Biological and chemical interactions with U(VI) during anaerobic enrichment in the presence of iron oxide coated quartz

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Abstract

Microcosm experiments were performed to understand chemical and biological interactions with hexavalent uranium (U(VI)) in the presence of iron oxide bearing minerals and trichloroethylene (TCE) as a co-contaminant. Interactions of U(VI) and hydrous iron oxide moieties on the mineral oxide surfaces were studied during enrichments for dissimilatory iron reducing (DIRB) and sulfate reducing bacteria (SRB). Microbes enriched from groundwater taken from the Test Area North (TAN) site at the Idaho National Laboratory (INL) were able to reduce the U(VI) in the adsorption medium as well as the iron on quartz surfaces. Early in the experiment disappearance of U(VI) from solution was a function of chemical interactions since no microbial activity was evident. Abiotic removal of U(VI) was enhanced in the presence of carbonate. As the experiment proceeded, further removal of U(VI) from solution was associated with the fermentation of lactate to propionate and acetate. During later phases of the experiment when lactate was depleted from the growth medium in the microcosm containing the DIRB enrichments, U(VI) concentrations in the solution phase increased until additional lactate was added. When additional lactate was added and fermentation proceeded, U(VI) concentrations in the liquid phase again returned to near zero. Similar results were shown for the SRB enrichment but lower uranium concentrations were seen in the liquid phase, while in the enrichment with carbonate a similar increase in uranium concentration was not seen. Chemical and biological interactions appear to be important on the mobilization/immobilization of U(VI) in an iron oxide system when TCE is present as a co-contaminant. Interestingly, TCE present in the microcosm experiments was not dechlorinated which was probably an effect of redox conditions that were unsuitable for reductive dechlorination by the microbial culture tested.

Keywords: Uranium; Groundwater; Iron-reducing bacteria; Sulfate reducing bacteria; Adsorption; Metal reduction

1. Introduction

Waste disposal associated with weapons production and energy research has resulted in widespread contamination of subsurface sediments and groundwater with chlorinated organics, metals and radionuclides. Nearly $3.2 \times 10^{12}$ L of water and $5 \times 10^7$ m$^3$ of soils and sediments have been contaminated at numerous Department of Energy (DOE) sites making up over 5700 individual contaminant plumes. Some of the more prevalent contaminants that have been co-disposed at DOE sites are chlorinated solvents (e.g.
perchloroethylene (PCE) and trichloroethylene (TCE)), metals (e.g., lead, chromium and mercury) and radionuclides (e.g., uranium, cesium and strontium) (Riley et al., 1992). These contaminants, especially the metals and radionuclides, can persist in the environment for extremely long periods. Since migration of contaminants within these plumes threatens regional aquifers and surface waters, understanding interactions of the contaminants in the water/rock environment is critical. Chemical and biological reactions of interest to our research included: formation of highly soluble solution species, adsorption of the hexavalent uranium (U(VI)) to the mineral surface through the formation of surface complexes, and biological metal reduction (e.g., U(VI) to reduced forms such as U(IV) and reduction of ferric iron on the quartz surface to ferrous iron).

In contaminated, but otherwise undisturbed aquifer material, migration of radionuclides, such as uranium will be significantly affected by the composition of the mineral phases found in the aquifer and the chemistry of the groundwater. Important reactions include adsorption to mineral phases on soil and sediment and/or formation of highly soluble solution species through coordination with water, carbonate or nitrate (Meinrath, 1996). Adsorption of U(VI) to minerals can best be described by the formation of surface species through chemical reactions between the surface functional groups and the aqueous species (Kohler et al., 1996). A large number of studies looking at the adsorption of U(VI) onto iron oxide minerals under environmentally relevant redox conditions have been reported. Minerals of particular interest have included goethite (Duff and Amrhein, 1996; Gabriel et al., 1998; Moyes et al., 2000; Giammar and Hering, 2001; Jacquier et al., 2001), hematite (Ho and Doern, 1985; Ho and Miller, 1986; Bargar et al., 1999; Murphy et al., 1999; Bargar et al., 2000), ferrhydrite (Waite et al., 1994; Arnold et al., 1998; Reich et al., 1998), uncharacterized Fe oxide minerals (Morrison et al., 1995; Sato et al., 1997; Abdelouas et al., 1998a, b; Rosentretser et al., 1998; Ferris et al., 2000; Kaplan and Serkiz, 2001), cleaned quartz (Kohler et al., 1996) and various clay minerals, including smectite (Morris et al., 1994; Turner et al., 1996), montmorillonite (Chisholm-Brause et al., 1994; Akcay and Kurtulmus, 1995; Akcay et al., 1996), and bentonite (Olguin et al., 1997; Boult et al., 1998).

