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ENVIRONMENTAL BIOTECHNOLOGY

Influence of bicarbonate, sulfate, and electron donors on biological reduction of uranium and microbial community composition

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Abstract A microcosm study was performed to investigate the effect of ethanol and acetate on uranium(VI) biological reduction and microbial community changes under various geochemical conditions. Each microcosm contained an uranium-contaminated sediment (up to 2.8 g U/kg) suspended in buffer with bicarbonate at concentrations of either 1 or 40 mM and sulfate at either 1.1 or 3.2 mM. Ethanol or acetate was used as an electron donor. Results indicate that ethanol yielded in significantly higher U(VI) reduction rates than acetate. A low bicarbonate concentration (1 mM) was favored for U(VI) bioreduction to occur in sediments, but high concentrations of bicarbonate (40 mM) and sulfate (3.2 mM) decreased the reduction rates of U(VI). Microbial communities were dominated by species from the *Geothrix* genus and *Proteobacteria* phylum in all microcosms. However, species in the *Geobacteraceae* family capable of reducing U(VI) were significantly enriched by ethanol and acetate in low-bicarbonate buffer. Ethanol increased the population of unclassified *Desulfuromonales*, while acetate increased the population of *Desulfovibrio.* Additionally, species in the *Geobacteraceae* family were not enriched in high-bicarbonate buffer, but the *Geothrix* and the unclassified *Betaproteobacteria* species were enriched. This study concludes that ethanol could be a better electron donor than acetate for reducing U(VI) under given experimental conditions, and electron donor and groundwater geochemistry alter microbial communities responsible for U(VI) reduction.

Keywords Uranium(VI) · Biological reduction · Electron donor · Groundwater · Microbial community · 16S rRNA analysis

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Introduction

Uranium mining and enrichment activities during the Cold War produced large amounts of radioactive wastes and contaminated significant volumes of soil and groundwater at the US Department of Energy's (DOE) complexes (Hazen and Tabak 2005). Uranium commonly exists in the environment either as oxidized, hexavalent U(VI) or as reduced, tetravalent U(IV) species, and its fate and transport are largely influenced by these oxidation states. Under oxic conditions, U(VI) forms carbonate complexes such as $UO_2(CO_3)_2^{2-}$ or $UO_2(CO_3)_3^{4-}$ when groundwater contains carbonate and bicarbonate anions at pH > 6.5 (Langmuir 1978). These U(VI) complexes are highly soluble and mobile in the subsurface environment. On the other hand, reduced U(IV) forms precipitates under

anaerobic conditions. Therefore, in the past decade, biologically mediated reduction of U(VI) to sparingly soluble U(IV) has been extensively explored as an alternative approach to remediate U(VI)-contaminated sites (Hazen and Tabak 2005).

Selection of electron donor source is essential for successful implementation of biological reduction of uranium in situ by stimulating the growth of indigenous microorganisms. Under anaerobic conditions, metal-reducing bacteria have limited ability to metabolize high-molecular-weight compounds such as proteins, celluloses, or long-chain fatty acids (Kourtev et al. 2006). Low molecular weight organics such as ethanol, acetate, lactate, glucose, and formate have been selected as preferred electron donors in U(VI) bioreduction studies (Finneran et al. 2002). In field trials, acetate was successfully used as an electron donor (Anderson et al. 2003; Holmes et al. 2002; Nevin et al. 2003; Vrionis et al. 2005), while Ortiz-Bernad et al. (2004) indicate that acetate has limited effect in stimulating the reduction of solid-phase U(VI). At the US DOE's Oak Ridge site, we found that ethanol stimulated the reduction of U(VI) at a faster rate than acetate and lactate (Wu et al. 2006). These observations were attributed to the fact that different electron donors have stimulated different microbial communities under site-specific geochemical conditions.

Among various factors, bicarbonate could significantly impact the microbial community structure and the biological reduction of U(VI). Bicarbonate concentration levels can lead to a change in groundwater pH and partitioning of U(VI) in the solid and solution phases (Phillips et al. 1995; Zhou and Gu 2005). High biocarbonate with calcium (0.45–5 mM) is also known to cause the formation of Ca–uranyl-carbonate complex, which inhibits the bioreduction of U(VI) by both Fe-reducing bacteria (FeRB) and sulfate-reducing bacteria (SRB)(Brooks et al. 2003). Sulfate is another geochemically important anion with controversial influences on the bioreduction of U(VI). Sulfate supports the growth of SRB such as *Desulfovibrio* spp., which are capable of reducing U(VI) (Lovley and Phillips 1992). However, sulfate could also support the growth of sulfate reducers such as *Desulfobacter* spp., which are unable to reduce U(VI) but utilize acetate, competing with U(VI)-reducing bacteria for electron donors (Lovley et al. 1993).

In this study, factorial microcosms were designed to investigate the interactions of the effects of electron donors (ethanol and acetate) and geochemical conditions (bicarbonate and sulfate) on U(VI) biological reduction. The associated microbial community was monitored during biostimulation using 16S ribosomal ribonucleic acid (rRNA) cloning library methods to better understand the microbial processes underlying U(VI) bioreduction.

