

Donor-dependent Extent of Uranium Reduction for Bioremediation of Contaminated Sediment Microcosms

Andrew S. Madden and Anthony V. Palumbo* Oak Ridge National Laboratory

Bruce Ravel Argonne National Laboratory

Tatiana A. Vishnivetskaya, Tommy J. Phelps, Christopher W. Schadt, and Craig C. Brandt Oak Ridge National Laboratory

Bioremediation of uranium was investigated in microcosm experiments containing contaminated sediments from Oak Ridge, Tennessee to explore the importance of electron donor selection for uranium reduction rate and extent. In these experiments, all of the electron donors, including ethanol, glucose, methanol, and methanol with added humic acids, stimulated the reduction and immobilization of aqueous uranium by the indigenous microbial community. Uranium loss from solution began after the completion of nitrate reduction but essentially concurrent with sulfate reduction. When electron donor concentrations were normalized for their equivalent electron donor potential yield, the rates of uranium reduction were nearly equivalent for all treatments ($0.55\text{--}0.95\ \mu\text{mol L}^{-1}\ \text{d}^{-1}$). Uranium reduction with methanol proceeded after a 15-d longer lag time relative to that of ethanol or glucose. Significant differences were not found with the inclusion of humic acids. The extent of U reduction in sediment slurries measured by XANES at various time periods after the start of the experiment increased in the order of ethanol (5–7% reduced at 77 and 153 d), glucose (49% reduced at 53 d), and methanol (93% reduced at 90 d). The microbial diversity of ethanol- and methanol-amended microcosms in their late stage of U reduction was analyzed with 16S rRNA gene amplification. Members of the Geobacteraceae were found in all microcosms as well as other potential uranium-reducing organisms, such as *Clostridium* and *Desulfosporosinus*. The effectiveness of methanol relative to ethanol at reducing aqueous and sediment-hosted uranium suggests that bioremediation strategies that encourage fermentative poisoning of the subsurface to a lower redox potential may be more effective for long-term uranium immobilization as compared with selecting an electron donor that is efficiently metabolized by known uranium-reducing microorganisms.

METAL and radionuclide contaminants have infiltrated water and sediment at many facilities under the management of the Department of Energy (DOE). More than \$50 billion has been spent on remediation of DOE facilities, with an estimated 151 to \$195 billion expected in the future with existing treatment technologies (NRC, 2000). One major research effort focuses on the stabilization of metal contaminants, such as uranium and chromium, through in situ stimulation of natural microbial communities to reduce the metals to less soluble forms (NABIR, 2003). These communities in the subsurface have the capacity to immobilize several metal contaminants but are often limited by the lack of suitable electron donors. As electron donors (e.g., methanol, ethanol, pyruvate, glucose) are added, oxygen is consumed through aerobic respiration. Then, alternate electron acceptors are used by bacteria, typically in a sequence determined by community metabolic potential, electron donor bioavailability, and decreasing energy yield of reaction. Oxidized soluble metals, such as U(VI) and Cr(VI), may be transformed to reduced, insoluble forms through various metabolic and coupled biogeochemical processes.

Previous studies, summarized in Suzuki and Suko (2006) and Wall and Krumholz (2006), have shown that although uranium bioremediation through subsurface electron donor addition is possible, significant challenges persist. For example, electron donors shown to stimulate aqueous uranium reduction may reduce only 51% or less sediment-bound uranium (Ortiz-Bernad et al., 2004; Wu et al., 2006). Often, the electron donor is chosen to target organisms known to rapidly reduce uranium in laboratory settings. Nonlinear relationships between microbial community structure and subsurface biogeochemistry have been demonstrated for immobilization of metals (Palumbo et al., 2004; Schryver et al., 2006), suggesting that, although enrichment of specific target organisms has been documented in field experiments (Anderson et al., 2003; Chang et al., 2005; Holmes et al., 2002; Istok et al., 2004; Nevin et al., 2003; North et al., 2004; Wu et al.,

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*Corresponding author (palumboav@ornl.gov).

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677 S. Segoe Rd., Madison, WI 53711 USA

A.S. Madden, Oak Ridge National Lab., Biosciences Division, P.O. Box 2008, Oak Ridge, TN 37831-6038. Present address: Univ. of Oklahoma, School of Geology and Geophysics, 100 East Boyd St., Norman, OK 73019. A.V. Palumbo, T.A. Vishnivetskaya, T.J. Phelps, C. Schadt, and C.C. Brandt, Oak Ridge National Lab., Biosciences Division, P.O. Box 2008, Oak Ridge TN 37831-6038. B. Ravel, Argonne National Lab., Molecular Environmental Science Group, Building 203, Argonne, IL 60439. Present address: National Institute of Standards and Technology Building 535A, Room M7 c/o Brookhaven National Lab., Upton, NY 11973.