While abiotic reactions such as adsorption and precipitation will dominate the interactions between contaminants and the water rock environment, microbial activity associated with the oxidation of natural or anthropogenic carbon sources have a significant effect on the redox chemistry in contaminated aquifers. Electrons liberated during the oxidation of organic substrates are coupled with anaerobic terminal electron acceptors such as nitrate, Fe(III), Mn (IV), sulfate or carbon dioxide. The sequence of redox reactions associated with microbial activity will lead to redox potentials amenable to the reduction of Fe (III), U(VI) and sulfate, under conditions of excess organic carbon (Stumm and Morgan, 1996). These reactions will play an important role in secondary mineral distribution in these aquifers and may also significantly impact radionuclide migration in subsurface systems via direct reduction of the radionuclide or formation of colloids (Wang and Papenguth, 2001).

In efforts to develop a biological treatment system for radionuclide contaminated aqueous streams, microbial reduction of U(VI) to the less soluble tetravalent form (U(IV)) by dissimilatory iron reducing bacteria (DIRB) and sulfate reducing bacteria (SRB) has been widely reported. Abdelouas et al. (1998a, 1999, 2000) identified bacteria capable of reducing nitrate and U(VI) in groundwater and Navajo sandstone from the Tuba City mill tailings site. U(VI) was reduced and precipitated as uraninite following complete reduction of residual nitrate and oxygen in the samples. Reduction of the U(VI) coincided with precipitation of iron sulfide via the reduction of Fe(III) on the sandstone and sulfate in the groundwater. In separate experiments, direct enzymatic reduction of U(VI) to U(IV) by SRB was noted in sediments from two additional mill tailings sites in Germany and from dairy and farm soil in New Mexico (Abdelouas et al., 1999). Studies indicated that SRB capable of U(VI) reduction are ubiquitous in nature. Pure strains of SRB such as Desulfovibrio desulfuricans (ATCC 7757) have been shown to reduce U(VI) under variable sulfate concentrations (Spear et al., 2000) and in the presence of the heavy metals zinc and nickel (Ganesh et al., 1999). Reductive precipitation of U(VI) appeared to be inhibited by copper. In unrelated experiments, the SRB D. desulfuricans and DIRB Shewanella alga were able to reduce U(VI) that had been complexed to the organic ligands acetate, maleonate, oxalate and citrate (Ganesh et al., 1997). D. desulfuricans rapidly reduced the monodentate aliphatic complex with acetate, while S. alga appeared to reduce U(VI) in multidentate aliphatic complexes with the other ligands. Iron reducing bacteria recovered from contaminated groundwater were also capable of reducing U(VI). S. putrefaciens was able to utilize U(VI) as a terminal electron acceptor in concert with sulfate reduction after oxygen and nitrate had been removed from the growth medium (Abdelouas et al., 1998b). Fredrickson et al. (2000) using the DIRB S. putrefaciens CN32 demonstrated the reduction of U(VI) in soluble carbonate complexes (i.e., UO2(CO3)34− and UO2(CO3)52−) as well as the U(VI) mineral metaschoepite in the presence and absence of goethite.

The purpose of our research was to investigate the effect of anaerobic enrichments on U(VI) adsorption to
iron oxide coated sand when TCE was present as a co-contaminant. Experimental conditions employed were designed to simulate aquifer conditions during chemical shifts that would be expected when accelerated anaerobic bioremediation is employed for the treatment of a chlorinated solvent plume. Microbial enrichments utilized included iron oxide coated quartz, iron oxide coated quartz plus sulfate, and iron oxide coated quartz plus bicarbonate. Atmospheric carbon dioxide was excluded during the experiments. DIRB and SRB were expected to be enriched from the environmental culture used for the testing. Information from the experiments may provide insight into developing a bioremediation technology that would be effective on mixed radiouclide/chlorinated solvent contaminant plumes, because a variety of DIRB and SRB have been implicated in both reductive dechlorination of chlorinated solvents and utilization of aqueous or solid phase minerals (e.g., oxide and oxyhydroxide minerals) (Picard et al., 1993, 1995) and radionuclides such as uranium (Ganesh et al., 1997; Fredrickson et al., 2000).

2. Materials and methods

2.1. Culture selection

The culture used for testing was enriched from groundwater obtained from the Test Area North (TAN) site at the Idaho National Laboratory where a field test is being performed to evaluate anaerobic reductive dechlorination of TCE using lactate as the carbon and energy source (Martin et al., 2001). This culture was of particular interest because of its ability to reductively dechlorinate TCE as well as reduce iron and sulfate in the aquifer.

