboratory Column"

17 mL of tap water and 35.45 g of FRC sediment were added to a beaker. The mixture was stirred continuously until the sediment was suspended. Next, 320.41 g of Hanford were moistened with 34 mL of tap water. The suspended FRC and moistened Hanford sediment were combined in a separate beaker, and stirred until the FRC uniformly coated the Hanford sediment.

132.12 g of the Hanford/FRC mixture were packed in a glass column (2.5 cm inside diameter x 25 cm long), which was covered with tin foil to prevent light exposure. A 'feed' solution was prepared by adding 10 mM bicarbonate to GW835 groundwater, and 10 mM ethanol to serve as the electron donor. Each week approximately three pore volumes of the feed solution were passed through the column using a peristaltic pump at a flow rate of ~ 1 mL/min.

The pore volume was calculated as ~ 31 mL using the column dimensions and assuming 40 % porosity. Triplicate samples were taken from the influent, and from the first pore volume of effluent. Additional samples were collected from the effluent during the third pore volume. The difference in concentration was calculated as the average of the uranium concentration in the triplicate feed solution samples less that of the uranium concentration in the first pore volume passing. This difference in concentration was then divided by the number of days between feedings (typically 7 days) to determine the rate of uranium reduction. During the first weeks of column feedings, the effluent concentrations were consistently less than the influent, which was due to less than three pore volumes being flushed through the column. When this problem was identified the pore volumes passing were no longer measured by flow rate setting and time, but with total volume of effluent collected. Thus, when ~ 95 mL of the feed solution had passed, an additional triplicate sample was taken. All samples were analyzed for nitrate, nitrite, sulfate, ethanol, and uranium concentrations. Several samples were analyzed for technetium as well.

2.6 Sorption Experiments

2.6.1 Batch Experiments with Hanford Sediment

141.9 g of FRC sediment and 70.0 mL of tap water were combined in a beaker. The mixture was stirred continuously until the soil was suspended. Next, 136 mL of tap water were added to 1281.6 g of Hanford sediment in a separate container. This mixture was stirred to ensure moistening of the Hanford sediment surfaces. Finally the suspended FRC sediment was combined with the moistened Hanford sediment and stirred until the FRC sediment uniformly coated the Hanford sediment.

This mixture was evenly divided into four 500 mL Nalgene bottles. 200 mL of reversed osmosis (RO) treated water was added to two bottles. 200 mL of FW21 water and GW835 water were added separately to the two remaining bottles. All bottles were hand shaken twenty times to ensure good initial contact with the soil and water. Samples were taken from the source waters and from the shaken solutions after settling of particles.

On the following day, the supernatant was decanted from the four bottles. 10 mM bicarbonate and 100 mM bicarbonate were added separately to the two RO supernatants. The FW21 and GW835 supernatants were adjusted with 100 mM and 10 mM bicarbonate concentrations respectively. The bicarbonate containing water was then replaced in their respective containers which were hand shaken 20 times. The bicarbonate adjusted supernatants were sampled the following day. All samples were analyzed for uranium and inorganic carbon.

2.6.2 Sorption to Limestone in pH Adjusting Column

A column experiment, using the setup shown in Figure 6, was performed to determine the magnitude of uranium sorption to limestone in the pH adjusting column. Triplicate samples were collected from the FW21 water flowing into the limestone and bicarbonate layered pH adjusting column, as well as from the column effluent and analyzed for uranium.

2.6.3 Batch Experiments with Limestone and Hanford

An additional batch experiment was performed to compare uranium sorption to Maynardsville Limestone and to Hanford sediment. Two bottles were prepared using

the same ratios of sediment and groundwater as described previously (section 2.6.1), only in this case one batch contained Maynardsville Limestone. 200 mL of FW21 groundwater were added to both containers. Again, triplicate samples were collected from the FW21 source water, from the settled supernatant after shaking, and finally from the supernatant after adding 100 mM bicarbonate and contacting over night. All samples were analyzed for uranium concentrations.

2.7 Reoxidation of U(IV) by NO_3^-

The laboratory column described previously (section 2.5) had been stimulated by sequential additions of GW835 groundwater containing 10 mM bicarbonate and 10 mM ethanol for eleven weeks. This experiment began by pumping the feed solution through the column using a peristaltic pump. Though the pump was set to deliver a flow rate of ~ 1.3 mL/min, the resulting flow rate was much slower presumably due to biomass clogging the pore space. After 76 hours, the feed solution was changed to tap water containing 120 mM nitrate. Triplicate samples were collected from the feed solution, and from the 120 mM nitrate solution. The column effluent was sampled periodically for ~ 100 hours. All samples were analyzed for nitrate, nitrite, and uranium.

2.8 Bio-immobilization experiment with GW835 groundwater: "The Area 2 Physical Model"

A larger scale version of the laboratory column was constructed for deployment at FRC Area 2. The column was constructed of 15.24 cm diameter PVC pipe, with a total length of 254 cm (Figure 8). The column is equipped with nine manometers, 5 ethanol injection ports, and eight sample ports.

14 kg of crushed limestone were moistened with 1.5 L of tap water, and set aside. 7.7 kg of dry FRC background sediment were combined with 3.8 L of tap water in a separate container, and was stirred continuously until the sediment was suspended. The contents of the two containers were combined and stirred well to ensure uniform coating of the limestone with FRC sediment. The column was packed in five layers of the FRC/limestone mixture in the proportions described above. Each

layer was topped with 47.6 grams of sodium bicarbonate. When the packing procedure was complete, the column was secured to a wooden stand (Figure 9).

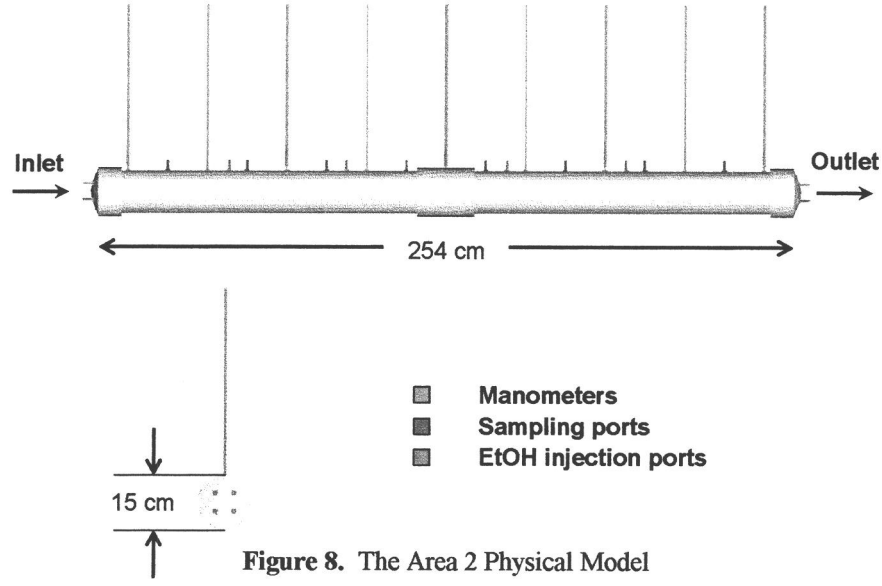


Figure 8. The Area 2 Physical Model

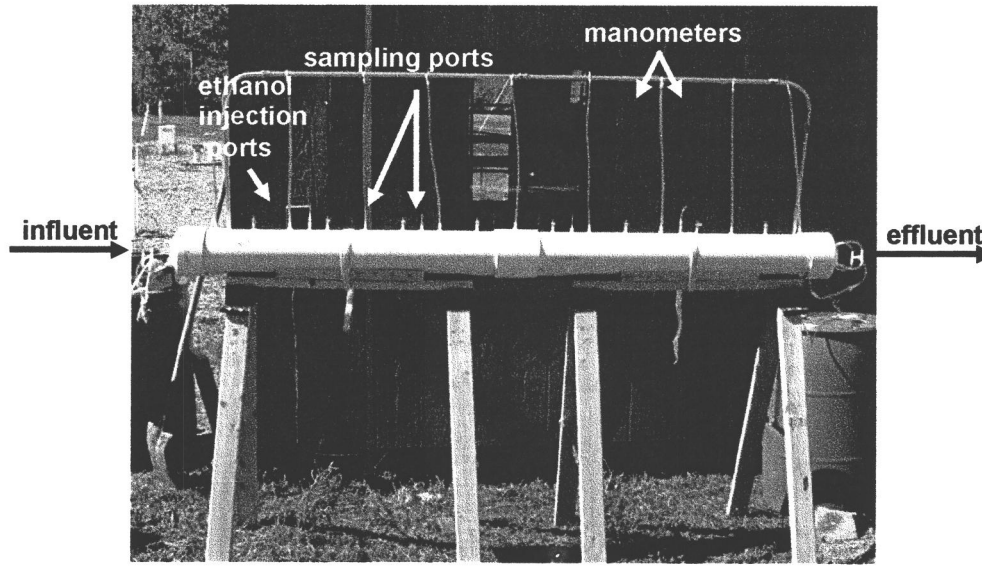


Figure 9. Intermediate-scale Area 2 physical model.

Table 10. Mass of materials used in packing the Area 2 physical model.

Material	Mass (kg)
Maynardsville Limestone	70
Background FRC sediment	8
$NaHCO_3$	0.238

10 mM bicarbonate and 50 mM ethanol were added to GW835 groundwater in a 200 L drum. This feed solution was pumped though the column using a peristaltic pump to saturate the column with uniform ethanol and bicarbonate concentrations. After pumping 200 L, the influent was switched to a drum containing GW835 groundwater without added bicarbonate or ethanol. The groundwater was pumped though the column using a peristaltic pump at a rate of ~ 2.5 mL/min. The estimated pore volume is 18 L (assuming a porosity of 40 %). Therefore the average residence time with a flow rate of 2.5 mL/min is ~ 5 days. The bulk density of the Maynardsville Limestone and FRC background sediment are 1.57 g/cm^3 and 1.08g/cm^3 respectively.

0.3 mL of neat ethanol is injected into the inlet daily, and into four ports spaced in ~ 50 cm increments from the inlet (Figure 8), for a total ethanol injection of 1.5 mL each day. 15 mL samples are collected from the inlet, outlet, and sample ports 3 days each week. The manometer readings and flow rate are recorded with each sampling.

2.9 Bio-immobilization experiment with FW21 groundwater: "The Area 1 Physical Model"

Rectangular sheets of 1.3 cm acrylic (trade name Plexiglas G) were used to construct a large scale version of laboratory chamber (Figure 10). The model was configured with 14 sample ports and 1.9 cm diameter perforated PVC wells were positioned along the centerline of the model every ~ 25 cm prior to placing the FRC/limestone material. The wells were secured to the top of the model while packing using duct tape. Ethanol injection lines were attached to four wells at three depths in the saturated region.

FRC sediment was collected in the form of soil cores and background sediment. Batches of ~ 1.4 kg sediment were stored in 2 L bottles with 1 L of solution containing 120 mM nitrate and 100 mM ethanol to stimulate growth of soil microorganisms. The bottles were kept in sealed containers to minimize oxygen exposure, and in a dark cabinet to prevent algal growth. The time of incubation in the bottles ranged from 2 weeks to several months.

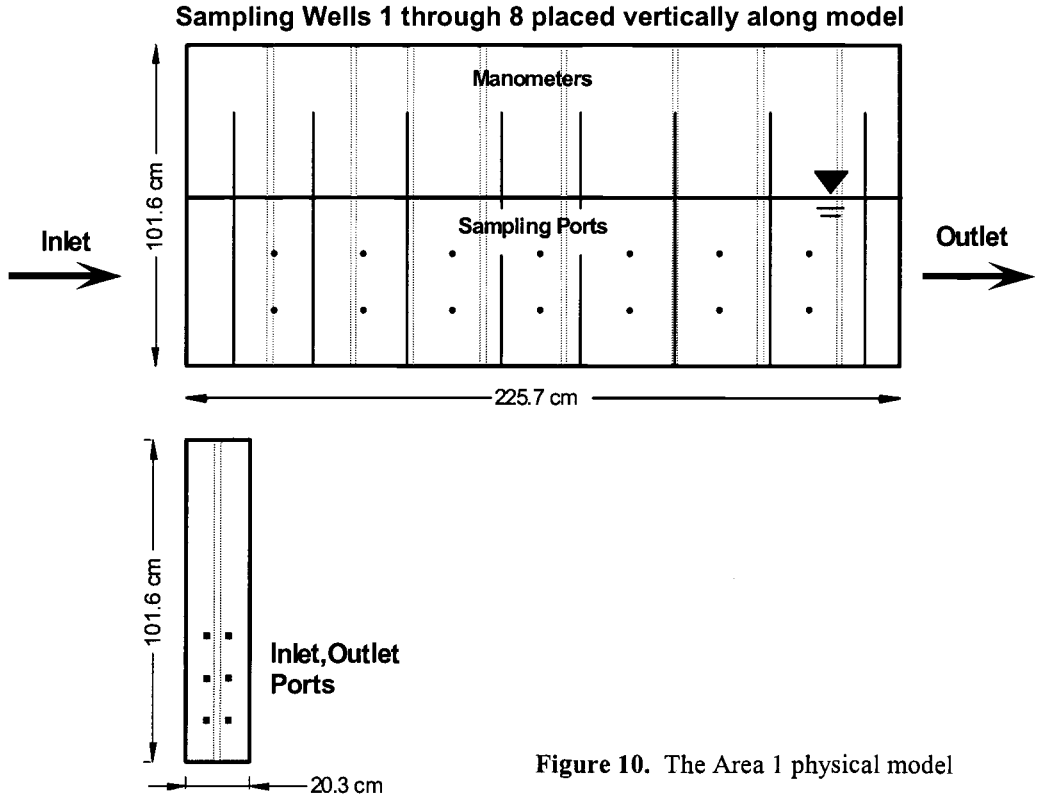


Figure 10. The Area 1 physical model

18 kg of stimulated sediment were emptied from the bottles into one container. Next 17.7 kg of rain moistened limestone, and 0.9 kg of stimulated FRC sediment were added to two large buckets. The contents of the two containers were mixed well, emptied into the bottom of the model, and packed into place. 1.25 kg of sodium bicarbonate were then placed on top of the packed layer. This procedure was repeated 10 times to pack the model to a total depth of ~ 54 cm - i.e. only the bottom of the model contains stimulated sediment. The remainder of the model was packed in the same manner, only without the stimulated sediment (Figure 11), and was covered completely with tin foil to prevent light exposure. The bulk density of the Maynardsville Limestone and FRC sediments were 1.57 g/cm^3 and 1.08 g/cm^3 respectively.

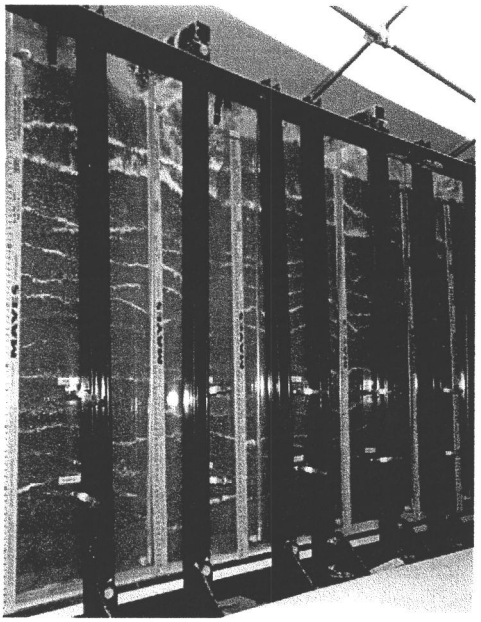


Table 11. Mass of materials used in packing the Area 1 physical model.