Abbreviations: cgDNA, community genomic DNA; DOE, Department of Energy; ORFRC, Oak Ridge Field Research Center; XAS, X-ray absorption spectroscopy.

2006), the prevalence of individual taxonomic groups may not be sufficient to describe the potential for long-term uranium immobilization. Sorbed or mineral U(VI) forms may continue to release uranium to ground water after cessation of electron donor addition, especially as carbonate complexes.

Microcosms and columns containing contaminated sediments and ground water amended with electron donor have provided significant insights into the relationships between microbial communities and aqueous geochemistry of uranium bioremediation (Edwards et al., 2007; Finneran et al., 2002a; Gu et al., 2005; Liu et al., 2005; Nyman et al., 2006). However, none of these studies has directly compared the aqueous uranium concentrations with the extent of uranium reduction for a suite of electron donors. In this study, we relate the electron donors to the rate and extent of uranium reduction in biostimulated sediments using microcosms. The pattern of electron acceptor use, the rate of U removal from solution in microcosms, the extent of reduction in sediment slurries, and the members of the microbial community are considered.

Materials and Methods

Microcosm Experiments

Microcosms were prepared with materials collected from the Environmental Remediation Sciences Program Oak Ridge Field Research Center (ORFRC) located on the Department of Energy Oak Ridge Reservation in Oak Ridge, Tennessee. Adjacent to a historical liquid waste disposal area for low-level radioactive waste, the ORFRC is contaminated with acidic uranium, nitrate, and various minor inorganic, radionuclide, and organic constituents. An uncontaminated background area served as a source of humic materials. Sediment coring, isolation of humic acids, and ground water collection were accomplished by ORFRC staff.

Sediment and ground water manipulations, microcosm construction, and sampling occurred within an anaerobic chamber (97:3 N₂:H₂). Sediment samples were homogenized and added to serum bottles. Four replicates were performed for each electron donor treatment, and each microcosm used 20 g of sediment (Area 2) and 80 mL of ORFRC ground water. The pH of the microcosms was adjusted to 7.0 using sodium bicarbonate. Electron donors were added at equivalent electron donor potential, as determined by balancing of half-reactions (e.g., Thauer et al., 1977): methanol (40 mmol L⁻¹), ethanol (20 mmol L⁻¹), and glucose (5 mmol L⁻¹). An additional duplicate microcosm set was performed with 10 mmol L⁻¹ glucose. Unamended controls were also included in each experiment. The microcosms were kept in an anaerobic chamber during the experiment. Additional uranium or other solutes were not added beyond that found in the ORFRC sediment and ground water.

Aqueous Chemical Measurements

Samples for chemical analysis were withdrawn using sterile needles and syringes within the anaerobic chamber. Samples for U(VI) determination were centrifuged, diluted 50-fold in 0.1 mol L⁻¹ HNO₃, and analyzed by kinetic phosphorescence

analysis (Chemchek Instruments, Inc., Richland, WA). Nitrate and sulfate were analyzed by ion chromatography (IonPac AS-14 analytical column and an AG-14 guard column; Dionex DX-120; Dionex, Sunnyvale, CA) after dilution in double deionized/ultrafiltered water.

X-ray Absorption Spectroscopy

X-ray absorption spectroscopy (XAS) was used to determine the average valence state of U in microcosm samples. Measurements were performed at the Advanced Photon Source on two different occasions using two different beamlines. For all samples except ethanol (77 d) and glucose (5 mmol L⁻¹), sediment slurries were transferred within an anaerobic chamber to 0.8 × 2 cm polycarbonate sample holders. Hydroxy-sulfate green rust was synthesized and reacted with a U(VI)-acetate solution (O'Loughlin et al., 2003) for use as a U(IV) standard. Within the anaerobic chamber, sample holder openings were covered by sections of Kapton film, sealed from air with Kapton tape, and placed in an additional bag of Kapton film as secondary containment. The samples were stored in N₂-flushed centrifuge tubes until measurement. Most spectra were collected at PNC-XOR, beamline 20BM (Heald et al., 1999). Simultaneous measurements in the transmission and fluorescence were recorded. An yttrium foil was placed between the transmission and reference ionization chambers and used as an energy reference standard. Several scans were averaged for each measurement, and periodically the Y K-edge was measured for subsequent scan alignments. Spectra were collected using an unfocused beam on the samples as prepared. On a separate occasion, ethanol (77 d) and glucose amended microcosm sediments were analyzed at MRCAT, beamline 10ID (Segre et al., 2000). Data processing, including alignment, scan averaging, and linear combination fitting, was performed using Athena software (Ravel and Newville, 2005). The averaged spectrum for ethanol (153 d) was smoothed with three iterations of interpolative smoothing before linear combination fitting analysis.