Groundwater samples for enrichment were taken from monitoring well TAN-37 at a depth of 116 m below ground surface using a variable speed submersible pump at a flow rate of 3.8 L/min. TAN-37 was approximately 46 m down gradient from the lactate injection point for a site being used to evaluate bioremediation for the treatment of a chlorinated solvent plume beneath TAN. Prior to sampling, three pipe volumes were purged and then a 1-L sterile polypropylene sample bottle was filled and shipped to the lab at 4 °C. Samples were held overnight at 4 °C and then filtered the next day. A 500 mL sample of ground-water was filtered through a 0.2 μm filter membrane and the filter was then added to a nitrogen/phosphate (N/P) (1 mM NH₄NO₃/1 mM phosphate buffer) growth medium containing 500 mg L⁻¹ lactate, 40 mg L⁻¹ sulfate, and 180 g of Fe oxide coated sand for enrichment. The final pH of the growth medium was 7.3. The culture was incubated in an anaerobic glove box with a 4% hydrogen in nitrogen atmosphere.

2.2. Iron oxide coated quartz

Sediments utilized during experimentation were obtained from the US Department of Energy, Office of Health and Environmental Research, Program for Natural and Accelerated Bioremediation Research (NABIR) field site for research into bacterial transport. The site is a sand borrow pit on the Delmarva Peninsula in Virginia, near the village of Oyster and has undergone extensive geologic and hydrologic characterization. The sediments found in the aquifer at the site are composed of quartz, feldspar, clays and iron and aluminum hydroxides (Johnson et al., 2001). Metal hydroxides occur as coatings on the fine-grained quartz, comprising between 0.4% and 1.2% of the mineralogy.

Uranium sorption experiments showed that at a pH of 7 the partition coefficient (Kd) ranged between approximately 1.5 and 2.5 mL g⁻¹ and correlated well with surface area and extractable Fe(III) and Al for these sediments (Rosentreter et al., 1998). These same studies showed that reducible iron, which was determined using the bicarbonate buffered diethionite-citrate extraction process, spanned a range from 2.6 to 25.96 μmol Fe g⁻¹ of sediment with a mean value of 7.93 μmol Fe g⁻¹. The average surface area of the sediment was 1.17 m² g⁻¹.

2.3. Microbial enrichment

Testing was performed in 250 mL amber bottles closed to the atmosphere using Mininert™ valves (Supelco, Inc., Bellefonte, PA), which had been disinfected using a dilute bleach solution since autoclaving ruined the integrity of the seal with the bottles and the function of the valves. Approximately 4.8 g of iron oxide coated quartz was added to each vial. Iron oxide coated quartz particles 1 mm in diameter or less were used for testing. The mean iron content of the batch of quartz used for testing was 2.15 μmole iron per gram of sediment. Microcosm bottles containing only iron oxide coated quartz were covered with aluminum foil and were autoclaved for 20 min at 121 °C at a pressure of 20 psi.

Microbial enrichment was initiated by preparing 4 L of N/P growth medium which contained 2 mg L⁻¹ resazurin as a redox indicator. Residual fixed gases (i.e., oxygen and carbon dioxide) were removed from the growth medium by sparging with nitrogen gas for approximately 2 h. Uranyl nitrate was added to the medium for a final U(VI) concentration of 1 mg L⁻¹ prior to dispensing into bottles containing the sterile iron oxide coated quartz. After the medium was dispensed into the bottles, each bottle was re-sparged for 15 min with nitrogen. During the second nitrogen sparging, the remainder of the medium constituents, with the exception of TCE, were added. The constituents added depended on the desired enrichment, which will be explained in detail below. Lactate, at a target
concentration of 800 mg L⁻¹, was used as the carbon and energy source for growth and was included in all enrichments except the blanks (See below). Following lactate addition, bottles were sealed with the Miniert™ valves. TCE was added to the N/P growth medium through the Miniert™ to yield a final concentration of 10 mg L⁻¹. Enrichments were respiked with lactate on day 22 of the experiment.

Chemical and biological interactions with the U(VI) were studied in the presence and absence of carbonate. The experiment consisted of seven treatments: one blank containing the enrichment medium, one blank with medium plus TCE, a killed control containing enrichment medium and inoculum that had been killed by autoclaving plus TCE, an enrichment containing inoculated enrichment medium plus sulfate (40 mg L⁻¹) plus TCE, an enrichment containing inoculated enrichment medium and TCE, an enrichment containing inoculated enrichment medium, carbonate (5 mM), and TCE and a carbonate control which contained enrichment medium, TCE, but no inoculum. The initial pH for each experiment was approximately 7.0, with the exception of the enrichment with carbonate which was started at pH 8, but equilibrated quickly to a pH near 7.