Materials and methods

Microcosm setup

The U(VI)-contaminated sediment was collected from well FW026 at the DOE Field Research Center at Oak Ridge, TN. The sediment sample was stored in a glass bottle filled with site groundwater at 4°C for 2 months before use. A factorial experimental design was employed for the microcosm study, and three factors were considered, including bicarbonate (1 vs 40 mM), sulfate (1.1 vs 3.2 mM), and electron donors (ethanol, acetate, and control). The low concentration level of bicarbonate was to simulate the condition used in our field pilot-scale test (Wu et al. 2006; 2007), while the high level was to test if U(VI) reduction may be accelerated through the extraction or desorption of solid-phase U(VI) by bicarbonate (Phillips et al. 1995; Ortiz-Bernad et al. 2004; Zhou and Gu 2005). Two concentration levels of sulfate were used to test if an elevated sulfate concentration may inhibit the reduction of U(VI). Therefore, microcosms were performed in the following four buffer solutions: S1C1 contained 1.1 mM Na_2SO_4 and 1 mM $KHCO_3$ (pH = 7.7), S2C1 contained 3.2 mM Na_2SO_4 and 1 mM $KHCO_3$ (pH = 7.8), S1C2 contained 1.1 mM Na_2SO_4 and 40 mM $KHCO_3$ (pH = 9.3), and S2C2 contained 3.2 mM Na_2SO_4 and 40 mM $KHCO_3$ (pH = 9.4). Note that a relatively high pH observed in the 40 mM bicarbonate buffer was due to degas or a loss of CO_2 because samples were purged with nitrogen and experiments were performed in a N_2-filled glove box. A relatively high pH (>7.5) used in these studies is also to minimize the abiotic reduction of U(VI) to U(IV) by sulfide (Hua et al. 2006).

Table 1 Geochemical properties of the FRC FW026 well sediment and the microcosm solution

Analyte	Sediment (g/kg)	Microcosms (mg/L)			
		S1C1	S2C1	S1C2	S2C2
Fe	101.4	0.3	0.5	0.1	0.1
Al	163.2	0.6	1.0	0.4	1.1
Mg	22.8	2.2	2.5	2.3	1.2
Mn	1.6	0.7	0.7	0.3	0.1
Ca	5.6	16.0	18.0	10.7	3.4
Si	3.8	0.9	1.0	0.6	0.6
P	5.8	0.0	0.1	0.2	0.1
U	2.8	1.8	2.2	8.2	9.1
SO_4^{2-}	ND	107.0	255.0	143.5	307.5
TOC	5.98	10.8	10.2	108.7	119.6

S1C1 1.1 mM Na_2SO_4 and 1 mM $KHCO_3$, *S2C1* 3.2 mM Na_2SO_4 and 1 mM $KHCO_3$, *S1C2* 1.1 mM Na_2SO_4 and 40 mM $KHCO_3$, *S2C2* 3.2 mM Na_2SO_4 and 40 mM $KHCO_3$, *ND* not determined

The microcosms were established in an anaerobic glove bag (N_2/H_2 = 98:2) by adding 6 g of sediment (dry weight) into 160-mL serum bottles and adding 120 mL of one of the prepared buffer solution, which was prepurged with N_2 for 1h to remove dissolved O_2. Next, 110 μL of 2.2 M ethanol or 2.2 M Na-acetate or water (as no-electron-donor control) was added to the serum bottles. The initial concentrations of ethanol and acetate were approximately 2 mM. Microcosms without addition of electron donors were used as controls, although the sediment sample contains soil organic matter at about 6 g total organic carbon (TOC)/kg, which could also be used as an electron donor source. The serum bottles were sealed with thick rubber septa and aluminum crimp caps and placed upside down on a rotary shaker at 23°C at a speed of approximately 30 rpm. The microcosms were kept in the dark by covering them with aluminum foil. Duplicates were prepared for each treatment. At days19, 26, 37, and 47 into the incubation, more ethanol or acetate was added (110 μL).

Sampling and chemical analysis

The aqueous chemical compositions of microcosm were determined with inductively coupled plasma–mass spectrometry (ELAN 6000; Table 1). To determine the elemental composition, the sediment was digested with addition of concentrated nitric acid and 30% hydrogen peroxide according to US EPA SW-846 Method 3050B. The TOC content was determined by combustion technique using US EPA Method 9060A.

At various time intervals, samples in serum bottles were taken by removing the crimp caps and rubber septa in the anaerobic glove bag. Four-milliliter samples were taken from each bottle and centrifuged at 12,000 × *g* for 5 min. The supernatant was immediately analyzed for U(VI) using the steady-state phosphorescence method (Gu et al. 2005a). Ferrous iron in the supernatant was determined promptly by a modified ferrozine method (Lovley et al. 1993). The supernatant was acidified with 0.1 mL of 1 M HCl and stored at 4°C before analyzing for sulfate, ethanol, and acetate content with an ion chromatograph or gas chromatograph, respectively (Gu et al. 2005b). After centrifugation, the sediments were stored at −80°C for 16S rRNA cloning library analysis.