16S Clone Libraries

Detailed microbial community analyses were performed in duplicate on sediments from ethanol- and methanol-amended microcosms. Community genomic DNA (cgDNA) was extracted from approximately 1.5 mL of ground water/sediment (v/w, 4/1) using the PowerSoil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA). Approximately 0.1 to 0.6 µg of cgDNA was isolated from each sample. The purified cgDNA was amplified using Taq polymerase and bacteria-specific primers targeted to *Escherichia coli* 16S rRNA positions 8 to 27 (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1510 to 1492 (5'-GGT TAC CTT TTA CGA CTT-3'). The resulting polymerase chain reaction product of approximately 1.5 kb contained essentially the complete 16S rRNA gene. Polymerase chain reaction products were purified from UltraPure Agarose (Invitrogen, Carlsbad, CA) using QIAquick Gel Extraction kit (QUAGEN Inc., Valencia, CA). Polymerase chain reaction products were ligated in pCR 2.1-TOPO vectors (Invitrogen), transformed into One Shot Mach1-T1 chemically competent *E. coli*, and plated onto LB agar containing 50 µg mL⁻¹ kanamycin and X-gal. Transfor-

ments were incubated overnight at 37°C and 96 white colonies were selected. Those colonies were grown separately overnight in LB broth containing 50 µg mL⁻¹ kanamycin at 37°C with aeration. Clones were then sequenced using the BigDye Terminator v3.1 Cycle Sequencing kit and a reverse primer targeting positions 536 to 519 of *E. coli* 16S rRNA gene (5'-G(A/T)A TTA CCG CGG C(G/T)G CTG-3') (Lane, 1991). Sequences were determined by resolving the sequence reactions on an Applied Biosystems 3730 automated sequencer. Clone libraries were not checked for chimeric sequences because of the short length of sequenced 16S rRNA fragments (<500 bp). Sequences were aligned against the most similar sequences in the Ribosomal Database Project II and assigned to a set of hierarchical taxa using a Naïve Bayesian rRNA classifier (<http://rdp.cme.msu.edu/classifier/classifier.jsp>) version 1.0.

Results and Discussion

Time Course Aqueous Geochemical Data from Microcosm Experiments

The evolution of nitrate (Fig. 1), uranium (Fig. 2), and sulfate (Fig. 3) concentrations followed expected patterns for electron-accepting processes with nitrate used first. Significant reductions in electron-acceptor concentrations were not observed in unamended control samples. Nitrate was completely consumed in all microcosms with added electron donor by 9 to 14 d (Fig. 1), depending on the donor. Microcosms with added methanol and methanol with humic acids reduced nitrate more slowly than those with added glucose or ethanol and required an additional 2 to 3 d before the onset of nitrate reduction. During this initial period of oxygen and nitrate depletion, uranium concentrations increased (Fig. 2). Various processes likely contributed to the initial increase in uranium concentrations within the microcosms. After addition of the ORFRC ground water to the microcosm sediments, changes in the ionic strength and solid/solution ratio were expected to cause the establishment of a new equilibrium between solution and solid. Although some small effect was seen in the control sample, the large effects observed in the donor-amended samples indicated that the increased uranium in solution was likely the result of a biologically mediated process, most likely the production of CO₂ during aerobic respiration and denitrification. At pH ~7, the addition of aqueous carbonate species would result in the formation of aqueous uranyl-carbonate complexes and would be expected to increase the concentration of aqueous U(VI) species (Langmuir, 1978). There may also have been an increase in pH during nitrate reduction, which could have resulted in desorption of U(VI) from sediment and biomass surfaces; the adsorption edge for U(VI) on similar ORFRC sediments was found to be extremely sensitive to pH in the range of 7 to 8 (Barnett et al., 2000). Uranium bound within sediment micropores or microprecipitates would likely have taken days to respond to increases in solution carbonate/bicarbonate species (Giammar and Hering, 2001; Liu et al., 2004; Mason et al., 1997; Phillips et al., 1995; Qafoku et al., 2005).