Material in pack	Mass (kg)
Maynardsville Limestone	700
FRC sediment	18
$NaHCO_3$	25

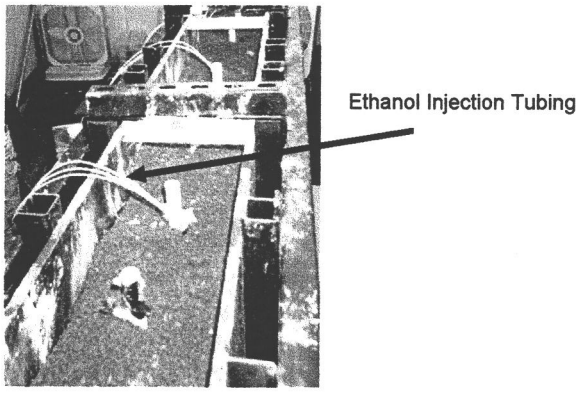


Figure 11. Side view of the Area 1 physical model- the white layers are bicarbonate (left). Sampling wells with ethanol injection tubing (right).

A 200 L drum full of FW21 groundwater was prepared to contain 50 mM ethanol and 100 mM bicarbonate. This solution was poured down the eight sampling wells until the model was saturated to a thickness of ~ 78 cm. The model remained saturated under no-flow conditions for 5 days, while samples were collected from the 14 sample ports.

On day 5, a peristaltic pump was used to deliver groundwater from FW21 at a flow rate of approximately 5 mL/min. 6 mL of neat ethanol are injected into the inlet and into five injection tubes fastened to the wells once daily using a programmable syringe pump. Thus, a total of 36 mL of ethanol are injected into the saturated region each day to reach the target ethanol concentration of 100 mM in the saturated region. Presently well one and five are used for ethanol injection (Figure 11), while wells 3 and 7 are equipped for ethanol injection as well. Daily samples are collected from the inlet, outlet, and the 14 samples ports. Temperature, manometer levels, and flow rate are all logged with each sampling. All collected samples are analyzed for nitrate, nitrite, ethanol, sulfate, and uranium and technetium.

The peristaltic pump was set to deliver a flow rate of ~ 5 mL/min (though an average of 3.7 mL/min was measured) for the first 10 weeks, but was increased to ~

8.5 mL/min on the 11th week. The average saturated thickness during the first 10 weeks results in a total saturated volume of 278 L. The estimated pore volume during that time was ~ 111 L (assumes a porosity of 40 %). Therefore the average residence time during the first 10 weeks was ~ 21 days, while a flow rate of 8.5 mL/min results in an estimated residence time of ~ 10 days.

3.0 ANALYTICAL METHODS

NO_3^-, NO_2^-, Cl^-, Br^-, and SO_4^{2-} concentrations were determined by ion chromatography (DX-120) using a AS9-HC Dionex column (4 mm diameter x 250 mm), with a runtime of 25 minutes per sample. 9 mM Na_2CO_3 was used as column eluent (flow rate of 1 mL/min). The sample vials were filled with 600 μL. The injected volume was 25 μL.

A kinetic phosphorescence analyzer (KPA-11, CHEMcheck Instruments) was used to measure U(VI). The samples were diluted 1:100 in 0.1 N nitric acid for two reasons. First, the groundwater samples contained elevated chloride concentrations, which can quench the KPA laser signal. Secondly, the complexing agent URAPLEX™ (CHEMcheck) binds to U(VI) most effectively under acidic conditions in the presence of nitrate. 1.5 mL of URAPLEX was added to each sample prior to analysis.

Tc(VII) was measured using a liquid scintillation analyzer (Tri-Carb 2900TR, Packard Instruments). Each 10 mL sample was combined with 10 mL of Ultima Gold XR Beta scintillation cocktail. The count-time was 20 minutes per each sample, which sums to .

Ethanol was measured using a Hewlett Packard 5890A Gas Chromatograph with flame ionization detection (FID). The oven temperature was set to 150 °C, which resulted in a peak time of 4 minutes. The total run time was 8 minutes for each sample. Hydrogen gas and 'breathing quality' air were used for the FID detector at 20 psi and 36 psi respectively. Helium at 50 psi was used as the column carrier gas. The column (2 m x 1/8") was packed with 80/100 Porapak Q.

Total Inorganic Carbon was measured using a Dohrmann DC-190 Carbon Analyzer (Rosemount Analytical). The carrier gas was oxygen at 30 psi pressure.

Fe (II) and Mn (II) were both measured using colorimetric kits (CHEMetrics, Inc.).

pH was measured using a glass electrode (Thermo Orion, Fisher Scientific, Inc.) and meter (Accumet® model 25 pH/ion meter).

4.0 RESULTS

4.1 pH Adjustment

4.1.1 Density of pH Adjusted FW21 Groundwater

Titration of FW21 groundwater with NaOH resulted in a well defined equivalence point near 1 meq of base added (Figure 12); Titration of FW21 groundwater with $NaHCO_3$ resulted in a less defined equivalence point (Figure 12). Titration with NaOH produced a brown precipitate (likely metal oxides), while titration with $NaHCO_3$ produced a white cloudy precipitate (likely calcium and aluminum carbonate precipitates). Though no analysis of the precipitates was performed, the densities of the precipitate containing solutions were calculated. The approximate densities of solution formed with NaOH and $NaHCO_3$ were 1.04 g/cm^3 and 1.02 g/cm^3, respectively. Again, the density of solution is defined as the sum of mass per volume water and mass of solids formed per volume of solution.

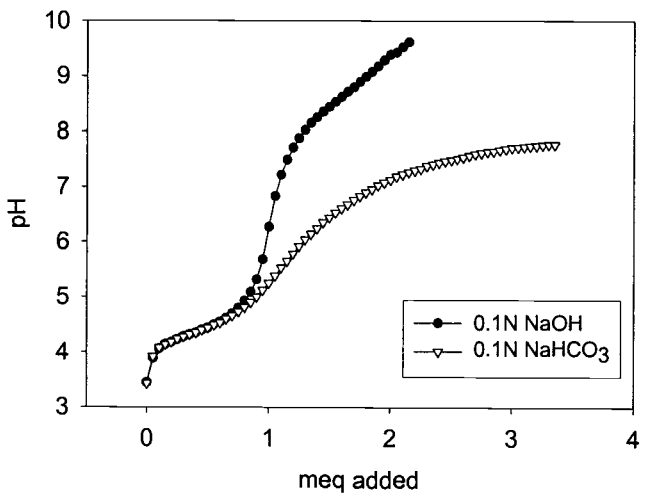


Figure 12. Titration of FW21 with 0.1N NaOH and 0.1N HCO_3

The mass of precipitate formed increased during the titration process (Figure 13). The plotted values represent total solids measured per 10 mL of titrant solution extracted. Both $NaHCO_3$ and NaOH produced ~ 20 mg of precipitate mass as pH increased from ~ 3.4 to ~ 7 (Figure 13). The mass of precipitate formed when the pH of FW21 water was increased to near neutral was required in assessing the extent to which pore space in packed sediment may clog.

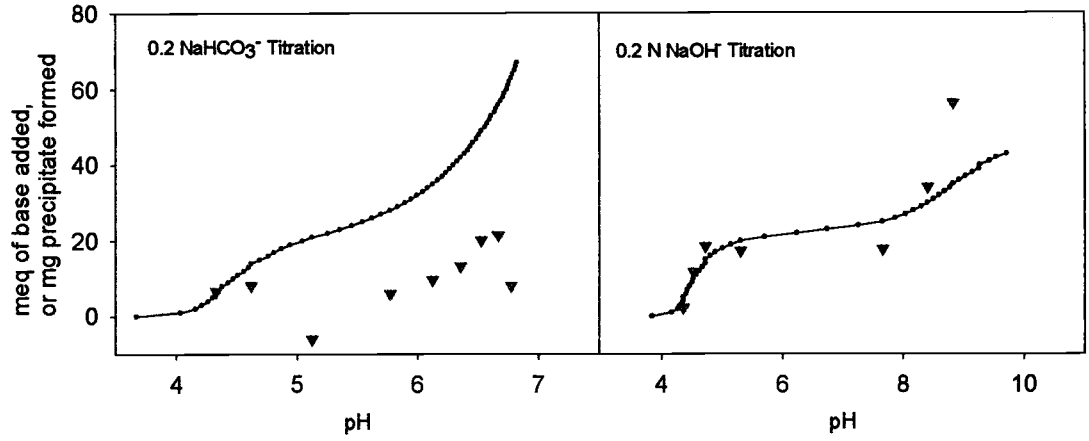


Figure 13. Mass of precipitate formed per 10 mL of titrant, either $NaHCO_3$ (left) or NaOH (right), added to FW21 groundwater, where ▾ represents mg of precipitate formed.

4.1.2 Precipitate Transport

Because the pH of FW21 groundwater was expected to increase from ~ 3 to ~ 7-8 in the intermediate-scale physical model, it was important to predict the likely effects of precipitate deposition and transport on hydraulic conductivity. A column experiment was conducted to assess the extent to which precipitate formed during pH adjustment of FW21 groundwater would clog pore space and decrease hydraulic conductivity of packed sediment.

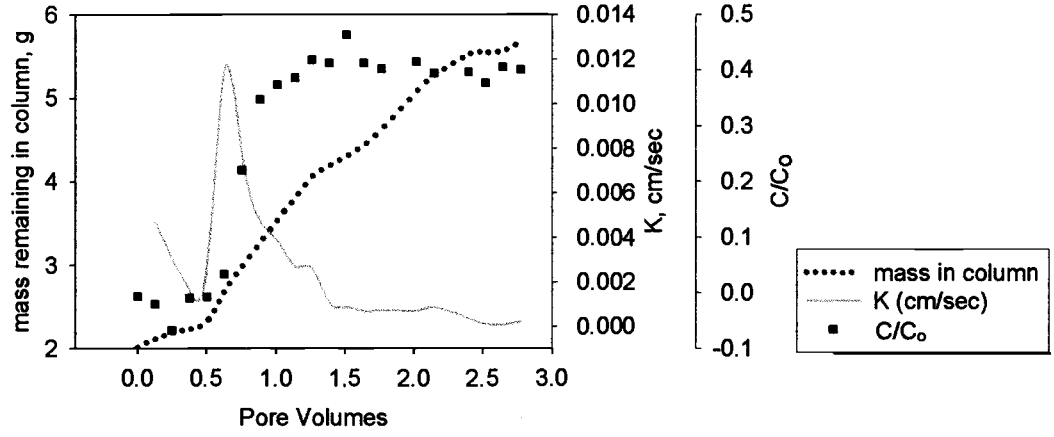


Figure 14. Relative precipitate concentration in column effluent (C/C_o), precipitate mass remaining in the column, and corresponding hydraulic conductivity during precipitate transport experiment.

Hydraulic conductivity initially decreased then rapidly increased to a maximum value of 0.011 cm/sec before gradually decreasing to 2.06×10^{-4} cm/sec

(Figure 14). The rapid changes in hydraulic conductivity observed during the first pore volume were likely due to trapped air passing through the column. The maximum hydraulic conductivity occurred when the column reached nearly-complete water saturation. Although some precipitate was rapidly transported through the column, a portion remained trapped in the column pore space. The decrease in hydraulic conductivity occurred concurrently with an accumulation of precipitate mass in the column (Figure 14). The conductivity dropped rapidly after ~ ¾ of a pore volume, which seems to correspond with the maximum precipitate concentration in the effluent. This value is represented as C/C_o, were C_o is the solids concentration in the pH adjusted FW21 influent. The decrease in hydraulic conductivity also appears to correspond with the mass of precipitate deposited in the column. These trends suggest that the column was in fact clogging due to precipitate filling the pore space.

4.1.3 pH Adjustment Experiments

Though several variations of this experiment were conducted, two were run for extended time periods and thus provide the most complete information. These two variations were conducted in columns packed with Hanford sediment layered with bicarbonate and limestone layered with bicarbonate. Both experiments begin with sharp increases, followed by sharp decreases in hydraulic conductivity (Figure 15). Again, this was likely because the columns initially contained trapped air. The maximum hydraulic conductivity likely corresponded with the sediment pack reaching maximum water saturation.

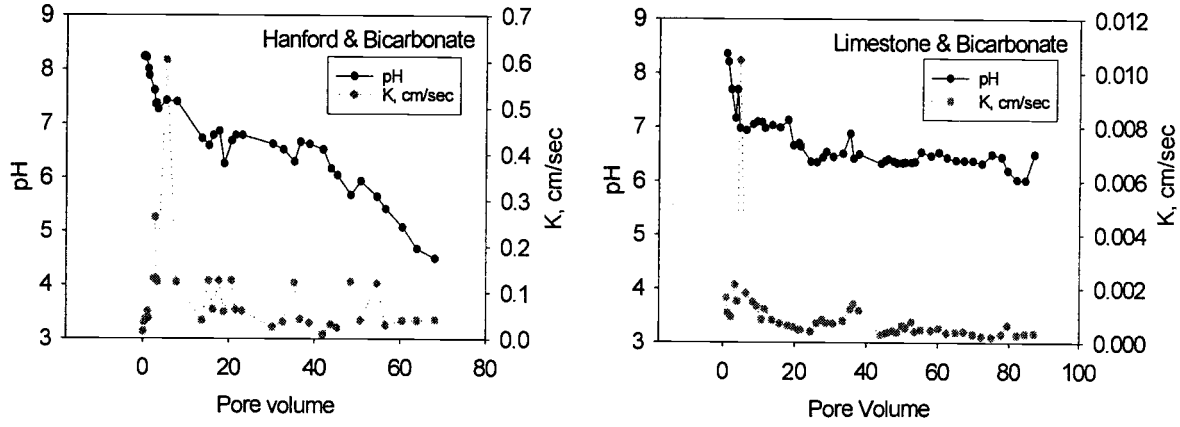


Figure 15. Change in hydraulic conductivity and pH observed during two pH adjustment experiments.

Although the columns were packed with similar amounts of bicarbonate and sediments (Table 12), the conductivities were quite different. The likely explanation is the varying content of fine grained particles. The limestone used in the laboratory column experiment was shipped from Nebraska in gravel sized particles (~ 2 cm). Thus the limestone was crushed to attain particles similar in size to the Hanford sediment. The crushing process produced fine grained particles (silt and clay sized) in addition to the target particle size (diameter ~ 0.64 cm). These fines were incorporated into the sediment pack, which likely produced the difference in magnitude between the conductivities of the extended bicarbonate column and the limestone and bicarbonate column.

Table 12. Mass of materials used in pH adjustment columns.

Column	Mass $NaHCO_3$ (g)	Mass sediment (g)	ρ_{bulk} sediment (g/cm^3)	Average K (cm/sec) after 20 pore volumes.
Extended Bicarbonate	7.98	263.98	Hanford: 1.6	0.052636
Limestone & Bicarbonate	8.51	247.82	Limestone: 1.55	0.000553

The pH adjustment capabilities of the limestone sediment pack are clearly more effective and longer lasting relative to the Hanford sediment pack. After nearly 90 pore volumes the limestone maintained a circum-neutral pH, whereas the Hanford exhibited little to no pH adjustment. This experiment demonstrated that limestone is the preferable media for pH adjustment in the intermediate-scale physical models.