16S rRNA analysis

Sediment nucleic acids were extracted with the UltraClean soil deoxyribonucleic acid (DNA) kit (MoBio Laboratories, Solana Beach, CA) following the manufacturer's protocol.

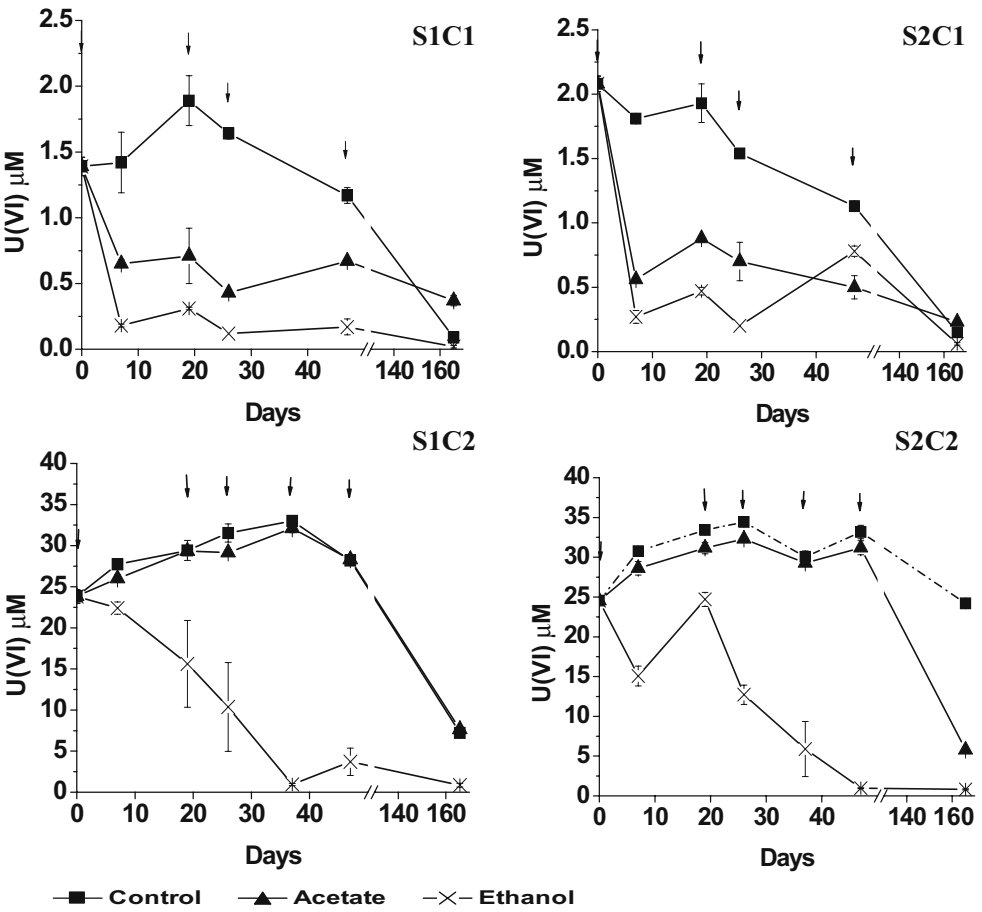

Fig. 1 The effect of electron donor amendments on U(VI) biological reduction at different bicarbonate and sulfate concentrations. S1C1 (1.1 mM Na_2SO_4, 1 mM $KHCO_3$), S1C2 (1.1 mM Na_2SO_4, 40 mM $KHCO_3$), S2C1 (3.2 mM Na_2SO_4, 1 mM $KHCO_3$), S2C2 (3.2 mM Na_2SO_4, 40 mM $KHCO_3$). *Arrows* indicate additions of ethanol or acetate

Community 16S rRNA genes were amplified by polymerase chain reaction (PCR) in 30-μL mixtures containing 10 ng DNA template, buffer containing 50 mM KCl, 1.5 mM $MgCl_2$, and 10 mM Tris–HCl (pH8.4), a 0.25 mM 4× deoxyribonucleotide triphosphate, 250 mM each of the forward and reverse primers, and 1U *Taq* DNA polymerase. The forward primer was the bacteria-specific 27F (5′-AGA GTT TGA TCC TGG CTC AG-3′), and the reverse primer was the universal 1492R (5′-GGT TAC CTT GTT ACG ACT T-3′)(Lane 1991). The PCR temperature program was initiated with a 30 s hot start at 80°C, then 94°C for 4 min, followed by 30 cycles at 94°C for 1 min, 57°C for 30 s, and 72°C for 1.2 min, and completed with an extended period of 10 min at 72°C with a GeneAmp 9700 thermocycler (Applied Biosystems, Foster City, CA)(Fields et al. 2005).