For microcosms with equivalent donor potential (excluding the 10 mmol L⁻¹ glucose sample), the onset of uranium reduc-

tion did not begin until 20 d or more after donor addition (Fig. 2). The onset of reduction was significantly delayed for methanol-amended microcosms by as much as 15 d. Slight decreases in the U(VI) concentrations between days 12 and 28 were likely due to decreases in pH from fermentation reactions, such as $4\text{CH}_3\text{OH} + 2\text{HCO}_3^- = 3\text{CH}_3\text{COO}^- + \text{H}^+ + 4\text{H}_2\text{O}$ (Adamse and Velzeboer, 1982), that produce acetate and other low-molecular weight organic acids. For all donor-amended microcosms, the total aqueous U(VI) concentrations reached relatively steady values after 34 d (ethanol and 5 mmol L⁻¹ glucose) or 42 d (methanol, methanol with humic acids) in the range of 0.4 to 1 µmol L⁻¹. After the period of rapid reduction of most aqueous U(VI), concentrations continued to fall slowly over the period of 42 to 92 d, reaching final concentrations below the EPA MCL of 30 ppb.

Despite the offset in initiation of U(VI) reduction, the rates of reduction vary by less than a factor of two, in the order 0.55 µmol L⁻¹ d⁻¹ (5 mmol L⁻¹ glucose), 0.8 µmol L⁻¹ d⁻¹ (ethanol), and 0.95 µmol L⁻¹ d⁻¹ (methanol), based on the instantaneous linear slope of the U(VI)(aq) concentration data (Fig. 2). Estimates of total biomass were made for the two treatments subjected to microbial community analysis based on total DNA (Ellenbroek and Cappenberg, 1991). When the instantaneous rates were normalized on an estimated “per bacterial cell” basis, the biomass-adjusted uranium reduction rate was significantly higher for the ethanol-amended microcosms as compared with the methanol-amended microcosms (39.4 nmol h⁻¹ cell⁻¹ vs. 3.4 nmol h⁻¹ cell⁻¹). Biomass estimates are not available from the 5 mmol L⁻¹ glucose microcosms due to the lack of DNA extracted.

In many laboratory and field studies, uranium reduction has been linked to or coincident with iron reduction (e.g., Abdelouas et al., 1998; Anderson et al., 2003; Finneran et al., 2002b; Nevin et al., 2003) and/or sulfate reduction (e.g., Nevin et al., 2003; Nyman et al., 2006; Suzuki et al., 2003; Wu et al., 2006), often with sulfate reduction not occurring until after a time where iron reduction appeared to be the dominant terminal electron accepting process. The time-course data for sulfate in these microcosm experiments did not provide such a straightforward relationship to uranium reduction. Sulfate concentrations ranged from ~0.6 to 0.8 mmol L⁻¹ initially and reached final values <0.1 µmol L⁻¹ (Fig. 3) and varied significantly in their evolution depending on the electron donor. In the case of glucose as the electron donor, sulfate reduction occurred rapidly between days 20 and 25 and took place slightly before and perhaps concurrent with the start of uranium reduction for the 5 mmol L⁻¹ glucose microcosms but after uranium reduction for the 10 mmol L⁻¹ glucose microcosms (Fig. 4). On the other hand, sulfate reduction appeared nearly coincident with uranium reduction for the ethanol-amended microcosms. In the methanol and methanol with humic acid treatments, the bulk of the decrease of U in solution occurred after sulfate reduction (Fig. 4).

One possible explanation for the relationship between sulfate and uranium in these microcosms was that freshly formed metal sulfides, observed in dark color development in the sediment, subsequently reduced U(VI). An accumulation of Fe(II) would be required before the precipitation of iron sulfides, yet uranium reduction did not likely coincide with the timescale for Fe(II)

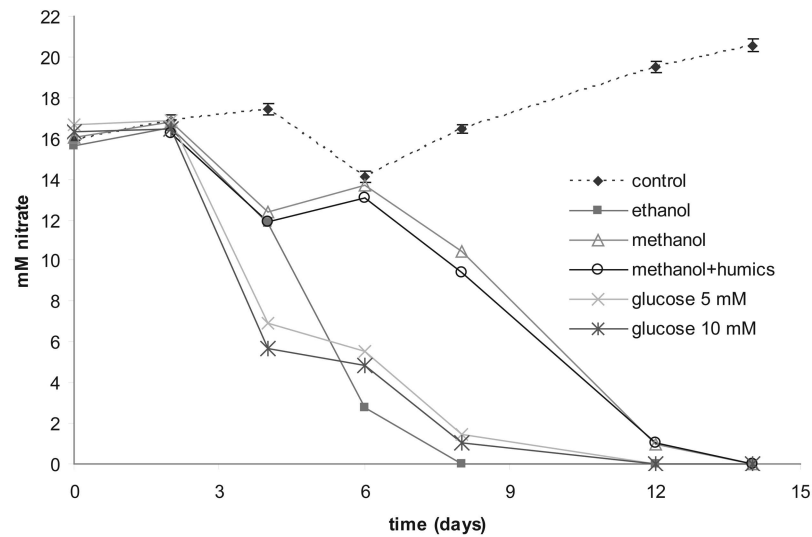


Fig. 1. Aqueous nitrate concentrations from microcosm experiments. Standard errors from replicates are shown as error bars. In many cases, the error bars are smaller than the size of the symbol and cannot be seen.