4.2 Bio-immobilization experiment with pH adjusted FW21 groundwater: "The Laboratory Chamber"

Nitrate concentrations decreased and nitrite concentrations increased as pH-adjusted FW21 groundwater flowed through the laboratory chamber. Rates of nitrate reduction (defined as the influent nitrate concentration minus the effluent nitrate concentration divided by the chamber residence time) increased with subsequent feedings to a maximum of 15.25 mM/day. The maximum nitrite concentration

measured was 12.1 mM. Nitrate reduction rates represent an average over the entire saturated thickness (~ 21.5 cm), which includes portions of the sediment pack that were prepared without biostimulated sediment and likely had much less initial microbial activity than the portion of the sediment pack prepared with biostimulated sediment. Thus, nitrate reduction rates measured in the laboratory chamber likely underestimate the maximum achievable rates of nitrate reduction in this system.

Uranium concentrations increased ~ 14 % as the FW21 groundwater solution passed through the chamber, which was likely caused by flushing of the contaminated FRC sediment used in the sediment pack. The maximum rates of ethanol utilization and Tc(VII) reduction were 13.12 mM/day and 258 pM/day, respectively.

An additional point of consideration is that only a portion of the saturated zone was inoculated with sediment. The saturated depth in the column was 21.5 cm during the column feedings, but the feed solution was drained to approximately 10 cm after feeding. The lower 5 cm were inoculated with sediment, but the upper 5 cm were not. Denitrifying microorganisms in the un-inoculated Hanford sediment could have decreased apparent denitrification rates.

4.3 Bio-immobilization experiment with GW835 groundwater: "The Laboratory Column"

The maximum ethanol utilization rate was 1.34 mM/day and the maximum reduction rates were: NO_3^- at 0.81 mM/day, U(VI) at 0.5 μM/day, and Tc(VII) at 57 pM/day. Though the rates of Tc(VII) and NO_3^- reduction, and ethanol utilization appear to be much smaller than those measured in the laboratory chamber, the concentrations of these solutes in the two groundwaters used were quite different (Table 13).

Table 13. Species concentration in GW835 and FW21 groundwaters.

Species	GW835 Solution	FW21 Solution
Tc(VII)	370 pM	12,200 pM
NO_3^-	5.5 mM	120 mM
EtOH	10 mM	100 mM

The hydraulic conductivity of the laboratory column decreased rapidly after ~ 11 weeks, which made it difficult to maintain a constant flow rate. Upon completion of the column experiment, the tin foil was removed and the column was visually inspected. The sediment had changed from a rust brown to a light grey color, which suggests that Fe(III) had been reduced to Fe(II). Fe(III) reducing conditions in the sediment and measurable ethanol utilization rates suggest that metal reducing microorganisms associated with the sediment were capable of enzymatically reducing U(VI) and Tc(VII). The speciation of U(VI) in the groundwater was primarily $UO_2(CO_3)^{2-}$, thus sorption of U(VI) to column sediment was likely not significant. Decreases in U(VI) concentrations were therefore most likely due to increased biomass, microbial activity, and precipitate deposition.

4.4 Sorption Experiments

4.4.1 Batch Experiments with Hanford Sediment

Sorption experiments were performed to ensure that the decreases in uranium concentration observed in the laboratory column were due to reduction of U(VI) to immobile U(IV) precipitates rather than sorption of U(VI) to the sediment. Four different batches of Hanford sediment were prepared for this experiment. As both FW21 and GW835 groundwaters were used in systems packed with Hanford and FRC sediments, it was important to evaluate the effect of the bicarbonate additions to both waters in contact with the sediment mixtures. This experiment had two objectives. The first was to determine if bicarbonate additions were effective in preventing sorption of U(VI) to the sediments in the laboratory column and chamber experiments. The second was to determine whether or not sorbed U(VI) might be present in the FRC sediment used to pack the columns.

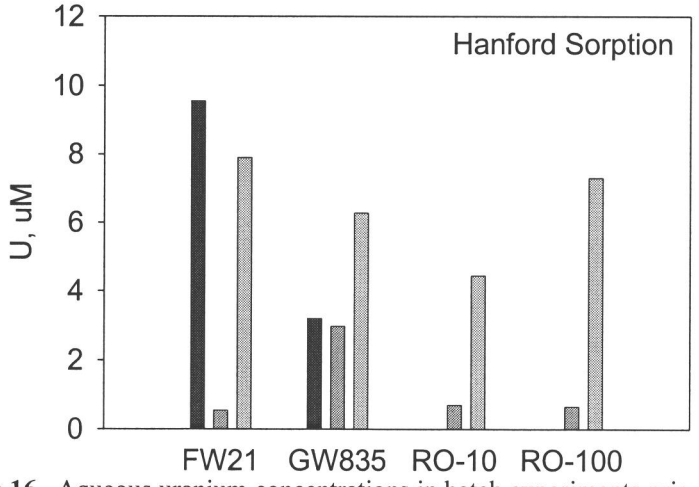


Figure 16. Aqueous uranium concentrations in batch experiments prior to contacting with sediment (▬), after contacting with sediment (▬), and after bicarbonate addition (▬). RO-10 and RO-100 represent reverse osmosis treated water with 10 mM and 100 mM bicarbonate additions, respectively.

Uranium concentrations decreased upon initial contact with the soil, presumably due to sorption (Figure 16). While very little sorption was observed with GW835 groundwater, ~ 94% of uranium sorbed to the sediment when FW21 groundwater was used (Figure 16). Uranium concentrations increased when bicarbonate-containing RO water was added to the sediment and resulted in a desorption of ~ 1.14 μg/g of uranium from the sediment.

100 mM bicarbonate reclaimed nearly 87.6% of the sorbed uranium from the FW21 sample. 10 mM bicarbonate proved to be a powerful extractant of sorbed U(VI), removing 10.2 μg/g of uranium from the GW835 soil sample. Extraction of previously sorbed uranium was also noted in both the RO with 10 mM and 100 mM bicarbonate concentrations. The RO samples indicate that 100 mM bicarbonate is a more powerful extractant than the less concentrated 10 mM. This is because additional bicarbonate in solution drives the uranium speciation in solution to contain primarily uranium-carbonate complexes, which are soluble in solution (Figure 3). Why then does the opposite seem to be true in the FW21 and GW835 groundwater samples?

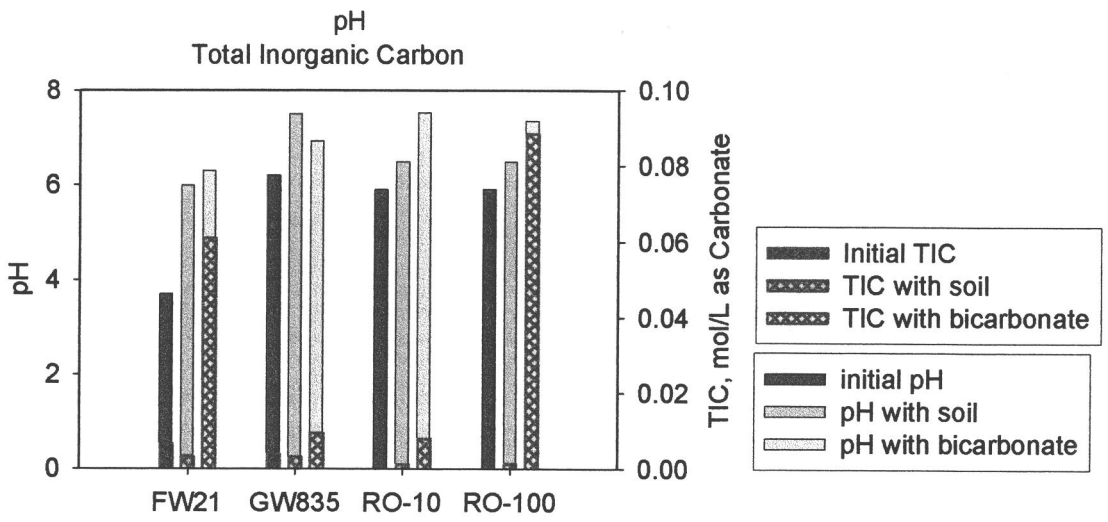


Figure 17. pH and TIC concentrations prior to contacting four solutions with sediment, after contacting with sediment, and after bicarbonate addition.

FW21 groundwater initially has ~ 1.7 times the total inorganic carbon (TIC) of the GW835 sample, and a lower pH as well (Figure 17). The low pH of FW21 water could dissolve carbonate from the aquifer sediments, and thus result in the higher TIC concentration. Under the initial conditions of FW21 groundwater (pH 3.7, TIC ~ 6.7 x 10^{-3} M), uranium is present as the uranyl ion (UO_2^{2+}) (Figure 2). This species has a high affinity for sorption to the negative surfaces of soil in the batch experiment. The initial conditions of GW835 water (pH 6.1, TIC ~ 5 x 10^{-3} M) resulted in the net negative $UO_2(CO_3)^{2-}$ ion dominating uranium speciation. The negative charge results in a low affinity for sorption to batch sediments. This difference in uranium speciation likely accounts for the significant difference in the amount of sorption from FW21 and GW835 groundwaters.

4.4.2 Sorption to Limestone in pH Adjusting Column

Samples were collected from the influent and effluent during the 70th pore volume of the pH adjusting column and were analyzed for uranium. A 98% decrease in uranium concentration was measured, which was clearly not due to bio-reductive precipitation as no electron donor was added. Decreased concentrations may have been caused by sorption of the uranyl ion to the limestone near the inlet or by co-

precipitation with calcite, aragonite, or aluminum hydroxides (38). It is important to distinguish sorption of U(VI) from reductive precipitation of U(IV), as the former may be less stable.

4.4.3 Batch Experiments with Limestone and Hanford

Limestone sorbed much less U(VI) upon initial contact than the Hanford sediment in batch experiments (Figure 18). 100 mM bicarbonate addition was also less effective at extracting U(VI) from the limestone than from the Hanford sediment (Figure 18). This could indicate that pH adjustment by the limestone results in unique sorption properties. Calcium carbonate may, for instance, re-crystallize as calcite upon equilibration with the pH adjusted groundwater. This could result in incorporation of U(VI) into the calcite layer, which may be less easily extracted.

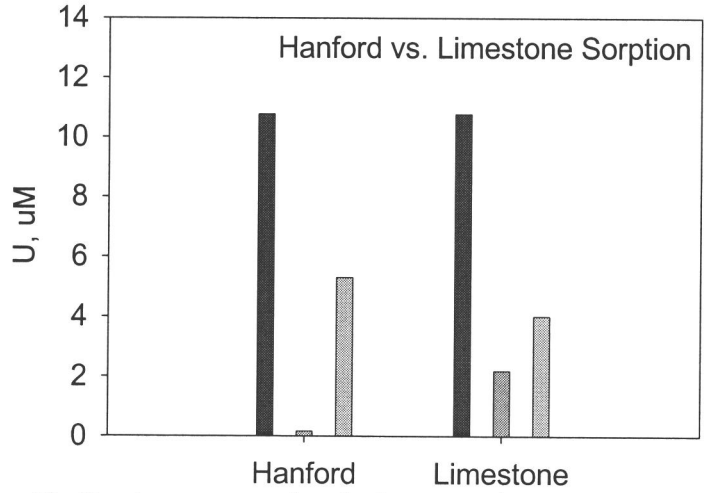


Figure 18. Uranium concentrations in the water prior to contacting with sediment (▬), after contacting with sediment (▬), and after bicarbonate addition (▬).

4.5 Reoxidation of U(VI) by NO_3^-

The GW835 feed solution (Table 14) was pumped through the previously stimulated laboratory column for 76 hours. Uranium concentrations remained low (less than 0.01 μM) for the first 75 hours of the experiment (Figure 19).

Table 14. GW835 feed solution

Species	SO_4^{2-}	NO_3^-	Ethanol	U(VI)
Concentration	0.94 mM	5.6 mM	10 mM	2.98 μM

Concentrations in the column effluent never reached those in the feed solution, which indicated that the microorganisms in the column sediment utilized the ethanol and nitrate quite rapidly. The downward sloping trend of the ethanol and sulfate concentration profiles after 21 hours indicates that the rates of transformation increased as additional feed solution was supplied.

However, upon switching the influent to tap water with 120 mM nitrate concentration, U(VI) concentrations rapidly increased to 0.5 μM (Figure 19). The spike in nitrite concentration indicated that microorganisms in the column were coupling oxidization of ethanol, other reduced carbon sources, or U(IV) to nitrate reduction. U(VI) concentrations begin to increase concomitantly with nitrite production, which suggests that microorganisms may have been coupling the reduction of nitrate to the oxidization of U(IV) to soluble U(VI). The U(VI) concentration decreased, as did nitrite, until 87 hours.

The final sample was collected at 100 hours. The nitrite concentration remained low but the U(VI) concentration increased to 0.75 μM. This could indicate that microbially mediated oxidation of U(IV) in the presence of high nitrate was faster than chemical oxidation of U(IV) by nitrate alone.

The peak in ethanol and sulfate concentrations at time 20 hours signifies the feed solution break through, which implies that the residence time in the column was ~ 20 hours. This is much longer than the ~ 25 minute residence time calculated in the first weeks of the experiment. The increase is likely due biomass clogging the pore space. The peristaltic pump used in this experiment was unable to compensate for the additional head loss and therefore, effluent flow rates were much smaller and sporadic.

Gasses produced during denitrification or fermentation likely contributed to clogging of the column. These gas 'pockets' were apparently passed during the column feeding, which resulted in a higher hydraulic conductivity. The feed solution was changed to the high nitrate tap water at 76 hours. Less than one hour later, the effluent contained elevated uranium, nitrite, and nitrate concentrations. This indicates that the residence time decreased from ~ 21 hours to less than 1 hour.