The quantified products were ligated with PCR 2.1-TOPO vector in the TA Cloning Kit (Invitrogen, Carlsbad, CA), and transformed into TOP10-competent *Escherichia coli*. The correct inserts were determined by PCR amplification with the 27F forward primer and 1492R reverse primer. The amplified 16S ribosomal DNA PCR products were used as templates for sequencing with the Prism BigDye terminator v3.1 Cycle sequencing kit (Applied Biosystems), and partial sequences were obtained with the universal primer 519R (5′-G(AT)A TTA CCG CGG C(GT)G CTG-3′) on an automated 3730XL sequencer (Applied Biosystems).

The obtained sequences were aligned with the BioEdit alignment program 7.0. Chimeric, and sequences were checked using the program Check Chimera. The resulting sequences were imported to Web-based Ribosomal Database Project II Release 9 to estimate the phylogenetic affiliations of the partial sequences with the program Classifier and establish the phylogenetic evolutionary tree with the neighbor-joining method using the Jukes–Cantor model. The inferred phylogeny was tested by bootstrap replicate 1,000 times. Rarefaction analyses were conducted with DOTUR-1.53 to estimate the diversity of the microbial community (Schloss and Handelsman 2005).

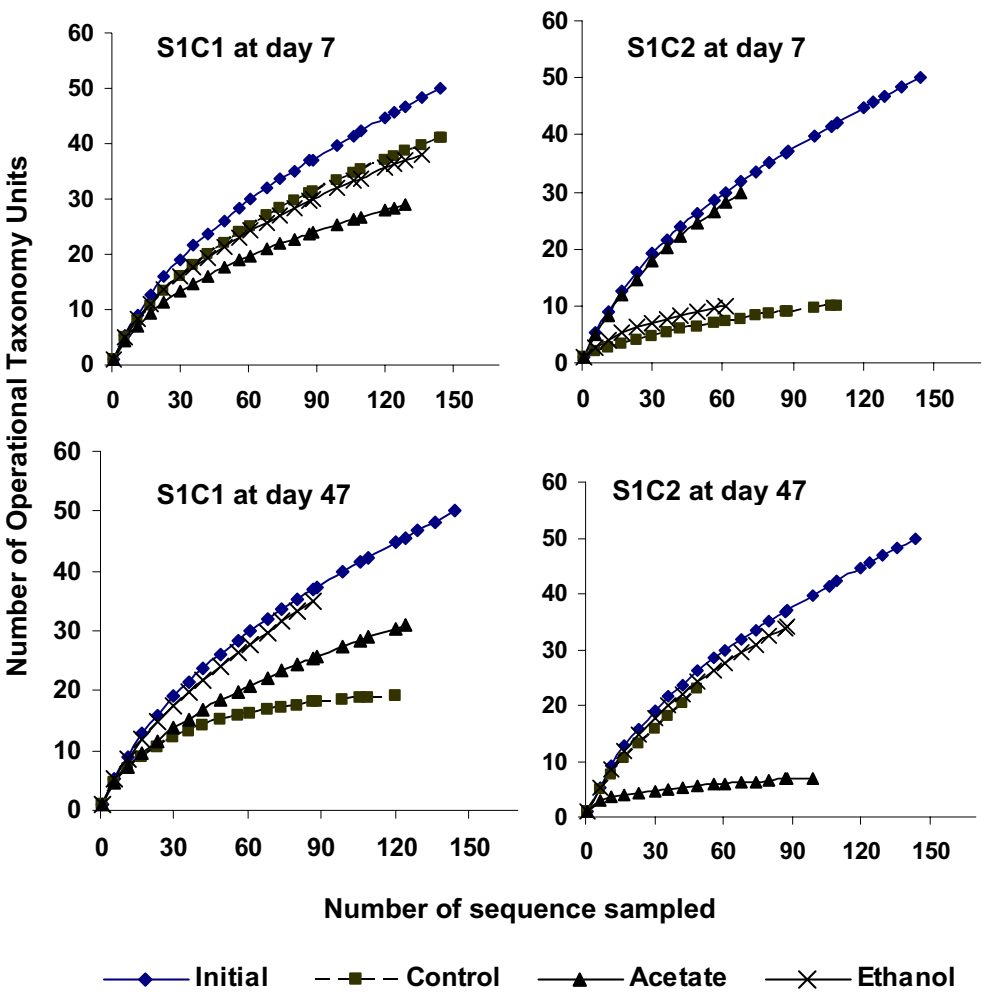

Fig. 2 Rarefaction curves generated using the furthest neighbor assignment algorithm with the 16S rRNA gene library. S1C1 (1.1 mM Na_2SO_4, 1 mM $KHCO_3$), S1C2 (1.1 mM Na_2SO_4, 40 mM $KHCO_3$)

Results

U(VI) bioreduction in microcosms

With electron donor amendments, the aqueous-phase U(VI) concentration in microcosms all decreased during the 47-day incubation with the exception of acetate-amended S1C2 and S2C2 (Fig. 1). In buffers containing the lower concentration of bicarbonate (S1C1 and S2C1), aqueous U (VI) decreased rapidly with ethanol (up to 90%) and acetate (up to 70%) in 7 days of incubation. However, in buffers with the higher bicarbonate concentration (S1C2 and S2C2), about 90% of U(VI) was removed in 47 days of incubation with ethanol amendment, but acetate amendment had no noticeable effect on U(VI) removal.