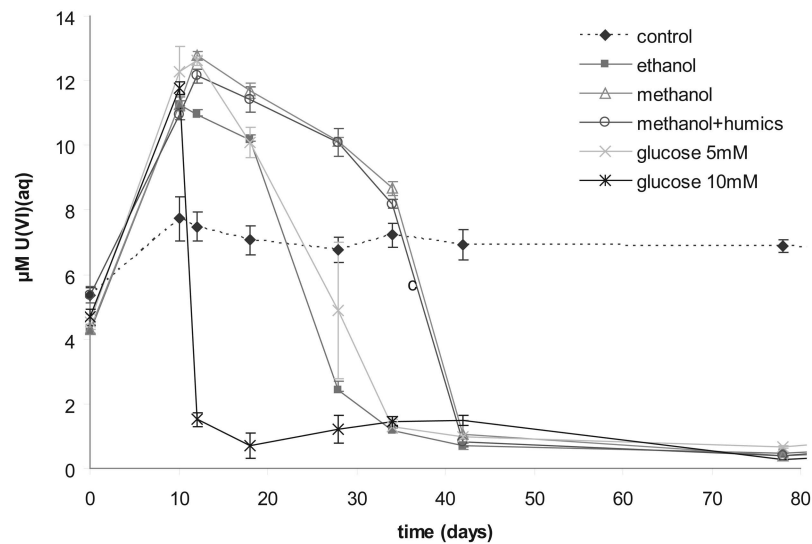


Fig. 2. Aqueous uranium concentrations from microcosm experiments. Standard errors from replicates are shown as error bars.

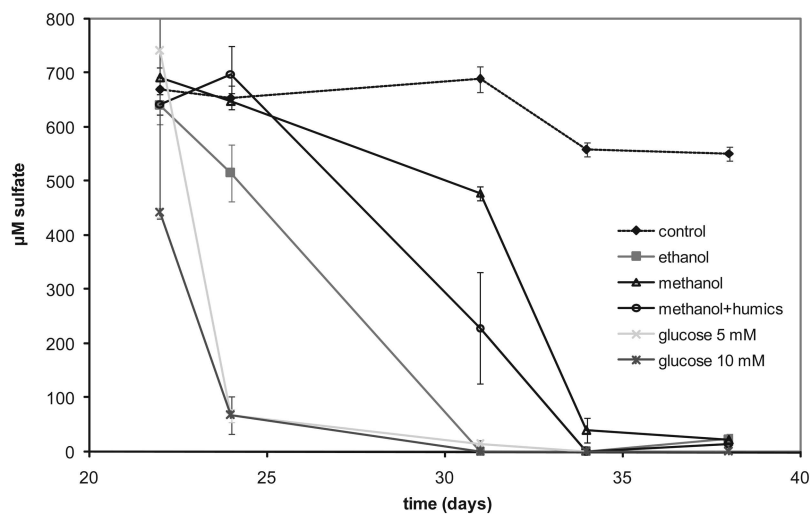


Fig. 3. Aqueous sulfate concentrations from microcosm experiments. Standard errors from replicates are shown as error bars.

buildup. In these microcosms, color changes indicative of iron reduction occurred before decreases in U in solution at day 12 for glucose, day 14 for ethanol, and days 20 to 24 for methanol. Experiments investigating the role of iron cycling on uranium reduction in ORFRC microcosms did not find Fe(II)-Fe(III) hydroxide mineral transformations to be directly related to the formation of U(IV) (Liu et al., 2005). U(VI) reduction by metal sulfides has been demonstrated in laboratory experiments (Eglizaud et al., 2006; Wersin et al., 1994). The rate of U(VI) reduction by sulfide is likely controlled by the proportion of uranyl species in the hydroxide form (Hua et al., 2006), suggesting that this process was not likely to proceed rapidly in complex ionic-biological systems. For example, methanol-amended enrichment cultures from ORFRC sediments were able to reduce uranium over similar timescales, whereas sterilized replicates containing cysteine did not (Madden et al., 2007); this finding is consistent with other studies where sulfide was a poor reductant for U(VI) in bioremediation-relevant systems (e.g., Abdelouas et al., 1998; Fox et al., 2006; Lovley and Phillips, 1992). The interactions between U and S depend on their exact and complicated distribution between various aqueous, complexed, surface-bound, and precipitated forms (e.g., Beyenal et al., 2004; Neal et al., 2004; Sani et al., 2005).