2.4. Sampling and analysis

2.4.1. Uranium

Kinetic phosphorimetry was used to monitor U(VI) over time during the adsorption experiments. These analyses were performed using a KPA-11 automatic kinetic phosphorescence analyzer (KPA) manufactured by Chemchek Instruments (Richland, WA). Calibration standards of known uranium concentrations were prepared from a 1000 ppm uranyl nitrate ICP standard (GFS Chemicals, Powell, OH) and were used to construct high- (25–500 μg L⁻¹) and low-range (0.1–10 μg L⁻¹) calibration curves from the initial luminescence intensity and U(VI) concentration. The instrument automatically switched between the high- and low-range U(VI) curves depending on luminescence intensity generated by the sample.

Unfiltered bulk liquid samples from the enrichments were diluted 1:10 in 4 mM HNO₃ to remove any interference from other ions in solution and to dissolve the uranium in the samples. Unfiltered samples were used for testing because initial filtration results indicated all of the U(VI) was removed when samples were filtered through a 0.2 μm filter.

Samples were covered with parafilm to decrease evaporation, and were placed in the autosampler for analysis. Samples near the instrument detection limit of 0.05 μg L⁻¹ were spiked with 10 μg L⁻¹ uranium to bring the response into the analytical range. Analytical results were compared to a U-free control.

Total U was determined at the end of the experiment using inductively coupled plasma mass spectrometry (ICP-MS). Samples were acidified to pH 2.5 using 5% HNO₃ and were then analyzed using a VG elemental plasma quad II ICP-MS. The instrument was calibrated and operated in accordance with manufacturer’s instructions. The range of the calibration for the analysis was 0–500 ng mL⁻¹. Indium was used as an internal standard for the ICP-MS analysis.

Neat samples were analyzed by spiking 10 mL of the sample with the internal standard prior to analysis. Dilutions were performed on other samples to bring them into the calibration range of the procedure. Sample dilutions were made using Nanopure water along with nitric acid and the internal standard. At least one sample was spiked with a known concentration of the analyte and a recovery was calculated to verify the accuracy of the procedure.

Uranium reduction in the enrichments was determined by subtracting the U(VI) concentrations determined using the KPA analysis outlined above from the total U determined using ICP-MS analysis. Microbiologically, tetravalent uranium (U(IV)) is the most significant valence state of the reduced forms of uranium (Ehrlich, 1996).

2.4.2. Volatile organic carbon

TCE concentrations in the adsorption bottles were measured via gas chromatography using a Hewlett-Packard 5890 Series II gas chromatograph (GC)(Hewlett-Packard, Co, Santa Clara, CA). Operation of the GC and data collection were automated using Hewlett-Packard 3365 Chemstation software. The GC was equipped with a Restek 30 m, 0.32 mm i.d. Rtx-35 column containing a 1.0 μm film thickness of cross-bonded 65% dimethyl–35% diphenyl polysiloxane (Restek Corp, Bellefont, PA). Sampling for TCE in the headspace of the bottles was accomplished using solid phase microextraction (SPME). The SPME unit was equipped with an 85 μm polyacrylate coated fiber which was inserted into the samples for 5 min followed by injection of the sample into the GC using a 5 min desorption phase.

A flame ionization detector (FID) held at 275 °C with an injector temperature of 250 °C was used for analysis of TCE. TCE and potential breakdown products were separated by running at 100 °C for 4 min, followed by a 40 °C min⁻¹ ramp to 220 °C to purge residual sample from the column. Helium was used as the carrier gas, at a volumetric flow rate of 7.8 mL min⁻¹ and a split flow of 20 mL min⁻¹.

2.4.3. Organic acids

Concentrations of volatile fatty acids (e.g., lactate, acetate, propionate, butyrate and formate) in the influent and effluent were determined using a
high-pressure liquid chromatograph (HPLC). The HPLC was equipped with a Linear™ UVIS200 UV/VIS detector (Linear Instruments, Fremont, CA) set at 210 nm. Samples were eluted using 0.01 N H₂SO₄ pumped using an Alltech 325 HPLC pump (Alltech Associates, Inc., Deerfield, IL) at a flow rate of 0.3 mL min⁻¹. The volatile fatty acids were separated using a Brownlee, Polypore H, 10 μ, 220 mm × 4.6 mm column (Alltech Associates, Inc., Deerfield, IL) held at 35 °C. Samples from the liquid feed reservoir and liquid effluent were diluted as necessary, acidified using 4 N H₂SO₄ and filter sterilized using 0.2 μm nylon filters (Gelman Sciences, Inc., Ann Arbor, MI) prior to injection.