After 47 days of incubation, the U(VI) concentration in ethanol-amended microcosms with S1C1 buffer had decreased to less than 0.18 μM. Without further electron donor amendment, ethanol-treated and control microcosms had U(VI) concentrations of less than 0.2 μM on day 166 in low-bicarbonate buffers (S1C1 and S2C1), whereas acetate-treated microcosms had U(VI) concentrations of 0.23–0.37 μM (Fig. 1). In high-bicarbonate buffers (S1C2 and S2C2), U(VI) in the ethanol-amended microcosms dropped to 0.8 μM, and acetate-amended microcosms had final U(VI) concentrations of 5–7 μM on day 166. The U (VI) concentration dropped to 7.2 μM in the S1C2 control but remained high at 24.2 μM in the S2C2 control.

Ethanol, acetate, Fe(II), and sulfate in microcosms

In a week, ethanol was consumed in all ethanol-amended microcosms, but acetate was generated and accumulated. In acetate-amended microcosms, accumulation of acetate was

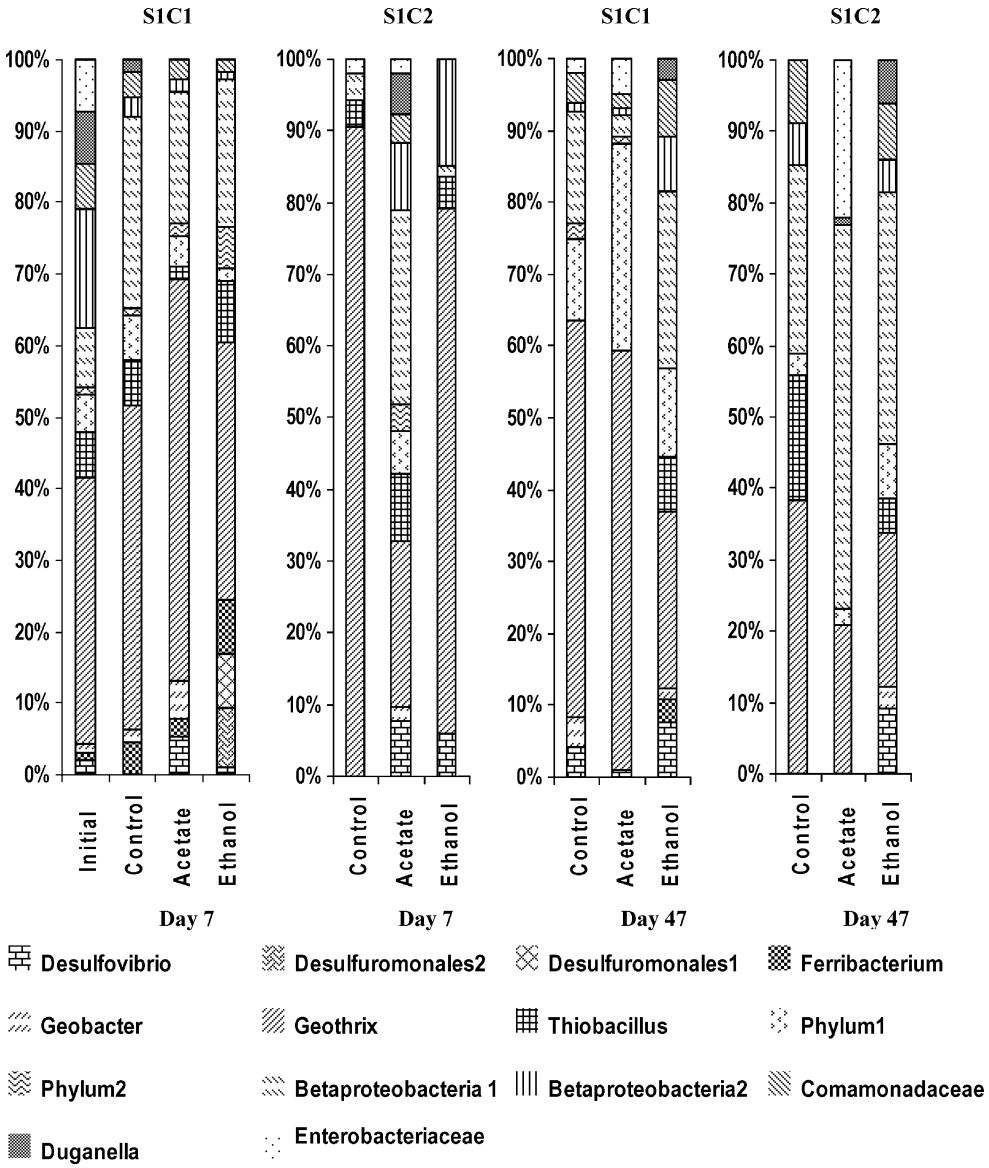

Fig. 3 Influences of ethanol, acetate, and bicarbonate amendments on microbial community changes at genus level with 16S rRNA clone library analysis. The samples were taken at days 7 and 47 of incubation in microcosms with S1C1 and S1C2 treatments. Components that accounted for at least 5% once in the cloning library are shown. The genus-unclassified clones were named according to their classification at family, order, or class. If the clones were not classified at phylum level, "phylum1" and "phylum2" were used