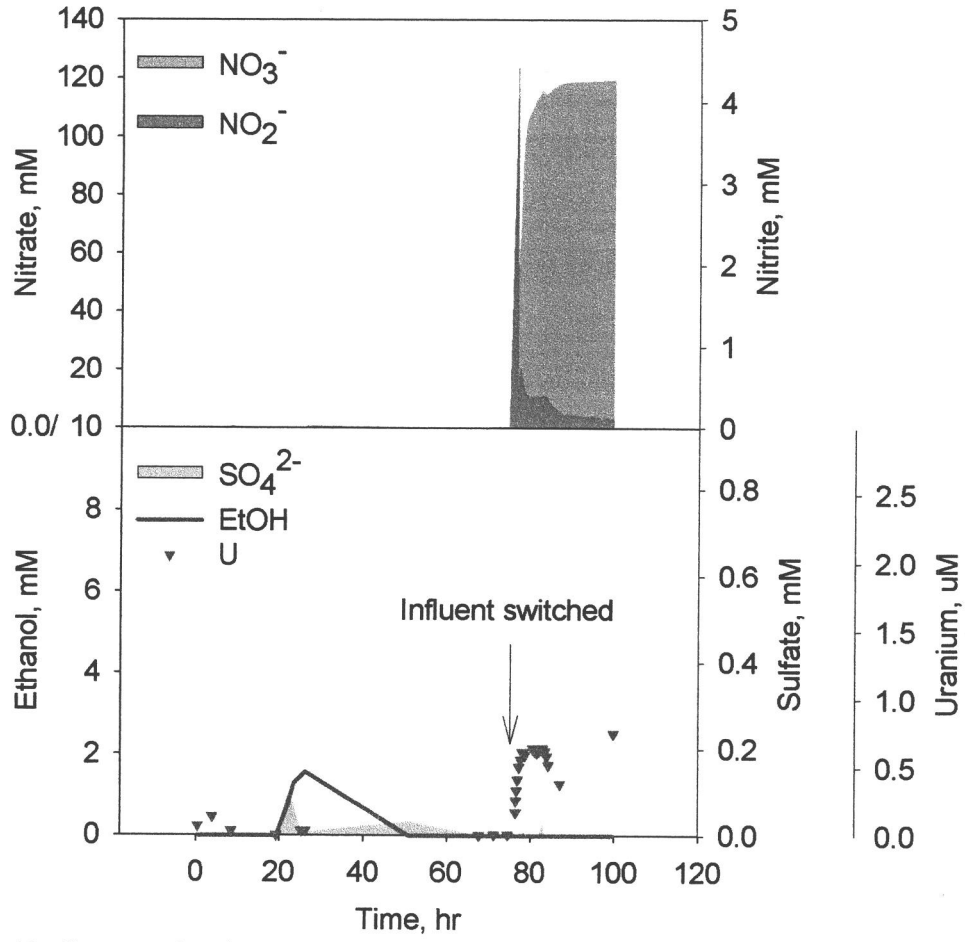


Figure 19. Concentrations in the column effluent measured over time. The arrow indicates the time when the influent was switched from the feed solution to 120 mM nitrate tap water.

4.6 Bio-immobilization experiment with GW835 groundwater: "The Area 2 Physical Model"

Although data was collected three times per week, three sets of concentration profiles were chosen to represent model performance during the first, third, and fifth week of operation (Figures 20 – 22 (p. 55 – 57)). The concentration profiles for the first week (Figure 20) show that nitrate concentrations decreased from 1.1 to ~ 0.55 mM. Nitrite concentrations increased from 0.03 mM to a maximum of 0.26 mM and ethanol concentrations decreased slightly from 44 mM near the inlet to 37 mM near the outlet. Concentrations of Tc(VII) and U(VI) both decreased by more than 83 % (Figure 20). Sulfate concentrations were highest near the outlet, which could be due to flushing of sulfate from the column. Whereas the inflowing groundwater contained

sulfate concentration of ~ 1 mM, the maximum concentration in the column was ~ 4 mM. The Maynardsville Limestone and FRC Background Area sediment used in the column pack are possible sources of elevated sulfate concentrations. The limestone and FRC sediment are presently being analyzed for sulfate and other chemical constituents.

Sulfate concentrations decreased to less than 1 mM by the third week of sampling (Figure 21). Tc(VII) concentrations changed only slightly, but nitrate, U(VI), and nitrite concentrations decreased significantly. Measured ethanol concentrations were highest in the inlet (~ 180 mM) as neat ethanol is injected at this location and at five additional locations throughout the model. Lower ethanol concentrations throughout the column may support more efficient utilization of ethanol for the reduction of nitrate and denitrification intermediates. More efficient utilization may help support complete denitrification, thereby avoiding the accumulation of the potentially toxic intermediate nitrite and potentially reoxidation of U(IV).

Ethanol utilization rates appear to be slightly higher during week five (Figure 22). Nitrate concentrations were reduced to zero and nitrite concentrations remained low. Peaks in sulfate, U(VI), and Tc(VII) concentrations were measured in water extracted from the sample port located 50 cm from the inlet. The ethanol concentrations at this location are quite low, and thus microorganism may have coupled the reduction of nitrate to the oxidation of U(IV), or other reduced precipitates in the sediment. Re-solubilization of reduced precipitates could explain the increased concentrations in the vicinity of this port, though this seems unlikely with such low nitrate concentrations.

U(VI) concentrations continuously decreased within the model, though Fe(II) production was not detected. Sorption could explain the decreased U(VI) concentrations. Substantial sorption of U(VI) to the sediment was not anticipated as U(VI) speciation, under the conditions of this experiment, was dominated by the highly soluble carbonate complex $UO_2(CO_3)_2^{2-}$ (Figure 3). Also, it is difficult to predict U(VI) sorption from GW835 groundwater to limestone because this combination was not previously tested. U(VI) sorption from GW835 was tested in

batch experiments using FRC and Hanford sediment only. Additional testing will be required to characterize sorption of U(VI) from GW835 groundwater to both the Maynardsville Limestone and FRC Background Area sediment.

The absence of detectable Fe(II) is not unequivocal evidence that Fe(III) reduction is not occurring. The sulfate concentration trends from week one to week 6 (data not shown), and the week 5 concentration profiles specifically (Figure 22), indicate that sulfate reduction was occurring in the model sediments. Thus, sulfide produced in model may have reacted with aqueous Fe(II) and precipitated it from solution.

The column flow rate was fairly stable at ~ 2.5 mL/min, though there were some issues with clogging of the outlet early on. Clogging was caused by outlet fittings rather than column dynamics. The average hydraulic conductivity of the column sediment remained relatively constant at ~ 0.018 cm/sec during the first weeks of operation (Figure 23).

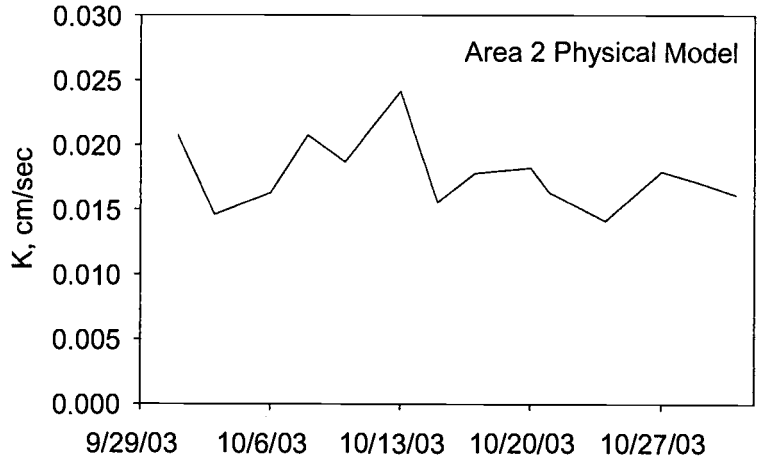


Figure 23. Hydraulic conductivity of the Area 2 physical model.

4.7 Bio-immobilization experiment with FW21 groundwater: "The Area 1 Physical Model"

Three sets of concentration profiles were chosen to represent model performance during the first, seventh, and eleventh week of operation (Figures 24 – 29 (p. 58 – 65)). The Area 1 physical model was equipped with two rows of sampling

ports, one row located in the upper portion of the saturated region (upper flow path) and another in the lower portion of the saturated region (lower flow path) (Figure 10). Each concentration profile was divided into two figures to represent data from the upper and lower flow paths separately.

Week One. It is important to note that some dilution of the FW21 groundwater used to saturate the model may have occurred during week one (Figures 24, 25 (p. 58, 59)). The model was leak tested with uncontaminated well water prior to packing. Although this water was partially removed, ~ 12 L remained in the bottom of the model. This volume represents ~ 11 % of the total pore water that was present during the initial, no-flow saturation phase. By the first week, 28 L (or ~ 26 % of the total pore volume) of FW21 groundwater had been pumped through the system.

Tc(VII) concentrations were significantly higher in the upper flow path (Figure 24) than in the lower flow path (Figure 25). Dilution effects may explain the differences in Tc(VII) concentrations between flow paths.

Uranium concentration profiles for the upper and lower flow paths were also different. This may have been caused by incorporating different amounts of stimulated sediment in each layer when packing the model. As the sorption experiments demonstrated, sorbed U(VI) was present in the FRC sediment. Therefore, if more FRC sediment was incorporated into the lower portion of the sediment pack, desorption of U(VI) from this sediment could result in initially higher U(VI) concentrations in the lower flow path.

Different amounts of stimulated FRC sediment in the upper and lower flow paths may also explain the nitrate concentration profiles. The stimulated sediment was supersaturated with 120 mM nitrate and ethanol containing solution during incubation. If the lower flow path was inoculated with more stimulated sediment, higher nitrate concentrations could have resulted. The influent water could have offset the higher concentrations by diluting the nitrate, to result in similar nitrate concentrations in the upper and lower flow paths.

Sulfate concentrations were higher in the lower flow path during the first week. Again, this difference in concentration could result if the lower layer was inoculated with more stimulated sediment and if this sediment contained high sulfate

concentrations. The pore water initially present in the model could have contained elevated sulfate concentrations as well. No samples were taken from the water, thus the sulfate concentrations are not known. However, both the Maynardsville Limestone and FRC sediment are presently being analyzed for sulfate.

The initially faulty ethanol injection pump likely caused the increased ethanol concentrations in the upper layer near the inlet. Higher ethanol concentrations could explain the higher nitrite concentrations in this region as well.

In summary, the concentration profiles for the first week of model operation suggests that more stimulated FRC sediment was likely placed in the lower flow path. Concentration profiles also indicate that the water initially present in the model diluted the FW21 groundwater solution used to saturate the model.

Week Seven. By the seventh week, the concentration profiles for the upper and lower flow paths remained dissimilar (Figures 26, 27 (p. 60, 61)). Rates of nitrate, Tc(VII), and U(VI) reduction in the different flow paths were calculated using measured concentrations along the model prior to any increases or rebound, or within the first ~ 160 cm (Table 15).

Table 15. Pseudo-first order reduction rates in upper and lower flow paths during week 7 (units of day^{-1}).

Species	Upper path	Lower path
Nitrate	0.33	0.76
Tc(VII)	0.22	0.28
U(VI)	0.12	0.03

Nitrate and Tc(VII) were both reduced but at a smaller rate in the upper flow path (Figure 26). Sulfate appears to have been flushed more completely from the upper flow path near the inlet but this trend reverses near the outlet. The sulfate and the Tc(VII) and nitrate trends suggest that residence times may be smaller in the upper flow path than the lower flow path.

U(VI) concentrations remained higher in the lower flow path, which could indicate that fewer pore volumes had passed through this region. In the case of sulfate, the initial concentration in the model was ~ 5 mM, whereas the sulfate concentration in FW21 was near zero. Thus, flushing effects are apparent. U(VI) concentrations in

FW21 range from 6 to 14 μM, which makes it difficult to distinguish between that which has accumulated or been flushed.

U(VI) may sorb to the limestone near the inlet during the pH adjustment process. If sorption of U(VI) to the limestone near the inlet is substantial, low U(VI) concentrations would be expected throughout the model. This is consistent with the concentration profile in the upper flow path (Figure 26). Higher U(VI) concentrations in the lower flow path near the inlet, similar to the sulfate concentration trends, suggests that fewer pore volumes were passed through the lower flow path.

Ethanol appears to have been completely utilized near the center of the model (Figures 26, 27). Nitrite concentration was reduced to zero at this location as well but increased near the outlet where additional ethanol was present. This trend suggests that nitrate and Tc(VII) reduction rates were likely limited by ethanol concentrations in this region.

Weeks Ten and Eleven. Apparent Tc(VII) and nitrate concentrations decreased slightly during week ten (Figures 28, 29 (p. 62, 63)). These rates were not calculated during week ten (due to the no-flow conditions discussed below). The more gradual concentration decreases could be due to changes in microbial communities in the model sediment. Sustained nitrite concentrations in the model (maximum concentration typically ~ 30 mM) may be gradually poisoning microorganisms, thereby reducing rates of Tc(VII) and nitrate reduction.

Ethanol concentrations were much lower in the lower flow path during the tenth week, while nitrite concentrations were higher in this region. Sulfate concentrations were slightly less in the upper flow path. Conversely, U(VI) concentrations were higher in the lower flow path.

Pumping of groundwater to the model was stopped for 5 days during the tenth week. Samples were taken and concentration profiles were generated for the upper and lower flow paths on the last day of non-flowing conditions (Figures 28, 29). On the following day, pumping resumed and the flow rate was increased to 8 mL/min. The last set of concentration profiles were generated using data collected on October, 29, five days after pumping resumed at the increased flow rate (Figures 30, 31 (p. 64, 65)).

When the flowing conditions resumed, the outlet flow rate measured 8.8 mL/min and the average saturated thickness was ~ 65 cm. The outlet clogged with sand during the next two days, during which time the average saturated head was ~ 85 cm. The outlet was unclogged when the problem was detected, and the average head was reduced to ~ 65 cm with a flow rate of 9.1 mL/min.

Approximately 65 L of FW21 groundwater were pumped through the model from October 23 (tenth week) to October 29 (eleventh week). Rates of nitrate, Tc(VII), and U(VI) reduction in the different flow paths were calculated using concentrations along the model prior to any increases or rebound (Table 16).

Table 16. Pseudo-first order reduction rates in upper and lower flow paths during week 11 (units of day^{-1}).

Species	Upper path	Lower path
Nitrate	0.18	0.44
Tc(VII)	0.24	0.29
U(VI)	2.04	1.56

Apparent Tc(VII) and nitrate reduction rates were much smaller, with a rebound in concentration near the outlet (Figures 30, 31). This was likely due to two factors. First, higher flow rates resulted in shorter residence times. Second, high concentration influent flushed some of the lower concentration porewater present during the no flow period from the model.

Conversely, U(VI) reduction rates appear to be higher during week eleven. The U(VI) concentration was reduced to near zero by the first sample port (~ 28 cm, or ~ one day). Again, Fe(III) reducing conditions were not detected in the model and nitrate concentrations are reduced to only ~ 100 mM. It is therefore improbable that reduced U(VI) concentrations are due to reductive U(IV) precipitation.

Sulfate appears to be nearly completely flushed from the model during week eleven. Slightly higher concentrations were detected in the lower flow path. Ethanol concentrations were near zero near the inlet, which could be due to increased utilization rates or to a clogged or broken ethanol injection tubes.

The FW21 groundwater was adjusted from pH ~ 3.5 to ~ 7.5 within the model. Hydraulic conductivity of the model sediment appears to be relatively constant after August, 25 (Figure 32).

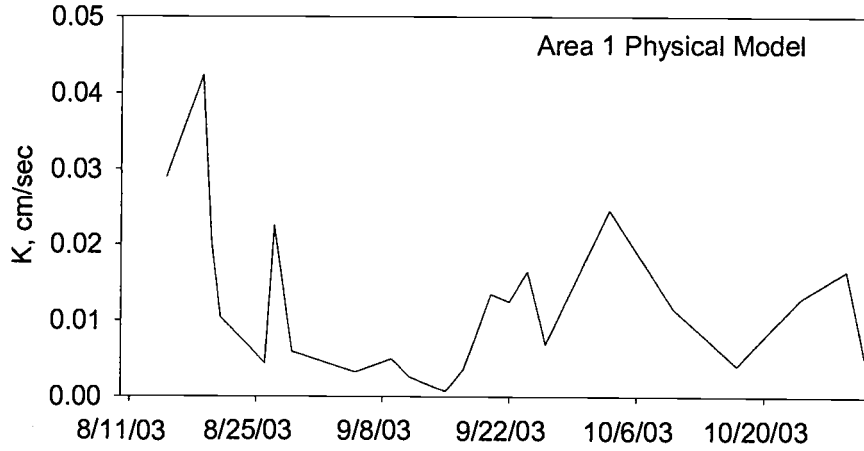


Figure 32. Hydraulic conductivity of the Area 1 column.