Humic materials have the potential to act as electron shuttles (e.g., Nevin and Lovley, 2000); however, the addition of 30 ppm ORFRC humic acid materials caused no significant differences in the evolution of U(VI)(aq) concentrations (Fig. 2) in these microcosms.

X-ray Absorption Spectroscopy and the Extent of Uranium Reduction

X-ray absorption spectroscopy was performed on microcosm sediment slurries. Samples for XAS were collected independently from microcosms sampled for aqueous chemical data. Due to the availability of beam time and logistical considerations, the number of days between donor addition and sampling for XAS varied for the various treatments (Fig. 5). Microcosm sediment slurries were found to contain a mixture of U(IV) and U(VI) (Fig. 5). The percentage of uranium as U(IV) relative to U(VI) was determined by linear combination fitting to be greatly dependent on the electron donor among: ethanol (7% reduced after 77 d and 5% reduced after 153 d), glucose (49% reduced after 53 d), and methanol (93% reduced after 90 d). The control sample was completely oxidized within the error of measurement after 90 d. Many factors contribute to the error in the linear combination fitting, such that the reported values can be considered estimates within approximately 10%.

It is likely that different geochemical pools of uranium were accessible to bioreduction depending on the electron donor. Sediment materials used in these experiments were homogenized core materials from ORFRC well FB088 in Area 2 over the depth range of 19 to 30 ft. For sediments from this area and depth, Moon et al. (2006) measured an average concentration of 2 mol L⁻¹ nitric acid–extractable (total) U to be approximately 97 mg kg⁻¹. Considering the microcosm solid:solution

ratio of 1:4 and highest values of approximately 3 mg L⁻¹ U(VI) measured by kinetic phosphorescence analysis in the microcosm solutions, at the start of the experiments approximately 11% of the U(VI) was likely in solution, whereas approximately 89% remained in the solid phase. Although almost all uranium was reduced with methanol as the electron donor, lower percentages of uranium reduced with ethanol and glucose amendment suggest that much of the solids-associated U(VI) remained inaccessible to microbial reduction during the time course of the microcosm experiments with those donors. For example, Moon et al. (2006) demonstrated that only approximately 40% of the total nitric-acid–extractable uranium could be recovered with oxalate or citrate-dithionate-bicarbonate extractions. Thus, it was likely in the ethanol samples that only aqueous uranium was reduced, whereas in the glucose sample a majority of both aqueous and sorbed U(VI) was reduced, leaving larger U(VI) precipitates and coprecipitates known to be found at the ORFRC (Stubbs et al., 2006) unreduced. It may be possible that with time, the glucose-amended microcosms would become more reduced. However, U XAS spectra of ethanol-amended microcosms after 77 and 153 d were not significantly different.

Characterization of Microbial Communities in Electron Donor–Amended Microcosms

The implications of similar reduction rates but vastly different extent of reduction have great significance for designing field bioremediation experiments. Despite the disadvantage of a significant lag time, methanol addition led to nearly complete uranium reduction. The lag time does not represent slower utilization of O₂ and nitrate because nitrate concentrations were below detection limit by 14 d. Rather, the lag may be interpreted as the time required for accumulation of acetate and other metabolic byproducts through the activities of fermentative methanol-using bacteria. The extent of reduction may be related to the relative proportion of electron donor converted to CO₂ vs. organic fermentation products. Edwards et al. (2007) demonstrated that in ORFRC microcosms, 14 and 44% of added ethanol and glucose were converted to organic fermentation products, respectively. It is perhaps coincidental that these numbers correspond closely to the trend in extent of U(VI) reduction in this study, as measured by X-ray absorption spectroscopy, or it might be that the buildup of fermentation products maintains a lower redox potential and facilitates the continuation of microbial electron flow that would have ceased on conversion of reduced carbon forms to CO₂.