observed, especially in buffers with the high bicarbonate concentration. Ferrous iron accumulated in the ethanol-amended S1C1 but not in the S2C1 (data not shown). Only minimal Fe(II) accumulation was detected in acetate and control treatments. The color of the sulfate-amended microcosms became noticeably darker during the experiment, which is attributed to the precipitation of ferrous sulfide because sulfate concentrations decreased in the order of ethanol > acetate > control in all microcosms.

Microbial community structure

The microbial community diversity in sediment samples varied at different incubation times and with different amendments, as indicated by the rarefaction curves (Fig. 2). The microbial community had the highest diversity just before incubation started. In the low-bicarbonate buffer (S1C1), the no-electron-donor-amendment control showed continuously decreasing diversity at days 7 and 47. In comparison, the diversity indexes of ethanol and acetate treatments were decreasing at day 7 but rebounded by day 47. In the high-bicarbonate buffer (S1C2), the control and ethanol treatments had a significant drop in diversity by day 7 but rebounded by day 47, while the diversity in the acetate-amended microcosms remained high at day 7 but then dropped at day 47.

The initial microbial community in the microcosms, as detected by 16S rRNA gene sequence analysis, was composed primarily of species within the *Proteobacteria* (55.9%) and *Acidobacteria* (36%) at the phylum level. The most abundant (36%) 16S rRNA gene sequences were 92–100% similar to the *Geothrix* genus within the *Acidobacteria* phylum. Another ~20% of 16S rRNA gene sequences had high similarity (91–100%) to established genera, including *Desulfovibrio*, *Thiobacillus*, *Ferribacterium*, *Geobacter*, *Nitrospira*, *Bradyrhizobium*, *Duganella*, *Aquabacterium*, and *Neochlamydia*. The remaining 50% of the 16S rRNA gene sequences had low similarity (<91%) to current genera.

At the lower bicarbonate concentration, species in the *Geothrix* genus accounted for a high percentage (34–55%) in all microcosms at day 7. However, a significant change of microbial community was detected as the *Deltaproteobacteria* component increased in the acetate (9.8%) and ethanol (14%)