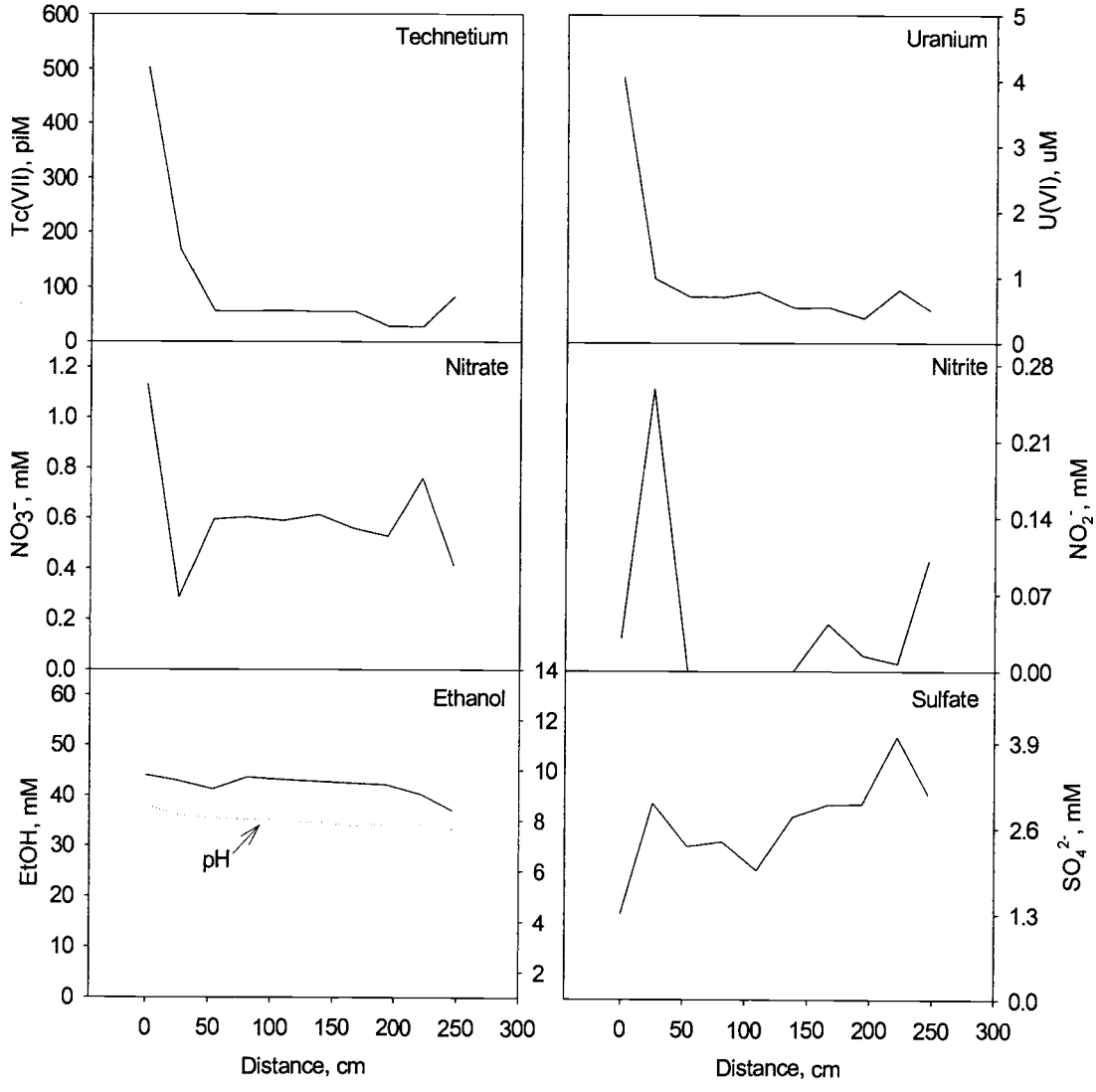


Figure 20. Area 2 column concentration profiles. Data measured 20 Sept. 2003 during first week of flowing conditions.

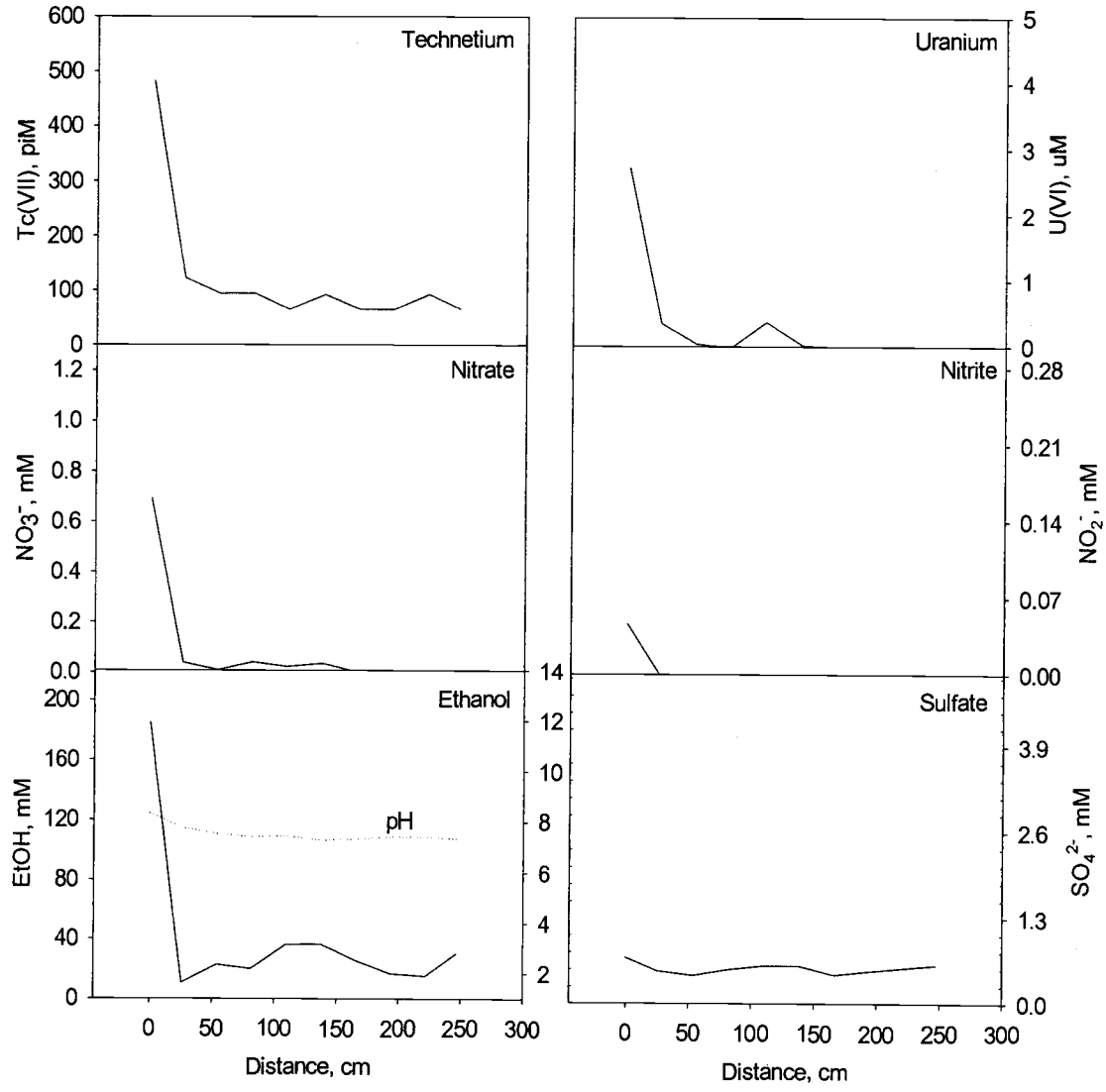


Figure 21. Area 2 column concentration profiles. Data measured 8 Oct. 2003 during third week of flowing conditions.

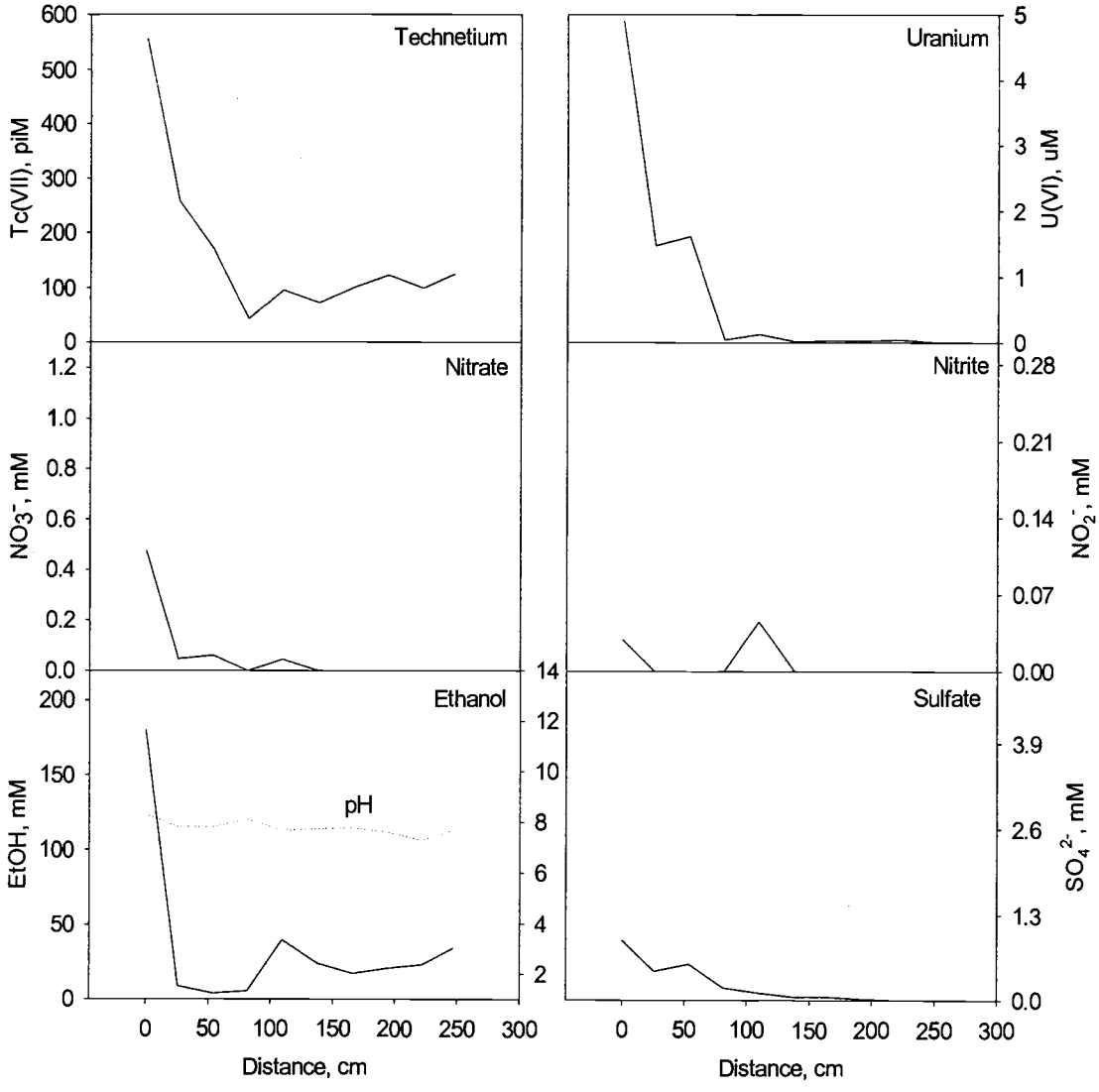


Figure 22. Area 2 column concentration profiles. Data measured 24 Oct. 2003 during fifth week of flowing conditions.

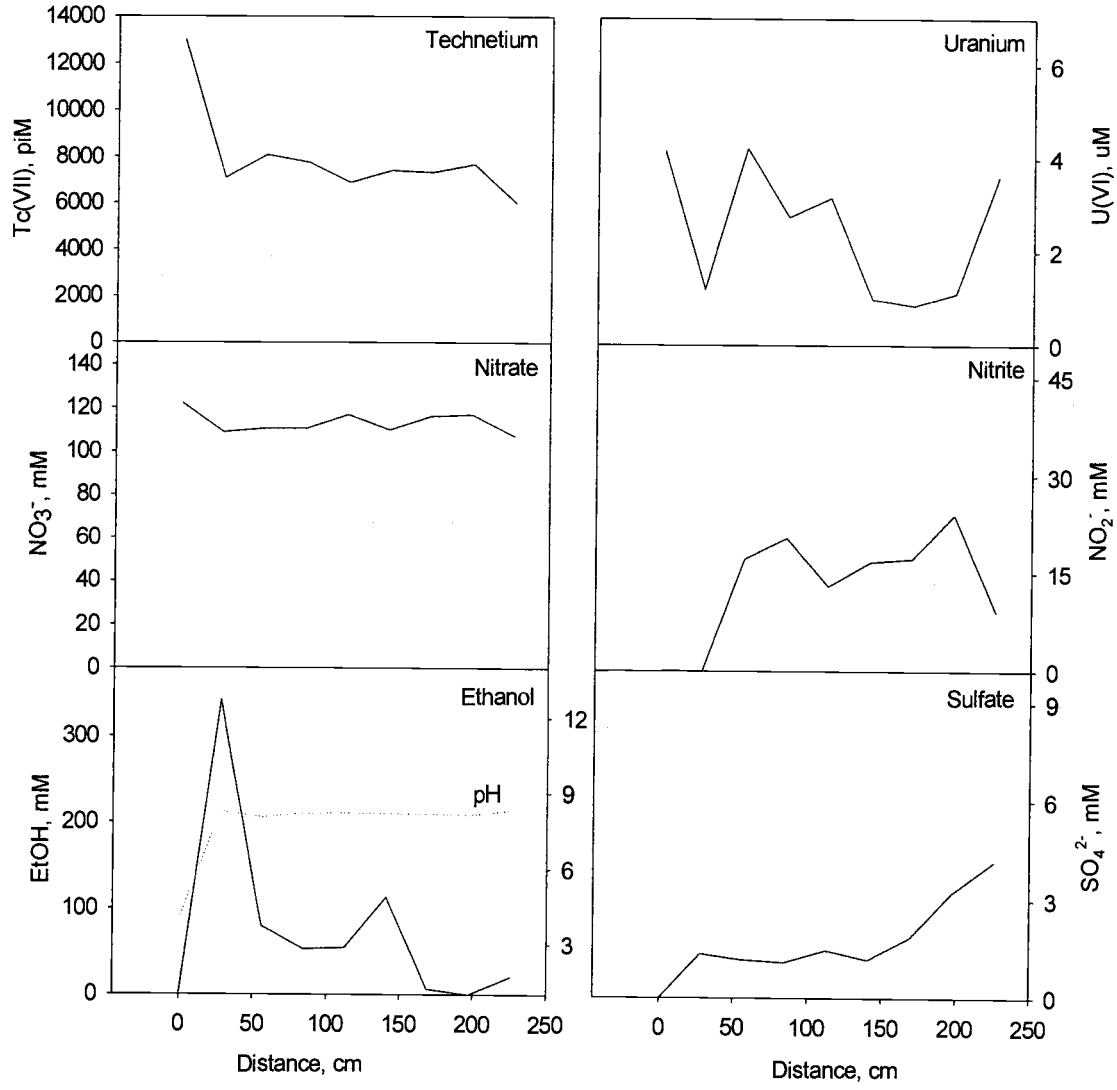


Figure 24. Area 1 model concentration profiles (Upper flow path). Data measured 19 Aug. 2002 during first week of flowing conditions.