2.4.4. Ferrous iron

Ferrous iron was measured using the ferrozine dye assay, using the variation of the Stookey method found in Kostka and Nealson (1998). Briefly, samples of the culture medium were extracted using HCl, added to 5 mL solution of 0.02% ferrozine (pH 7), incubated for 15 min and then analyzed by a spectrophotometer set at a wavelength of 562 nm. Fe concentrations were determined by comparing absorbance values of the unknown sample to standard concentrations made using ferrous ammonium citrate.

2.4.5. Eh and pH

Enrichment medium Eh and pH were measured using an Accumet Portable AP10 pH/mV meter (Fisher Scientific, Pittsburgh, PA). A 6-cm MI-414 pH combination electrode (Microelectrodes, Inc., Bedford, NH) was used to measure pH. Liquid samples that were pulled to analyze for organic acid concentrations were measured for pH prior to acidification. Redox potential was measured using an MI-800-413B Micro-Redox Electrode (Microelectrodes, Inc., Bedford, NH). The redox potential of the bulk solution was measured following the initial nitrogen sparge, while the redox potential of the individual enrichments was measured at the end of the experiment by inserting the electrode through the septum in the Mininert™ valve.

3. Results and discussion

3.1. Chemical interactions

Upon initial entry of U(VI) into an aquifer environment, abiotic chemical interactions will dominate. The primary chemical interactions that would be expected are formation of uranyl complexes with carbonate, hydroxide and other anions in solution leading to precipitation, adsorption or the formation of highly soluble solution complexes (Wang and Papenguth, 2001). The percent removal of U(VI) in the enrichment vials and associated controls during the first 8 h of the experiment can be seen in Fig. 1. U(VI) removal results shown were attributed to chemical interactions that would be expected as a function of the solution chemistry of test conditions employed. Removal of U(VI) in enrichments and the control containing carbonate was nearly double compared to the enrichments without carbonate, the killed control and blanks.

As stated in Section 2, U(VI) results reported are for the bulk liquid phase from the enrichments, because early in the experiment, results showed that the U(VI) fraction filterable through a 0.2 μm filter membrane was minimal. Formation of the >0.2 μm particulate may have been caused by the phosphate buffer added to the enrichment medium. Due to the low solubility of several uranyl–phosphate complexes, the existence of a precipitate is possible. Species formed at the beginning of the experiment were estimated using Visual MINTEQ 2.0. An average initial pH of 6.76 was used for the carbonate free enrichments, while a starting pH of 7.89 was used for the carbonate containing enrichment. Results indicated that 100% of the uranyl ion would be in the form of UO₂(HPO₄)²⁻, whether carbonate was present or not. Results from the experiment indicated that while some of the U(VI) may have been removed by precipitation onto the surface of the Fe oxide coated quartz, there was still a substantial particulate phase that remained suspended in solution that could be dissolved in nitric acid and analyzed.

![Fig. 1. Percentage of U(VI) removed during the first 8 h of a microcosm experiment designed to study chemical and biological interactions of U(VI) in an iron oxide system during enrichment for DIRB and SRB in the presence and absence of carbonate.](image-url)
The percentage of U(VI) removed during the remainder of the experiment for each enrichment can be seen in Fig. 2A. U(VI) in both enrichments without carbonate was completely removed within 10 days of initiating the enrichments. During the time period between days 10 and 20 the U(VI) concentration in the liquid phase began to increase, the U(VI) concentration in the enrichment containing only iron oxide coated quartz (▼) appeared to be higher than U(VI) in the enrichment with sulfate (●). On day 22 when additional lactate was added, the percent U(VI) removed decreased until day 28 when U(VI) concentrations in the liquid phase again began to increase. U(VI) removal in the enrichment that contained carbonate (○) reached ∼90% after 5 days and then the removal did not fluctuate after that point.

The percentage of U(VI) removed in both blanks (Blank + TCE (●); Blank−TCE (■)) and the carbonate free control (□) was less than that seen in the enrichments (Fig. 2B). By the end of the experiment, only 70% of the U(VI) in the blanks and control had been removed. Interestingly, the U(VI) concentration in the killed control (▲) also dropped to zero, indicating potential chemical carry-over from the autoclaved inoculum or chemical precipitation of uranium in solution. Causes for the loss of U(VI) were not determined, but by the end of the experiment the concentration of U(VI) removed in the killed control was similar to levels seen in the blanks with and without TCE and the carbonate control. Differences in U(VI) removal between the enrichments, blanks and killed control were not linked to changes in pH since the adsorption medium pH remained stable throughout the entire experiment (See Below).