The pathways of electron donor use are controlled by relationships between microbial community diversity and sediment/ground water biogeochemistry. In 16S rRNA clone libraries from DNA extracted from the methanol- and ethanol-amended microcosms after Day 77, sequences from δ -proteobacteria were dominated by *Geobacter* spp. (Table 1). *Geobacter* spp. previously tested in pure cultures were not able to metabolize methanol directly (e.g., Coates et al., 1996; Nevin et al., 2005; Sung et al., 2006). Enrichment cultures from ORFRC sediments containing 30 mmol L⁻¹ methanol

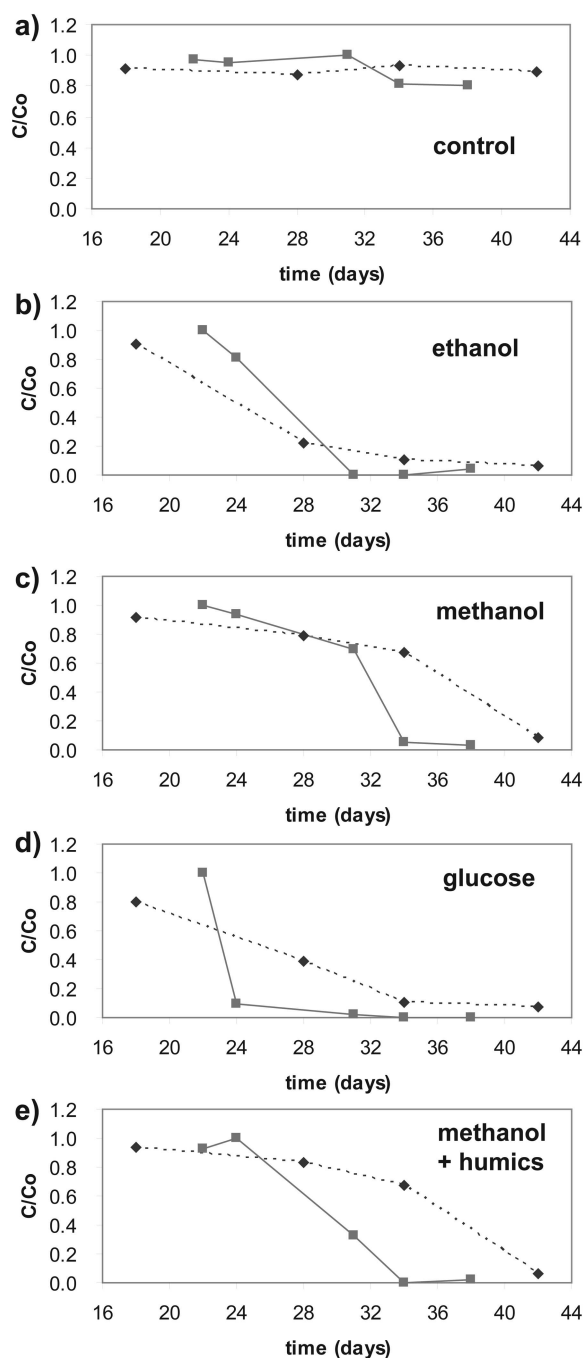


Fig. 4. Comparison between uranium (dashed line) and sulfate (solid line) aqueous concentrations, normalized to the highest concentrations in each time-course experiment. Only the 5 mmol L⁻¹ glucose data are included, containing equivalent electron donor potential to the other microcosms.

but without oxygen or nitrate produced 8.5 mmol L⁻¹ acetate in anaerobic basal medium after 4 d (data not shown), demonstrating the community capacity to rapidly generate significant acetate concentrations that may be further processed by *Geobacter*, which have been linked to uranium reduction at field sites where acetate was added directly (Anderson et al., 2003; Chang et al., 2005; Holmes et al., 2002). *Trichlorobacter*, found in both treatments, is closely related to *Geobacter*, with