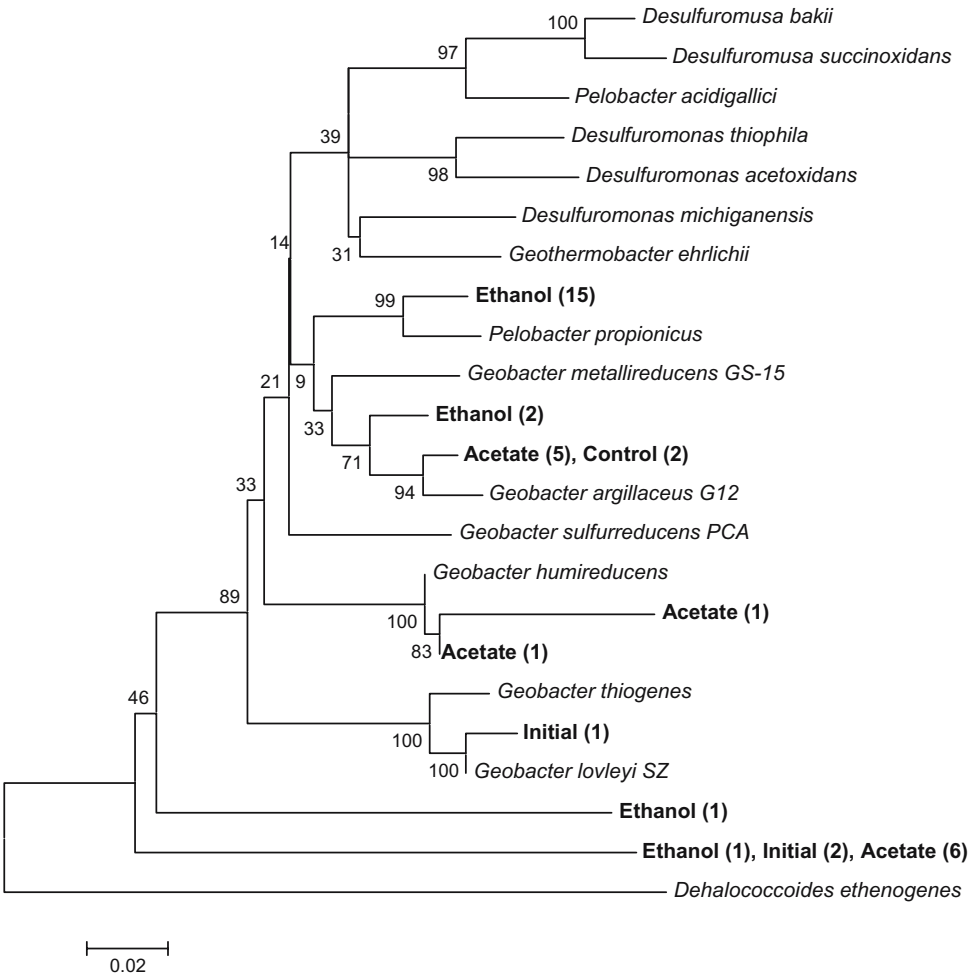

Fig. 4 Phylogenetic affiliation of *Geobacteraceae* clones from samples removed on day 7 from microcosms with low sulfate concentrations (S1C1 and S1C2). Reference sequences were downloaded from the Ribosomal Databases Project II. The sequences from this study (in *bold font*) are indicated by the treatment. *Dehalococcoides ethenogenes* was used as the outgroup of the tree. The numbers of clones are indicated in the *parentheses*

treatments relative to the control (1.4%; Fig. 3). However, different species were enriched in the acetate and ethanol treatments. For example, the *Desulfovibrio* genus in the acetate treatment increased from 0 to 4.5%, and the unclassified *Desulfuromonales* increased in the ethanol treatment from 0 to 12.5%. The closest relatives of the unclassified *Desulfuromonales* from ethanol treatment were *Geobacter* and *Pelobacter*, but they appeared at different positions in the phylogenetic tree (Fig. 4). At the higher bicarbonate concentration (S1C2), the control and the ethanol-treated microcosms had very high percentages of species in the *Geothrix* genus (90 and 80%, respectively) at day 7, but the acetate-treated microcosm had only about 22% *Geothrix* (Fig. 3).

At day 47 in low-bicarbonate microcosms, the percentage of *Geothrix* species was high in the control (56%) and in the acetate-amended treatments (52%) but was comparatively low in the ethanol-treated microcosm at 24% (Fig. 3). A significant decrease in the number of *Deltaproteobacteria* was also found in the acetate treatment, with a concomitant increase in the number of unclassified bacteria in phylum 1 (29%). On the other hand, in the high-bicarbonate microcosms, the once dominant species in the *Geothrix* genus had fallen relative to day 7 in the control and in the ethanol-treated microcosms, while the percentage of unclassified *Betaproteobacteria* 1 increased in all of the microcosms (Fig. 3). Archaea was not determined in this study because of low methanogenic most probable number counts (10^2 cells/g sediments or lower) found in the subsurface sediment (Wu et al. 2007) where the sample was taken.

Discussion

Influence of bicarbonate and sulfate on U(VI) bioreduction

Dependent upon electron donor types, bicarbonate had a significant impact on the bioreduction rate of U(VI). In both low and high concentrations of bicarbonate, ethanol effectively stimulated U(VI) biological reduction (Fig. 1). In contrast, acetate was not effective in enhancing U(VI) reduction at high concentrations of bicarbonate. A possible explanation is that high concentrations of bicarbonate increased the pH to 9, and the growth of acetate-utilizing, U(VI)-reducing microorganisms were inhibited because acetate was only minimally consumed in treatments with high bicarbonate. This observation was supported by studies of Phillips et al. (1995) who found that pure-culture *Desulfovibrio desulfuricans* with acetate as a carbon source reduced uranium at a much slower rate in 100 mM bicarbonate than in 30 mM bicarbonate buffer. While Küsel and Dorsch (2000) found that acetate was not used under acidic conditions (pH < 5) in freshwater lake sediment, we found that acetate was not effectively used in alkaline conditions (pH > 8.5). These results suggest that acetate may work in a narrow neutral pH range for U(VI) bioreduction but may not work well under either low or high pH conditions.

A high bicarbonate concentration resulted in higher U (VI) concentrations in the aqueous phase than a low bicarbonate concentration (Table 1). This is mainly caused by extraction of sediment-adsorbed or precipitated uranium into the supernatant by bicarbonate (Zhou and Gu 2005). Similarly, several research groups found that microbial respiration caused increases in bicarbonate concentrations, resulting in a rebound in the soluble U(VI) concentration (Anderson et al. 2003; Vrionis et al. 2005). It is thus suggested that an elevated bicarbonate concentration may be used during remediation to increase the bioavailability and therefore the reduction of U(VI) (Ortiz-Bernad et al. 2004) because reduced U(IV) is more stable than adsorbed U(VI) (Kohler et al. 2004). In this study, the U(VI) concentration at day166 in high bicarbonate was 0.8–7 μM, which was considerably higher than the 0.02–0.4 μM in low bicarbonate. These results suggest that an excessive bicarbonate concentration may lead to a decreased microbial activity to reduce U(VI), and relatively low bicarbonate concentrations are likely beneficial to enhance the biological reduction of U(VI).