3.2. Biological interactions

Microbial activity in the enrichments was monitored by following lactate removal and the production of fermentation products such as acetate and propionate. Organic acid concentrations in all of the enrichments and the killed control can be seen in Fig. 3. Time zero measurements of organic acids were not analyzed. Conversion of lactate to acetate, propionate and butyrate in the enrichments containing only the iron oxide coated sand (Fig. 3A) and the iron oxide coated sand and sulfate (Fig. 3B) were very similar. No microbial activity was apparent by day 3 of incubation as measured by utilization of the lactate in the bottles. Microbes within both enrichments began using lactate between days 3 and 10. Propionate to acetate ratios (P:A) produced by the microbes in these enrichments were very similar to the P:A demonstrated in the field (Martin et al., 2001). Additional lactate was added on day 22 and the concentrations were measured on day 24. Since no change in lactate concentrations were noted on day 24, there appeared to be a lag period before lactate utilization was reinitiated even though the microbes were already acclimated to the carbon source. This lag may have been caused by a shift in the physiology of the microbes present to utilize the other organic acids present, or a shift in the population to microbes that were able to utilize propionate and acetate. Following a short acclimation period after lactate addition on day 22, the microbes again began to ferment the lactate to

![Graph A](image1)

![Graph B](image2)

Fig. 2. Percentage of U(VI) removed during microcosm experiments designed to study chemical and biological interactions of U(VI) in an iron oxide system during enrichment for DIRB and SRB in the presence and absence of carbonate. Annotation: (1) initiation of lactate fermentation in enrichments; (2) complete removal of lactate from liquid medium; (3) lactate added to liquid medium; and (4) complete removal of lactate from second addition.
propionate and acetate; however there was no butyrate produced during this phase of growth.

Lactate conversion by microbes in the enrichment containing carbonate can be seen in Fig. 3C. Only small amounts of lactate remained after 8 days of incubation, but less acetate and propionate was produced, compared to the carbonate-free enrichments with and without sulfate (Figs. 3A and B). Decreased concentrations of propionate and acetate may be a factor of better conversion efficiency by this enrichment compared to the previous enrichments. After lactate was reintroduced on day 22, propionate and acetate concentrations in the adsorption medium more than doubled as the microbes in the enrichment began fermenting the carbon source.

Fig. 3D shows lactate, propionate, acetate, formate and butyrate concentrations in the killed control. As can be seen from the graph no lactate conversion was noted during the test period.

In an attempt to understand the effect of microbial activity, as measured by lactate conversion on U(VI)
removal; 4 events in the course of the experiment will be further discussed. Fig. 2A which shows the percent U(VI) removed during the experiment has been annotated to show these events.

1. On day 3 of the experiment, no lactate conversion was noted. During the time period between days 3 and 10, conversion of lactate to primarily propionate and acetate occurred. During this same time period, U(VI) removal (Fig. 2) in the enrichments with and without sulfate reached 100%.

2. After 10 days of incubation when no lactate was present in the enrichments, U(VI) concentrations began to increase as noted by the decreasing percent removal of U(VI). During this same time period considerable amounts of propionate and acetate were present in the enrichment medium (Figs. 3A and B).

3. On day 22 of the experiment, additional lactate was added to the bottles containing the enrichments with and without sulfate. From the data, microbes began to utilize lactate after day 24, which corresponded to a decrease in U(VI) concentrations in the bottles between days 21 and 25.

4. Finally, conversion of lactate to propionate and acetate occurred some time between day 24 and 30, as noted with the previous time point on which lactate was depleted, U(VI) once again began to partition back into the bulk liquid-phase.

One additional note, even though near 100% U(VI) removal was seen in the killed control, no lactate conversion was demonstrated. While the U(VI) concentration in the killed control increased after day 10 of the experiment, following this time point the U(VI) partitioning behavior in this bottle was more consistent with the blanks than the microbiologically active enrichments. This along with the U(VI) removal results in the enrichments, both the DIRB and SRB, may indicate that the process controlling removal of total uranium required a respiring microbial population. When no microbial activity was indicated by lactate conversion, U(VI) concentration in solution increased. This was seen in both enrichments and the killed control, but upon the addition of lactate to the enrichments, U(VI) was again removed from solution, but it is not known whether this was reduction or coprecipitation with other reduced phases in the medium (e.g., Fe(II) or sulfides).