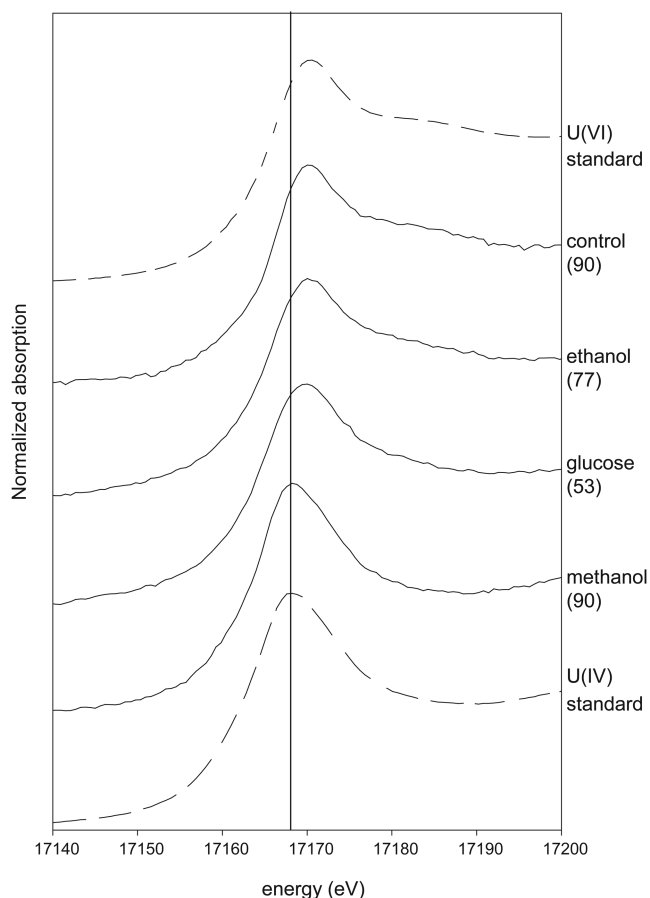


Fig. 5. U L₃-edge X-ray absorption spectra of microcosm sediments and sediment slurries, uranyl nitrate standard, and UO₂-green rust standard. The number in parentheses for each sample description represents the number of days between electron donor addition and sampling. The vertical line is a visual aid for comparison across the samples.

at least one member recently reclassified as *Geobacter* (Nevin et al., 2007). *Geothrix*-like sequences were recovered only in ethanol-amended microcosms. *Geothrix fermentans* has previously been associated with uranium reduction in contaminated sediments at the ORFRC (Brodie et al., 2006). Sequences similar to *Rhodospirillum rubrum* were recovered from the methanol-amended microcosms. Some strains have been shown to couple oxidation of acetate to metal reduction, although not uranium reduction, and could not grow with methanol (Finneran et al., 2003). Other known potential uranium reducing bacteria found in donor-amended sediments and known to directly use ethanol and methanol for acetogenesis include *Clostridium* and *Desulfosporosinus* (Table 1).

The results of this study indicate potential advantages of using metal remediation strategies that consider a broader view of the microbial community metabolic capacity for poisoning the redox potential toward metal reduction and also rapid enzymatic metal reduction. However, the generality of the results needs to be examined by additional sampling at other locations. We have seen in other experiments at the ORFRC site that methanol addition does not always lead to U reduction (e.g., Palumbo et al., 2005), and the underlying reasons for

Table 1. Presence of major taxonomic groups based on the development of 16S rRNA clone libraries contain approximately 100 clones per sample, along with prevalent subgroups.

Taxonomic unit	Ethanol	Methanol	Prevalent subgroups ethanol	Prevalent subgroups methanol
α-proteobacteria	+	+	<i>Rhizobium</i>	<i>Brevundimonas</i> , <i>Rhizobium</i>
β-proteobacteria	+	+	<i>Azoarcus</i> , <i>Acidovorax</i> , <i>Vogesella</i> , <i>Janthinobacterium</i> , <i>Diaphorobacter</i>	<i>Rhodoferrax</i> , <i>Polaromonas</i> , <i>Janthinobacterium</i>
δ-proteobacteria	+	+	<i>Geobacter</i> , <i>Trichlorobacter</i>	<i>Geobacter</i> , <i>Trichlorobacter</i>
γ-proteobacteria	+	n.d.†	<i>Escherichia</i> , <i>Rhodanobacter</i> , <i>Pseudomonas</i>	
Bacteroidetes	+	+	<i>Sphingobacteria</i> (<i>Roseivirga</i>)	<i>Sphingobacteria</i> (<i>Roseivirga</i>)
Firmicutes	+	+	<i>Cryptanaerobacter</i> , <i>Dehalobacter</i> , <i>Clostridium</i> , <i>Desulfosporosinus</i>	<i>Desulfosporosinus</i> , <i>Clostridium</i> , <i>Acetivibrio</i>
Actinobacteria	+	n.d.	<i>Coriobacteriaceae</i>	
Acidobacteria	+	n.d.	<i>Geothrix</i>	
Genera incertae sedis	n.d.	+		OP10

† Not determined.

these differences in response need to be better understood. Substrates such as ethanol and acetate, which are rapidly used by a diverse community and are more often converted directly to CO₂, do not provide as many long-term reducing equivalents to the subsurface, whereas methanol is used more slowly by fewer members of the community and is likely to be less often converted directly to CO₂. Previous studies have shown an inverse relationship between the extent of nitrate-dependent U(IV) oxidation and the system reductive capacity, whereas the rate of oxidation was constant (Senko et al., 2005). The production of a more completely reduced subsurface, including higher levels of organic fermentation products and biomass, will also lead to the production of Fe²⁺ and reduced S species that will buffer against infiltrating oxygen. The choice of electron donors may significantly influence the effectiveness of bioremediation and long-term immobilization of uranium.

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