High sulfate show negative effects on U(VI) bioreduction, especially at high bicarbonate concentrations and with a limited electron donor supply (Fig. 1). In studies with pure SRB cultures, Spear et al. (2000) found that U(VI) reduction was accelerated in the presence of sulfate. However, other studies (including this work) using sediment samples found that U(VI) reduction either was not affected or was decreased in the presence of sulfate (Anderson et al. 2003; Finneran et al. 2002; Nevin et al. 2003; Senko et al. 2002). A possible explanation is that relatively high concentrations of sulfate compete with U (VI) as electron acceptors when electron donor was limited in sediments. A previous study also showed that hydrogen sulfide produced by SRB may abiotically reduce U(VI) to U(IV), although this reaction is inhibited in the presence of high bicarbonate concentration (Hua et al. 2006). This may offer another explanation that U(VI) reduction is inhibited in the presence of high sulfate and bicarbonate.

Influence of electron donor on U(VI) bioreduction

In all microcosms, ethanol was found to be a better electron donor than acetate for stimulating U(VI) biological reduction. Similar results have been observed in pure-culture studies using *Desulfuromonas acetoxidans* (Roden and Lovley 1993), in sediments at low pH (pH = 4) (Shelobolina et al. 2003) and in groundwater (Geets et al. 2005). These

observations may be explained by the fact that energetically, ethanol has a higher number of electron equivalents than acetate; that is, ethanol can provide more energy to sustain U(VI) bioreduction than acetate. Ethanol can also be oxidized to acetate and H_2 (a widely used electron donor for uranium reduction) by several *Pelobacter* species (Schink 1985). This explanation is supported by the microbial community analysis, in which close relatives of *Pelobacter* were enriched by ethanol in S1C1 at day7 when Fe(II) was significantly accumulating and U(VI) was markedly decreasing.

After 166 days of incubation, substantial U(VI) reduction was also observed in microcosms without electron donor amendment controls. The electron donor source here was likely attributed to sediment natural organic matter. Certain microorganisms have been shown to reduce U(VI) by using soil organic matter as an electron donor (Elias et al. 2003; Suzuki et al. 2005). Because soil organic matter usually has relatively large molecules and is involved in complex metabolic pathways, the diverse microbial community present in the sediment may have a synergistic function in coupling organic matter metabolism with U(VI) bioreduction.

Geochemical conditions on microbial community change

Regardless of the amendments and incubation time, the 16S rRNA analysis revealed that a significant percentage of the microbes in the microcosm was related to microorganisms that have the ability to reduce U(VI), Fe(III), and sulfate. For example, species from the *Geothrix* genus are typical FeRB (Coates et al. 1999) that were predominant in the tested sediments, although, to date, no studies have shown their capability to reduce U(VI) to U(IV). In microcosms with low bicarbonate, the ethanol and acetate treatments led to a significant increase in the percentage of species from the *Geobacteraceae* family, which are known for their metal-reducing capabilities. These observations are consistent with results from other U(VI) bioreduction studies (Anderson et al. 2003; Holmes et al. 2002; Vrionis et al. 2005). Our study also revealed that ethanol and acetate enrich different microorganisms in the *Geobacteraceae* family and some other unclassified species.

Microbial community diversity decreased at some points in microcosms with high bicarbonate. Species in *Geothrix* and in unclassified *Betaproteobacteria* were significantly enriched. This result may be attributable to the significant increase in pH from 6.5 to 9 at high bicarbonate concentrations. There have been reports that *Geothrix* are widely distributed in subsurface environments and are capable of growing in bicarbonate buffer (Brodie et al. 2006; Coates et al. 1999; Senko et al. 2002). However, no information is available on the unclassified *Betaproteobacteria*, so their functions remain unknown. Several studies that involved extreme conditions, such as high salinity or acidity, showed that *Geobacteraceae* were not the primary microorganisms responsible for U(VI) reduction in those environments (Nevin et al. 2003; Petrie et al. 2003). In this study, we found that microorganisms from the *Geobacteraceae* family were not the most abundant species in alkaline sediment. Further characterization studies are warranted to elucidate the role(s) of non-*Geobacteraceae* in biological reduction of U(VI).

An important implication of this study is that electron donor choice and site geochemical conditions need to be considered in designing strategies of U(VI) bioreduction for remediating uranium-contaminated sediments and groundwater. The present study is consistent with recent field observations that at a relatively low bicarbonate concentration (1–3 mM), a rapidly decreased uranium concentration in groundwater can be achieved and maintained below the U.S. maximum contaminant level for drinking water (0.03 mg/L) during bioremediation using ethanol as an electron donor (Wu et al. 2007). Additionally, we note that: (1) a significant percentage of microbes in the microcosm is related to those having the ability to reduce U(VI), Fe(III), and sulfate, (2) ethanol and acetate enrich different microorganisms in the *Geobacteraceae* family, and amendments with acetate and ethanol significantly increase the *Geobacteraceae* population at the low bicarbonate concentration, and (3) species in the *Geothrix* genus and unclassified *Betaproteobacteria* are dominant and likely responsible for the reduction of U(VI) at high bicarbonate concentrations.

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