Total uranium was measured at the completion of the experiment to determine whether the microbes were able to reduce the U(VI) in the enrichment medium. ICP-MS analysis showed higher total uranium concentrations compared to U(VI) concentrations in the enrichments determined by kinetic phosphorimetry. The difference in concentration between total uranium and U(VI) was assumed to be reduced forms of uranium. Less U(VI) reduction was seen in the enrichment with carbonate compared to U(VI) in the enrichments with and without sulfate. Concentrations of reduced forms of uranium (U$_{\text{red}}$) in the enrichments were 52.2, 350, and 237 $\mu$g L$^{-1}$, respectively. The killed control showed a U$_{\text{red}}$ concentration of 24 $\mu$g L$^{-1}$ at the conclusion of the experiment. While uranium may have been present in other reduced forms, U(IV) would be the prevalent state expected from microbial reduction (Lovley et al. 1993a, b; Fredrickson et al., 2000; Chang et al., 2001). Differences between U(VI) and total uranium in the blanks and killed controls were not significant, indicating that abiotic uranium reduction was not occurring.

A substantial amount of the typically insoluble U(IV) was in the liquid phase. Since the U(VI) and total U analyses were performed on the bulk liquid medium, there was no differentiation between soluble and insoluble fractions. As previously stated, while formation of a uranyl–phosphate precipitate was apparent, these complexes behaved in a manner similar to soluble U(VI) since they remained suspended in the liquid phase. In any case, the microbes were able to reduce the U(VI) complexed in these precipitates.

In addition to demonstrating the ability to reduce the U(VI) in the enrichment, the microbial population was also capable of iron reduction. Fig. 4 shows the increasing ferrous iron concentrations in the enrichments compared to the two blanks and the killed control. Iron reduction in the enrichment containing the Fe oxide coated quartz appeared to be linked to the lactate utilization. When lactate was present and being oxidized, ferrous iron production was evident. After day

![Fig. 4. Ferrous iron production as an indicator of iron reduction in enrichments used to monitor chemical and biological interactions of U(VI) in a iron oxide system.](image-url)
10 when the lactate was depleted, ferrous iron production ceased. After lactate was added on day 22 and was again oxidized by the microbes in the enrichment, the ferrous iron concentration in the growth medium doubled. Ferrous iron production in the enrichment with sulfate continued even after the lactate was depleted, but began to drop after day 15. One possible explanation for this drop in ferrous iron was the precipitation of ferrous sulfide which was supported by the appearance of the black precipitate on the mineral surface. As with the enrichment containing only Fe oxide coated quartz, ferrous iron concentrations in the enrichment with sulfate began to increase after the second lactate addition. Ferrous iron production was not evident in the killed control or the blanks independent of the presence of TCE.

Microbes in the enrichment with carbonate were also capable of reducing the iron oxide on the quartz to ferrous iron. Unlike the other enrichments, ferrous iron production was gradual and steadily increased over the duration of the experiment.

Using the electron donor (i.e., lactate) and the electron acceptors provided, the microbes were able to decrease the redox potential during enrichment compared to the killed control and blanks. Redox potential was measured for the enrichment medium prior to dispensing into the adsorption vials, following the initial nitrogen sparge, and then when the experiment was completed. Changes in redox potential in the different treatments can be seen in Fig. 5. Results showed that microbes in the enrichments were able to decrease the redox potential in the adsorption medium. Redox potentials generated are within ranges for microbially mediated redox processes for DIRB (−30) and SRB (−181) (Stumm and Morgan, 1996). High redox potentials associated with the Blank with TCE and the Killed Control were more than likely due to the high oxidation reduction potential for TCE.

Solubility and speciation of U(VI) due to changes in pH were determined not to be a factor in the U(VI) removal noted in the enrichments because pH remained stable during the entire experiment. With the exception of the enrichment and control containing carbonate, the pH of the liquid medium in all of the test vials varied only slightly between 6.6 and 6.8 (Fig. 6). The pH in the enrichment and control containing carbonate was near 8.0 at the beginning of the experiment. By day 10 of the experiment, the pH in the enrichment dropped to near 7.0; whereas the pH in the carbonate control remained stable near 8.0 until after day 15, at which time the pH dropped to near 6.7.

In general, microbes used in the enrichments to determine the effect of microbial growth on U(VI) adsorption to iron oxide coated quartz were not able to reductively dechlorinated TCE (Data not shown). Enrichment using the iron oxide coated quartz with and without sulfate or in the presence of carbonate may have selected for metal reducing microbes in the culture, shifting the community away from TCE degraders that were prevalent in the field. In addition, reductive dechlorination of TCE in the accelerated remediation site at TAN was associated with methanogenic conditions and the fermentation of propionate (Martin et al., 2001). Redox potentials generated during the adsorption...
studies indicated that the Eh had not decreased to levels conducive for methanogenesis or TCE reduction to occur.