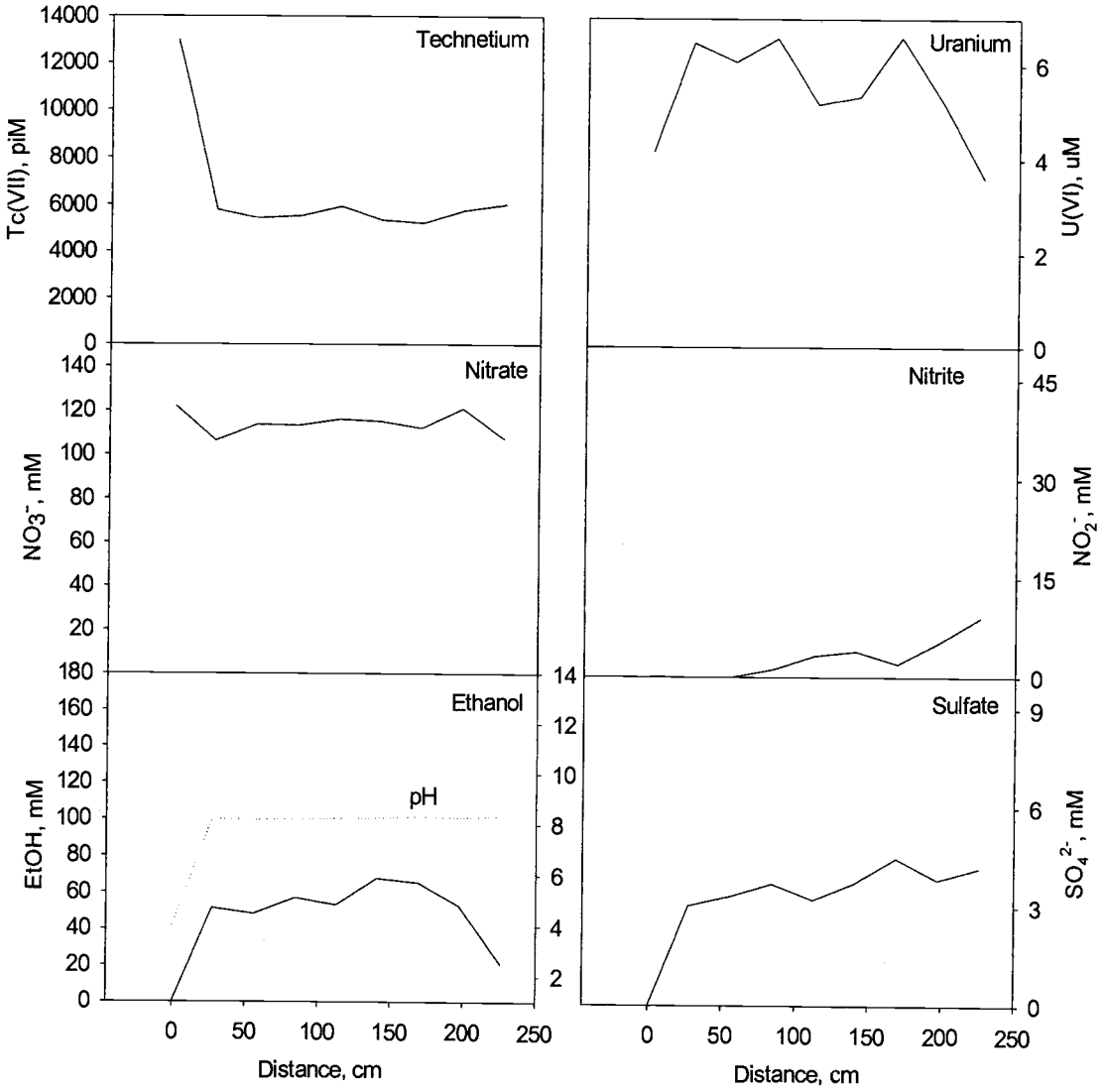


Figure 25. Area 1 model concentration profiles (Lower flow path). Data measured 19 Aug. 2002 during first week of flowing conditions.

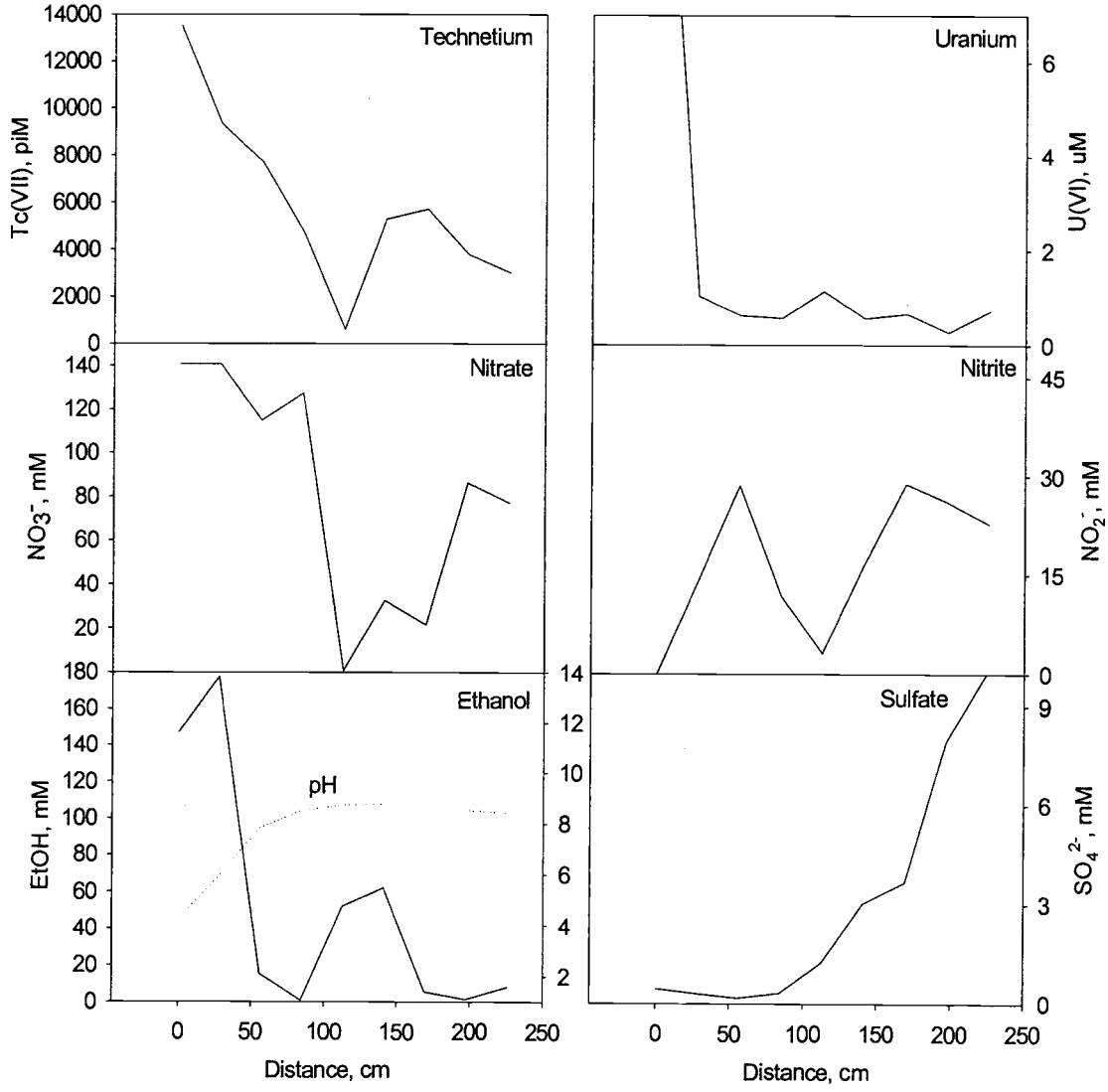


Figure 26. Area 1 model concentration profiles (Upper flow path). Data measured 16 Sept. 2003 during seventh week of flowing conditions.

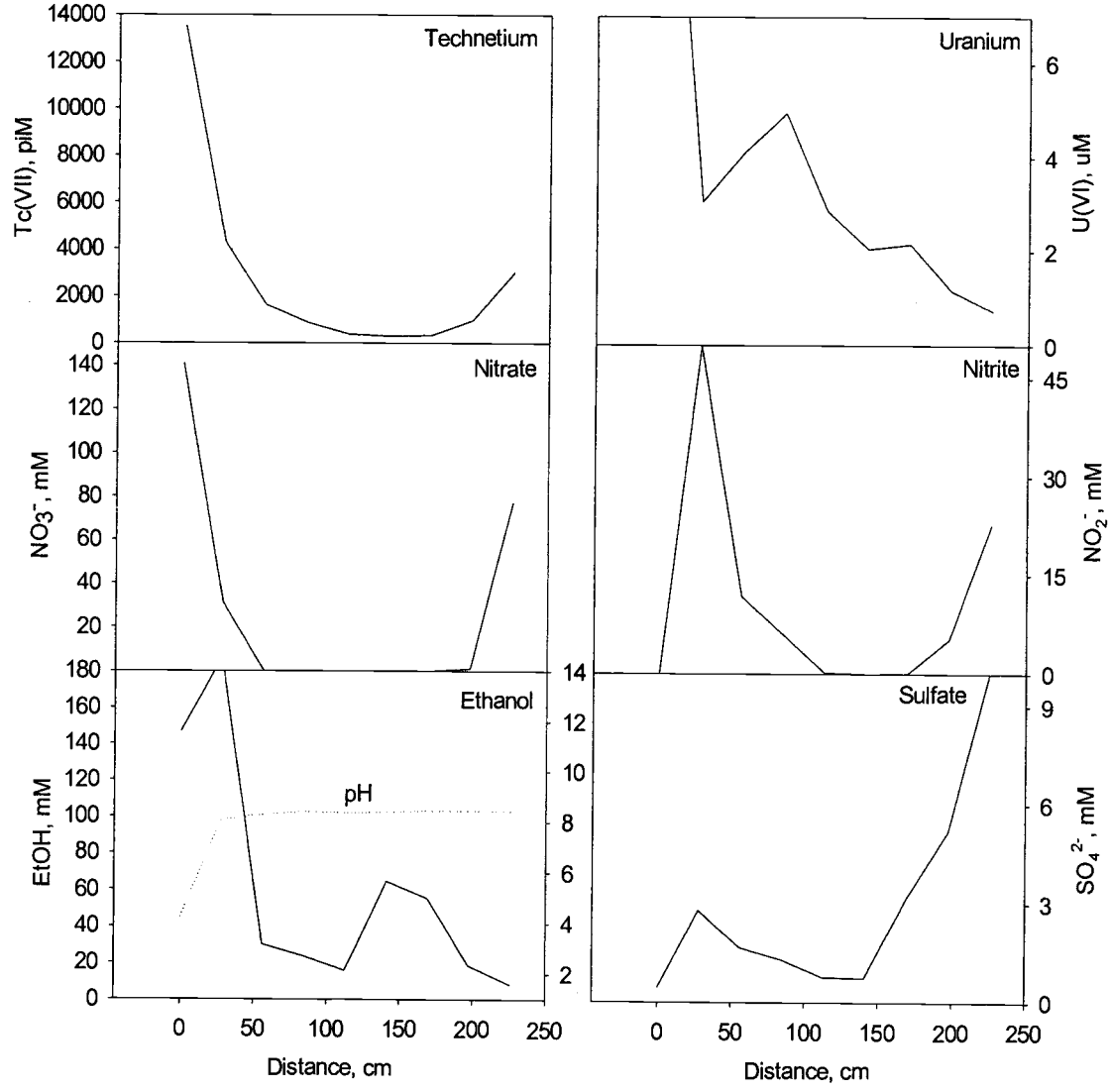


Figure 27. Area 1 model concentration profiles (Lower flow path). Data measured 16 Sept. 2003 during seventh week of flowing conditions.

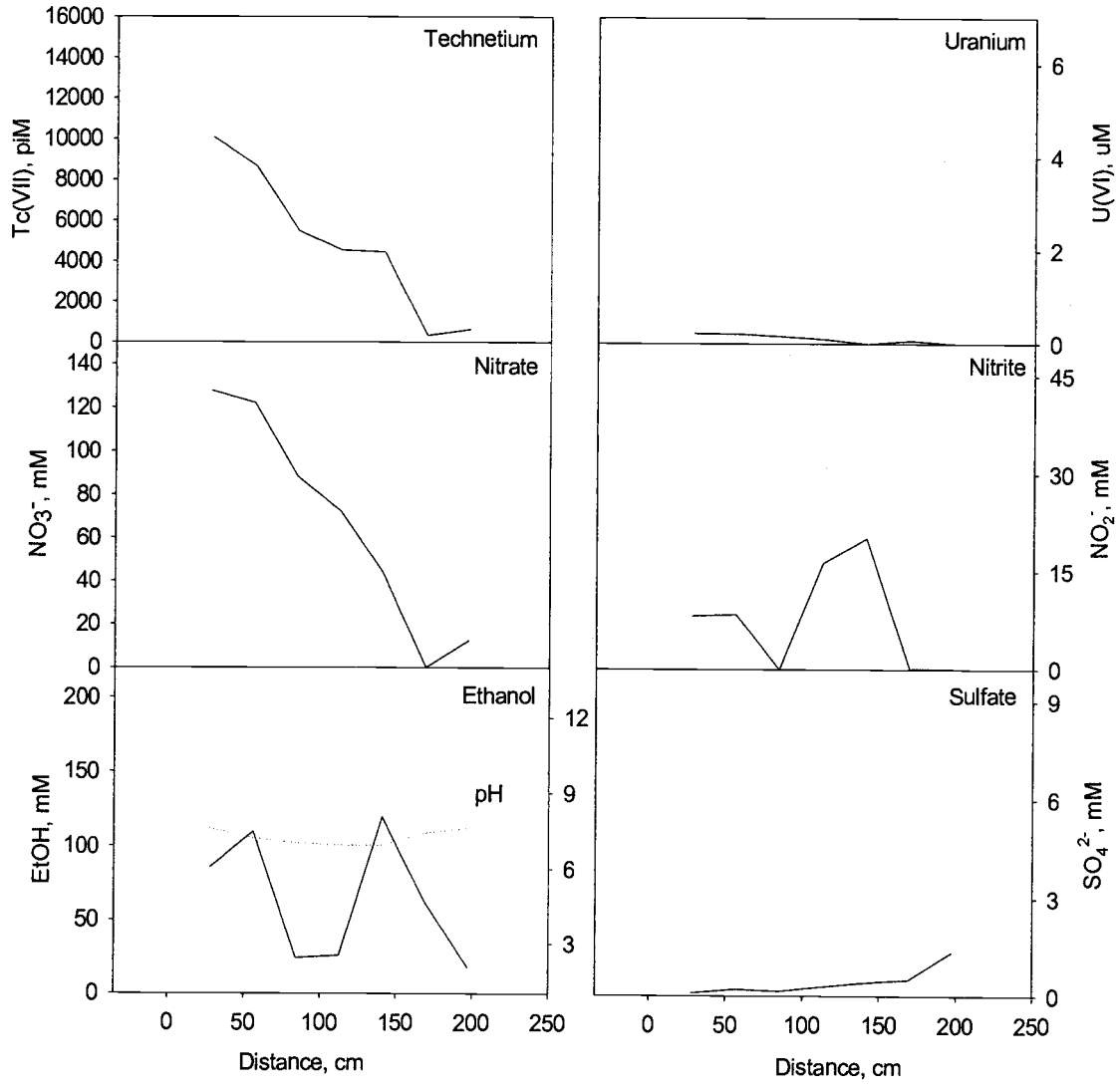


Figure 28. Area 1 column concentration profiles (Upper flow path). Data measured 23 Oct. 2003 during tenth week of flowing conditions.