4. Conclusions

Chemical and biological interactions with U(VI) occurred during the enrichment of anaerobic microbes from groundwater from accelerated bioremediation operations at the TAN site at the Idaho National Laboratory. Chemical interactions were dominant early in the experiment prior to the onset of microbial activity in the enrichments. Once lactate utilization was seen in the enrichments with and without sulfate, metal reduction was evident by the appearance of ferrous iron. The metal reducing ability of the microbes in the enrichments was also proven by the presence of reduced forms of uranium at the conclusion of the experiment. While metal reducing microbes were not specifically identified, these data indicated their presence in the enrichments. Specific conclusions that can be drawn from the research are:

1. Removal of U(VI) in the enrichments was divided into two distinct phases, whether carbonate was present or not. The first phase occurred within the first 8h of the experiment (Fig. 1) while the second phase took place over the long term, as the experiment proceeded. Uranium removal from solution during the first 8h of the experiment was thought to be a function of chemical effects such as adsorption and formation of less soluble species since the microbes had not begun using lactate. The second distinct phase observed in the enrichments was associated with microbial activity and the fermentation of lactate to propionate and acetate.

2. The long term (i.e., after 20 days) disappearance of U(VI) in the enrichments containing the inoculum from TAN was related to microbial reduction of the uranium. Reduction of the U(VI) as well as the Fe oxide in the carbonate-free enrichment without sulfate appeared to be linked to lactate oxidation, while Fe reduction in the enrichment with sulfate appeared to be independent of lactate oxidation. A small amount of reduced uranium was found in the killed control, but the reduction was attributed to chemical mechanisms because lactate was not oxidized in this treatment. Reduced forms of uranium were not observed in experimental blanks.

3. Carbonate had a substantial effect on U(VI) removal compared to carbonate free enrichments. When 5mM carbonate was present in the enrichment medium, substantially more U(VI) was removed during the first 8h of the experiment when chemical interactions would be expected to dominate. When microbes began to use lactate in the enrichments, U(VI) removal in the enrichment with carbonate was similar to the U(VI) removal seen in enrichments without carbonate. The U(VI) concentration in the liquid phase of the enrichments containing carbonate appeared to be affected less by fluctuations in the lactate concentration or the presence of other organic acids than the enrichments without carbonate. Enrichment of the inoculum from TAN with carbonate again lead to higher levels of U(VI) removal from the liquid phase compared to abiotic controls.

4. Speciation of uranium using Visual MINTEQ 2.0 indicated that phosphate levels in the adsorption medium for optimum growth of the enrichments may have led to the formation of uranyl–phosphate precipitates, especially in the enrichments performed without carbonate. The ability of the microbes in the enrichments to reduce the U(VI) did not appear to be inhibited by the formation of these uranyl–phosphate complexes. While the presence of these uranyl–phosphate complexes lead to a potentially mobile form of U(VI), these levels of phosphorus would not be expected in an aquifer under natural conditions but could be present during active bioremediation processes, such as the anaerobic bioremediation test at TAN.

5. Results generated from the above research showed that microbes in cultures originally utilized for reductive dechlorination of TCE were able to reduce metals such as iron and U(VI), as well as sulfate. Microbes enriched during the experiment were not able to reductively dechlorinate the TCE provided as a co-contaminant in the adsorption reactions. This lack of reductive dechlorination of the TCE, was more than likely due to redox levels in the adsorption medium that were not conducive to TCE dechlorination by the microbes in this particular culture. Results from the field demonstration at TAN site at the INEEL where accelerated reductive dechlorination is being applied indicated that reductive dechlorination of TCE was initiated under sulfate reducing conditions and was completed under methanogenic conditions where ethene and ethane were measured in the groundwater (Martin et al., 2001).

Results from the current experiments as well as the successful TCE remediation at the TAN site indicate that the potential of remediating contaminant plumes where radionuclides such as U(VI) have been co-disposed with the chlorinated solvent exists. An engineered approach in which aquifer redox chemistry is controlled within ranges conducive for microbial metal reduction and for the dechlorination of the chlorinated contaminant is envisioned. In such a system, metal
radionuclides, such as uranium would be immobilized as the redox level of an aquifer is decreased via the addition of an electron donor. Metal reduction would be maintained as redox conditions transitioned to highly reducing conditions for reductive dechlorination of the chlorinated solvent of interest. Of course, following re-establishment of oxic conditions in the aquifer being treated the U(VI) would require monitoring to determine whether the re-oxidation of immobilized uranium was occurring.

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