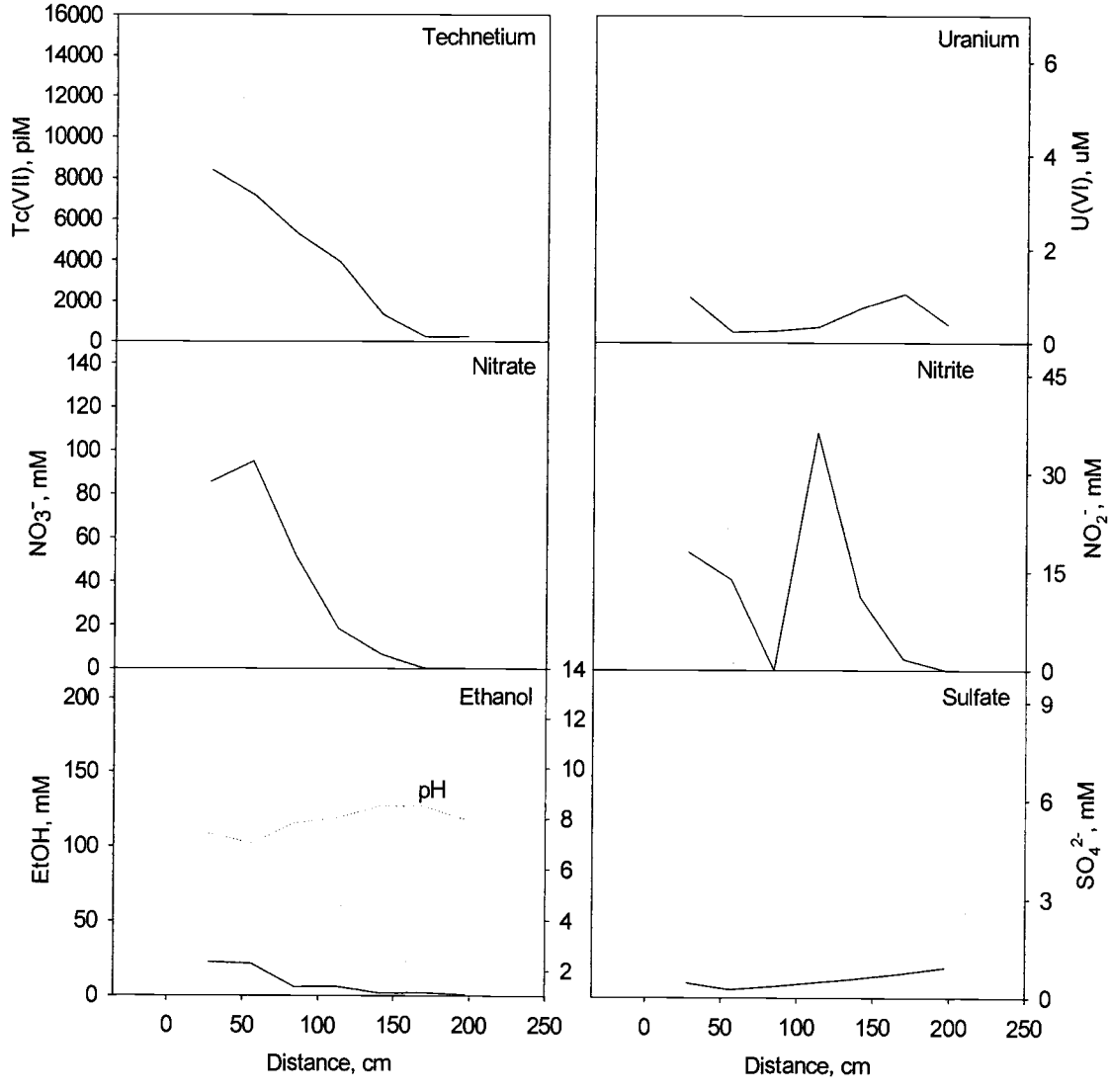


Figure 29. Area 1 column concentration profiles (Lower flow path). Data measured 23 Oct. 2003 during tenth week of flowing conditions.

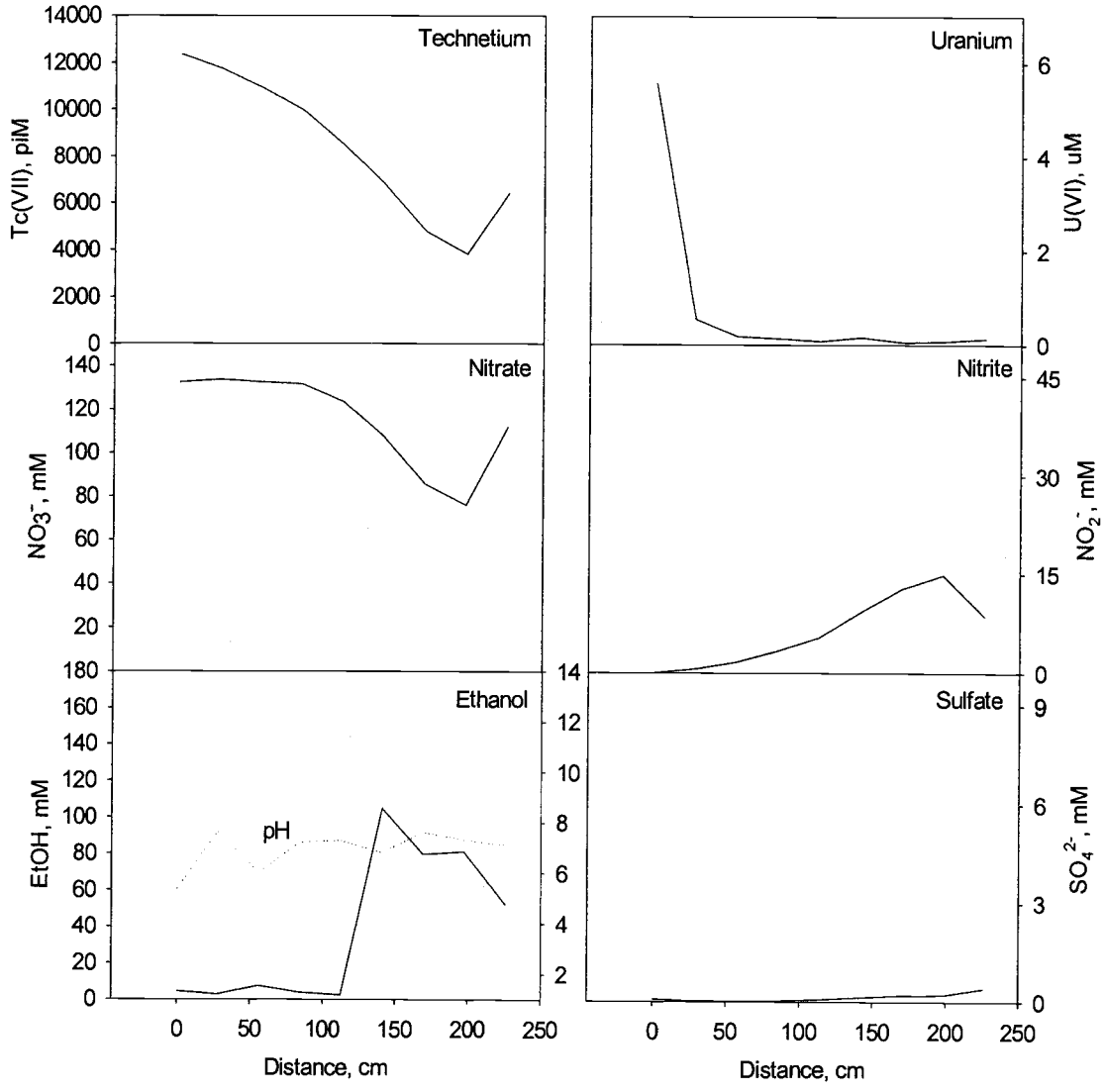


Figure 30. Area 1 model concentration profiles (Upper flow path). Data measured 29 Oct. 2003 during eleventh week of flowing conditions.

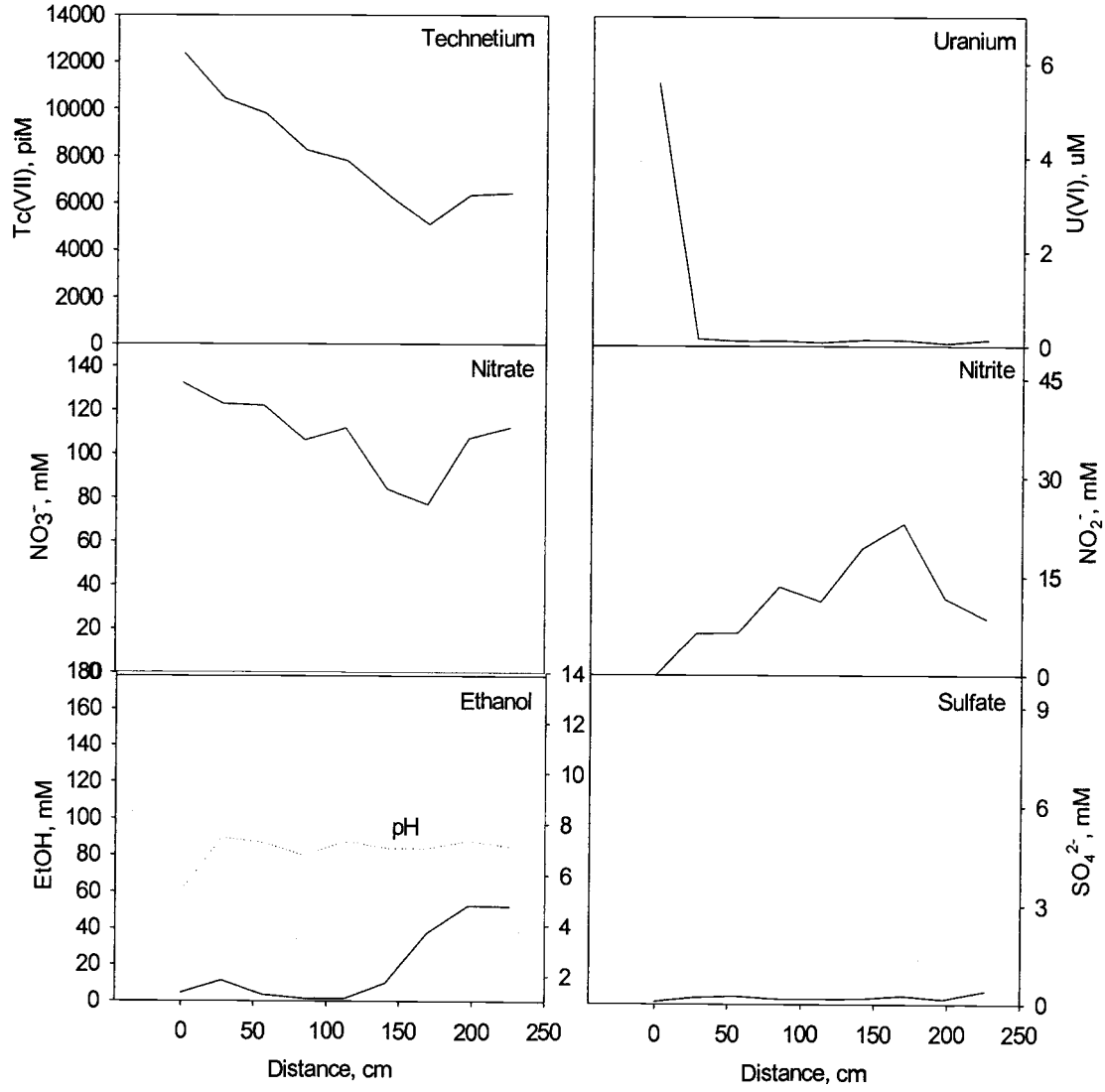


Figure 31. Area 1 model concentration profiles (Lower flow path). Data measured 29 Oct. 2003 during eleventh week of flowing conditions.

5.0 CONCLUSION

5.1 In situ treatment of FW21 and GW835 groundwaters is possible.

Laboratory column studies demonstrated that the pH of FW21 groundwater can be adjusted from ~ 3.7 to 7 – 8 in packed sediment layered with bicarbonate. Information obtained through the experiments presented in this thesis was used to estimate the mass of precipitate formed and deposited in the model from September, 15 to October, 29. Given that ~ 21.5 mg of precipitate forms per 1.12 L of FW21 groundwater when adjusted to pH ~ 7 with 0.2 N $NaHCO_3$ (Figure 12), the total amount of precipitate formed and deposited in the model sediments during this time was estimated to be ~ 6.6 g. Using the precipitate density value of ~ 1.02 g/mL (section 4.1.1), the portion of pore space filled with precipitates was estimated to be ~ 0.0065 L. Thus, presently less than 0.1 % of the total pore volume within the model likely contains precipitates.

The laboratory column transport experiment study showed that some portion of precipitate may be transported through packed sediment. The column experiment had a residence time of ~ 30 minutes and a total pore volume of only 74 mL, whereas the residence time in the Area 1 physical model is on the order of weeks and the total pore volume is ~ 110 L. Thus ample time is provided for precipitate entrainment.

Column studies also established a correlation between deposition of precipitate mass and decreased hydraulic conductivity (Figure 13). The study showed that when 4 g of precipitate accumulated in the pore space, which was estimated to account for ~ 5.3 % of the pore space in the laboratory column, the hydraulic conductivity decreased significantly. If a linear correlation exists between the percent of pore space filled and time (or pore volumes) until clogging occurs, it would take an estimated 191 years for the Area 1 physical model to clog. This suggests that in situ pH adjustment of FW21 groundwater will likely not be the inhibitive aspect of FW21 bio-immobilization treatment. As demonstrated in the 'laboratory column experiment', biomass accumulation and or trapped gasses could be the primary causes of much larger decreases in hydraulic conductivity.

The laboratory chamber experiment demonstrated that nitrate and Tc(VII) reduction are possible in stimulated sediments using ethanol as the electron donor. Nitrate and Tc(VII) were reduced concomitantly as expected based on redox potentials (Figure 4). The nitrate reduction rates observed in the Area 1 physical model were 43 % higher and Tc(VII) reduction rates were ~ 300 % higher than those measured in the laboratory chamber.

Fe(II) production was not measured in the small laboratory column, though it was detected qualitatively as the sediments changed from a rusty brown to a light grey color. Though Fe(II) was not measured, U(VI) reduction to insoluble U(IV) did occur. This was demonstrated during the "Re-oxidation with NO_3^- column experiment" (Section 2.7). U(VI) and ethanol containing water was passed though the previously stimulated column for 75 hours, during which time U(VI) concentrations were reduced from ~ 3 μM to near zero. Only when the influent was switched to tap water containing 120 mM nitrate were increased U(VI) concentrations detected. Though the mechanism responsible for the re-oxidation of U(IV) has several possibilities, it seems quite clear that the elevated U(VI) concentrations were due to re-oxidation of previously reduced U(IV).

By the same token, although Fe(II) production was not measured in the Area 2 physical model, sulfate reducing conditions demonstrate that the decreased U(VI) concentrations are likely due to bio-immobilization rather than sorption. Redox conditions within the Area 2 model sediment can support U(VI) reduction (Figure 4). The U(VI) reduction rates observed during the fifth week of Area 2 model operation were 50 % higher than those measured in the laboratory column.

5.2 Microbial Activity in Ethanol Amended FRC Sediments

Microorganisms within the model sediments couple oxidation of ethanol (and likely fermentation products) to the reduction of electron acceptors in order of their energetic potential, beginning with oxygen (Figure 31). When oxygen is no longer present in solution, facultative microorganism respire with the next most energetically favorable electron acceptors nitrate and Tc(VII). Strictly based on redox potentials,

Tc(VII) reduction should proceed prior to nitrate reduction. In both Area 1 and Area 2 physical models, nitrate and Tc(VII) reduction were observed to occur concomitantly.

Mn(IV) serves as the next most favorable electron acceptor after nitrate and Tc(VII). Mn(IV) concentrations in the Maynardsville Limestone and FRC Background area sediment are presently being analyzed. Mn(II) production has not been measured in the intermediate-scale physical models but will be in the future.

After Mn(VI), Fe(III) and U(VI) become favorable electron acceptors. Fe(III) reducing conditions indicate that the metal reducing organisms capable of U(VI) reduction are active. Thus, detection of Fe(II) in solution indicates that redox conditions within the saturated sediment can support U(VI) reduction. Though no Fe(II) production has been measured in the Area 2 physical model, sulfate reducing conditions have been observed. Thus any Fe(II) present in solution likely forms insoluble Fe(II)-sulfide precipitates. In the Area 1 physical model, nitrate and Tc(VII) were reduced to near zero in week seven. Fe(III) reduction could have occurred based on redox potentials during that time, though no Fe(II) production was detected. Any Fe(II) formed during week seven of area 1 model operation could have formed insoluble Fe(II)-carbonate species.

Finally, sulfate reduction has been measured in the Area 2 model but not in the Area 1 model. There are advantages to sustained sulfate reducing conditions. First, sulfide produced during sulfate reduction may directly reduce Tc(VII). Sulfide containing solid phases may also help control the redox conditions within sediment, helping to protect reduced U(IV) or Tc(IV) from re-oxidation.

Tc(VII) reduction can occur enzymatically or directly via sulfide or Fe(II)-containing magnetite. U(VI) reduction is a biologically mediated reaction that may be subject to reversal in the presence of nitrate or denitrification intermediates. Under Fe(III) or sulfate reducing conditions, U(VI) reduction may be directly coupled to ethanol oxidation (Figure 32). U(VI) reduction may be coupled to the fermentation of ethanol, which forms acetate, propionate, or benzoate. These fermentation products (especially acetate) may be oxidized as coupled to the reduction of U(VI). Fermentation is a process which yields little energy, which could potentially result in

more efficient microbial reduction of U(VI) with less bio-growth that can clog pore space. Fermentation products are suitable electron donors for sulfate reduction as well.

Microbial processes could also result in the reoxidation of U(IV). Lithotrophic organisms may couple the reduction of nitrate to the oxidation of Fe(II). Fe(III) is generated during this process which may chemically oxidize U(IV) (Figure 32). Lithotrophs may also couple the reduction of nitrate directly to U(IV) oxidation. Finally, nitrate may chemically oxidize U(IV) in reduced sediments but this reaction may occur more slowly than microbially mediated reactions.

5.3 Future Research

The laboratory pH adjustment column experiments and the bio-immobilization chamber and column experiments established that treatment of FW21 and GW835 groundwater in packed sediments is possible. The intermediate-scale models demonstrated that along with the increased size scale comes an increase in complexity. It was more difficult to pack the models uniformly, to distribute electron donor evenly, and even to interpret the data collected. These are important issues, which must be addressed before field scale bio-immobilization treatment schemes may be implemented. Seven research questions were developed during the course of this preliminary research. These questions summarize the next steps in characterizing the controlling processes in bio-immobilization barriers at the FRC.

(1) Could preferential flow paths control bio-immobilization barrier performance? Sulfate concentration profiles suggest preferential flow paths may exist as sulfate has apparently been flushed more extensively from the upper flow path than the lower flow path. Unidentified preferential flow paths could confound the interpretation of concentration profiles. Tracer tests are required to determine residence time distributions for each sampling port so that reaction rates may be determined accurately.

(2) Could observed decreases in U(VI) concentrations be due to sorption rather than to reductive precipitation? In laboratory columns, which were also packed

with layers of bicarbonate and limestone, 98% U(VI) removal was observed (assumedly due to sorption). This could indicate that U(VI)-carbonate complexes are co-precipitated with solids forming within the bicarbonate layers, or at the limestone interface. It is important to differentiate between sorption of oxidized U(VI) species and reductively precipitated U(IV) species as the former is potentially less stable. Additional batch and column studies are required to identify factors that control sorption of uranium.

(3) Is reduced U(IV) re-oxidized by NO_3^- or denitrification intermediates? Previous research has indicated that U(IV) is reoxidized during denitrification (Istok et al. (62), Finneran et al. (44)), findings which were corroborated by the results of the re-oxidation study presented in this thesis (p. 46). Identifying the causes and the rates of U(IV) reoxidation in the physical models could provide information critical to the efficacy and longevity of full scale bio-immobilization barriers.

(4) How will spatial gradients in microbial communities correlate with aqueous concentration profiles? Adaptation and function of microbial communities throughout the physical models likely control water chemistry dynamics. It is expected that acidophiles will likely dominate near the model inlet, followed by denitrifiers, and finally metal reducing microorganisms. Correlating the concentration profiles to microbial community structure and function over time could provide information about biogeochemical conditions that limit or control rates of NO_3^-, U(VI), and Tc(VII) reduction.

(5) Will reduced iron, sulfur, and manganese precipitates concentrate near the model outlet? Oxidized precipitates are likely concentrated near the inlet of the model due to the rapid increase in pH. As the conditions along the length of the model become more reducing, mineral precipitates containing reduced sulfur, iron, and manganese will form. These reduced precipitates can control redox conditions, which may help control the stability of U(IV) and Tc(IV) containing precipitates (38).

(6) Will hydraulic conductivity decrease significantly over time? Oxidized or reduced precipitates, microbial biomass, or nitrogen gas produced during denitrification may result in decreased hydraulic conductivity of the sediment pack. Quantifying the extent to which this occurs is important in assessing bio-

immobilization barriers as treatment alternatives for radionuclide contaminated groundwaters. FW21 water contains significant Al and Ni concentrations, which are precipitated near the model inlet in response to pH adjustment. These metal containing precipitates could not only clog the system, but could potentially effect microbial growth in this region (Ni and Al can be toxic to microorganisms).

(7) What will ultimately limit the rates of Tc(VII) and U(VI) reduction? Carbon and nitrogen will be continuously supplied to the physical model by ethanol injections and NO_3^-, but phosphorous or other trace elements could potentially limit microbial growth. Fe(III) may too become limiting under iron reducing conditions as Fe(II) is soluble and may be flushed from the system. A recent study has shown evidence that nitrate respiration in G. *metallireducens* is significantly decreased with Fe(III) concentrations less than 500 μM (57). These limitations may result in changes in the microbial community, or in reduced microbial activity. The limestone and FRC sediment used to pack the physical model will be sampled over time to monitor changing concentrations of trace nutrients and Fe(III).

Electron Acceptors in the Physical Models

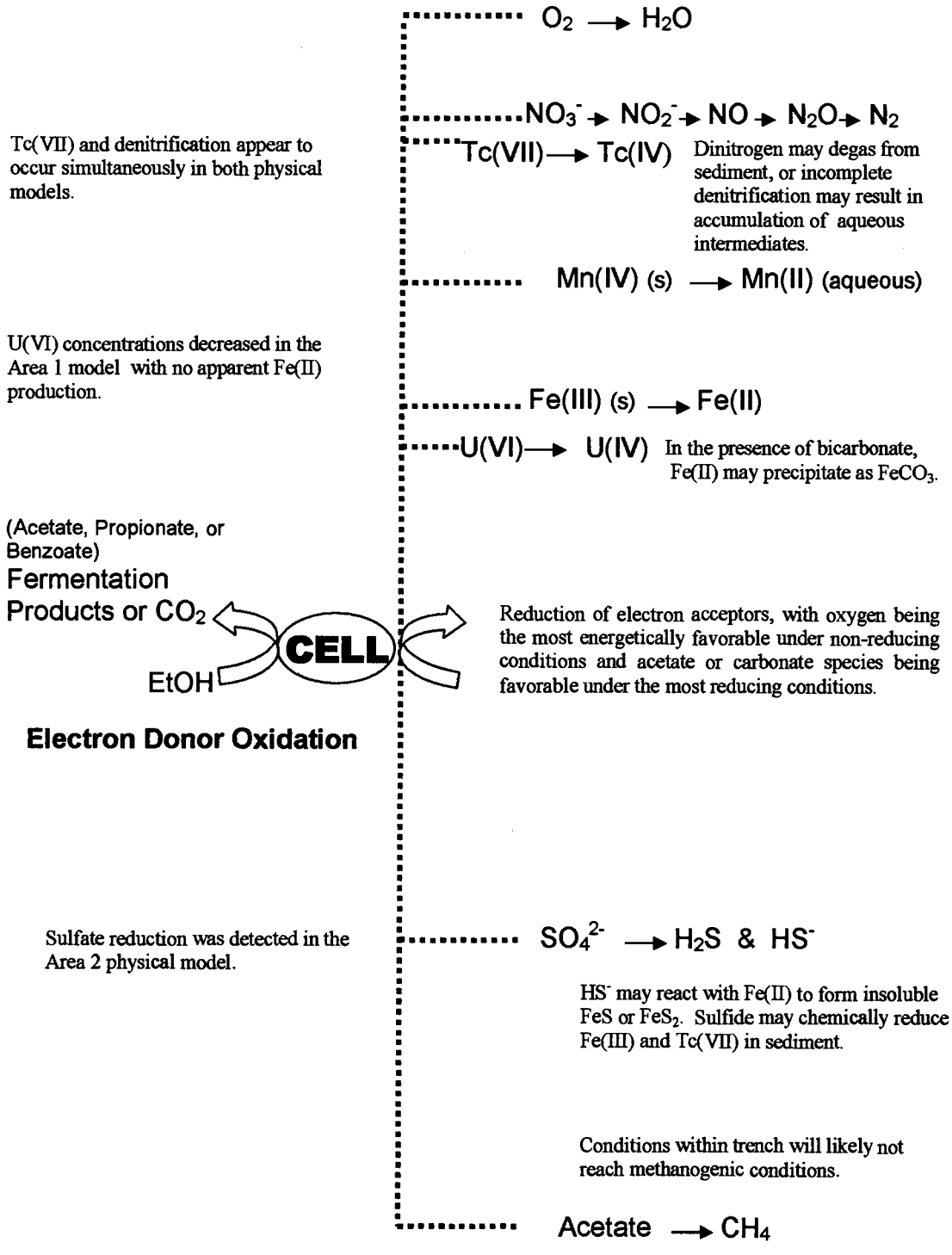
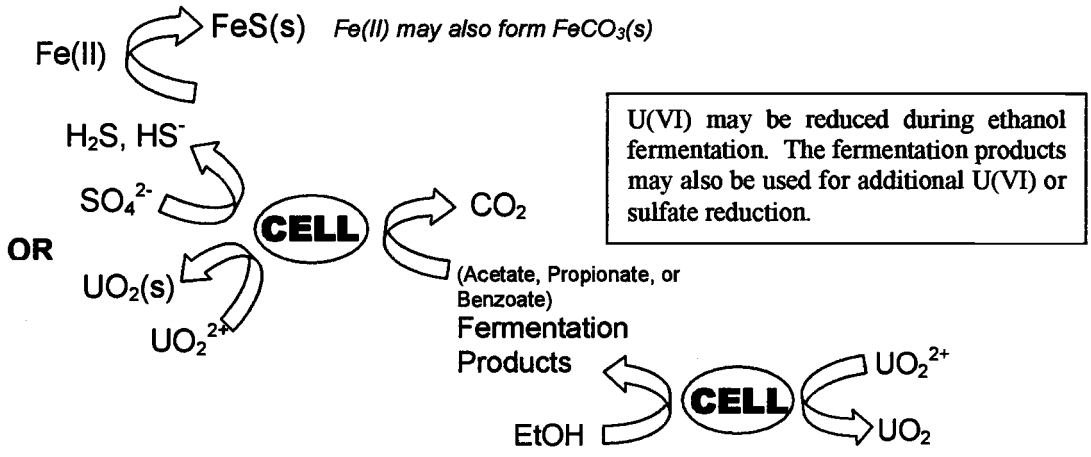
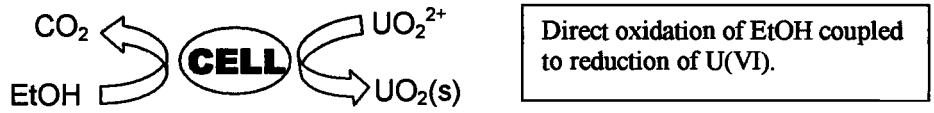
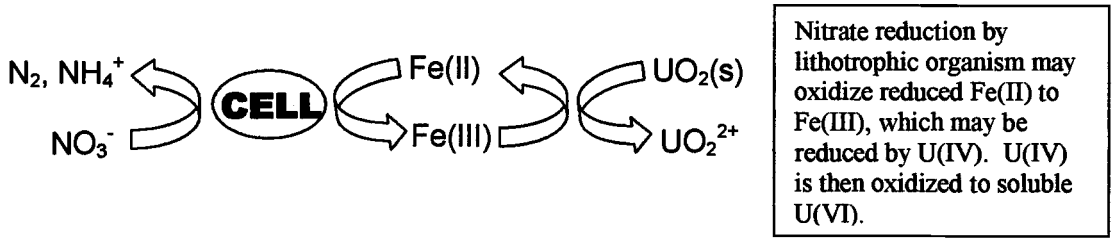


Figure 33. Conceptual diagram for principal redox reactions in the physical models.







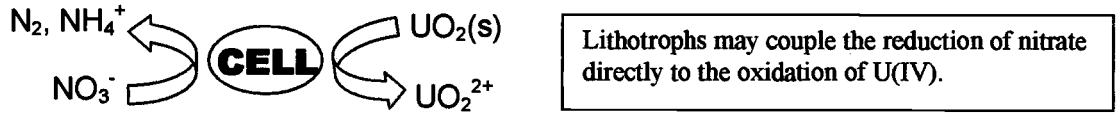


Figure 34. U(VI) reduction / re-oxidation cartoons.

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