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Uranium reduction and microbial community development in response to stimulation with different electron donors

Melissa Barlett · Hee Sun Moon · Aaron A. Peacock · David B. Hedrick · Kenneth H. Williams · Philip E. Long · Derek Lovley · Peter R. Jaffe

Abstract Stimulating microbial reduction of soluble U(VI) to less soluble U(IV) shows promise as an in situ bioremediation strategy for uranium contaminated groundwater, but the optimal electron donors for promoting this process have yet to be identified. The purpose of this study was to better understand how the addition of various electron donors to uranium-contaminated subsurface sediments affected U(VI) reduction and the composition of the microbial

community. The simple electron donors, acetate or lactate, or the more complex donors, hydrogen-release compound (HRC) or vegetable oil, were added to the sediments incubated in flow-through columns. The composition of the microbial communities was evaluated with quantitative PCR probing specific 16S rRNA genes and functional genes, phospholipid fatty acid analysis, and clone libraries. All the electron donors promoted U(VI) removal, even though the composition of the microbial communities was different with each donor. In general, the overall biomass, rather than the specific bacterial species,

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was the factor most related to U(VI) removal. - Vegetable oil and HRC were more effective in stimulating U(VI) removal than acetate. These results suggest that the addition of more complex organic electron donors could be an excellent option for in situ bioremediation of uranium-contaminated groundwater.

Keywords Subsurface Uranium reduction
Electron donors Microbial community structure

Introduction

Uranium contamination in subsurface environments is a widespread problem at numerous US Department of Energy (DOE) sites (Hazen et al. 2005; Marshall et al. 2009), and its fate and transport are largely influenced by its oxidation state (oxidized: U(VI) or reduced: U(IV)). Uranium can be immobilized in groundwater systems through the reduction of soluble U(VI) to insoluble U(IV) by indirect and direct (enzymatic) processes catalyzed by dissimilatory metal- and sulfate-reducing bacteria that are stimulated by the injection into the subsurface of an electron donor (Anderson et al. 2003; Lloyd et al. 2000; Lovley et al. 1991; Lovley and Phillips 1992; Lovley 1995; Wall and Krumholz 2006; Gorby and Lovely 1992).

Many electron donors have been shown to stimulate known uranium reducers and/or the biological reduction of uranium, including acetate (Finneran et al. 2002; Luo et al. 2007; Marshall et al. 2009; Shelobolina et al. 2008) lactate (Finneran et al. 2002; Shelobolina et al. 2008), formate (Prakash et al. 2010; Finneran et al. 2002), benzoate (Finneran et al. 2002), butyrate and butanol (Prakash et al. 2010), glucose (Finneran et al. 2002), ethanol (Shelobolina et al. 2008; Luo et al. 2007), pyruvate (Junier et al. 2010; Shelobolina et al. 2008), fumarate (Esteve-Núñez et al. 2004), aromatic hydrocarbons such as benzoate and toluene (Prakash et al. 2010), and hydrogen (Junier et al. 2010; Liu et al. 2002; Marshall et al. 2009). In field studies, acetate (Holmes et al. 2002; Anderson et al. 2003; Vrionis et al. 2005; Istok et al. 2004; Nevin et al. 2003; Williams et al. 2011), ethanol (Wu et al. 2006; 2007; Istok et al. 2004), and glucose (Istok et al. 2004) have been used successfully to stimulate the reduction of U(VI).

Only a few of these studies have compared the effectiveness of the electron donor on U(VI) reduction. For example, relative reduction rates of U(VI)

were reported to be higher for H₂ than lactate (Liu et al. 2002), and both acetate and glucose were reported to be more effective than lactate, benzoate, or formate (Finneran et al. 2002). Luo et al. (2007) reported that ethanol resulted in higher uranium reduction than acetate. The extent of U(VI) reduction in Oak Ridge sediments was also found to be higher with methanol than glucose and much higher with glucose as compared to ethanol (Madden et al. 2008).

Different microbial community compositions are typically associated with different added electron donors. *Geobacter* species, which have been shown to reduce U(VI) (Lovley et al. 1991), tend to be common in uranium bioremediation and have been found in abundance with various electron donors including acetate, lactate, glucose, benzoate, and formate (Snoeyenbos-West et al. 2000). In addition to *Geobacter*, unclassified *Desulfuromonales* were enriched by ethanol, and *Desulfovibrio* were enriched by acetate (Luo et al. 2007). Ethanol and methanol stimulated acetogens such as *Clostridium* and *Desulfohalobium*, as well as acetate-metabolizing *Deltaproteobacteria* such as *Geobacter* (Madden et al. 2008). Sometimes stimulation can have negative implications for U(VI) reduction, such as the growth of *Desulfobacter* spp., which are unable to reduce U(VI) but utilize acetate, often compete with U(VI)-reducing bacteria for electron donors supplied at limiting concentrations (Lovley et al. 1993; Williams et al. 2011). Also, excess growth can cause permeability reduction within the delivery zone resulting from biomass buildup (Li et al. 2009; Williams et al. 2011) thus increasing biofouling which may result in a zone in which biostimulation is diminished.

The discussion above indicates that the selection of an appropriate electron donor is important for a successful in situ biostimulation scheme for the purpose of uranium reduction and immobilization. However, no previous studies have been conducted to our knowledge that examine uranium bioreduction as a function of different organic carbon sources in which both geochemical changes as well as changes in the microbial community structure were tracked as a function of different electron donors applied for extended periods of time under field-relevant conditions. This study's objective was to do just that: use flow-through columns to stimulate uranium bioreduction using different electron donor sources (HRC, vegetable oil, acetate, and lactate), examine iron-,

sulfate-, and uranium-reduction, and link this information to the stimulation of microorganisms known to be directly associated with uranium bioreduction.

There is virtually no information on uranium reduction and community structure for commercial electron donors such as HRC^{TM} or edible oil (vegetable oil), which are slow-releasing electron donor sources that appear to have many advantages for in situ anaerobic bioremediation (Borden 2007; Haas et al. 2001). Neither edible oil nor HRC had been previously used to stimulate uranium reduction, but they have been used to stimulate Cr(VI) reduction (Hazen et al. 2005; Faybishenko et al. 2008), MTBE degradation (Haas et al. 2001), PCE dechlorination and degradation (Borden 2007; Long and Borden 2006), and acid mine drainage remediation (Lindow et al. 2005).

Materials and methods

Sediment and groundwater sample collections

The sediment and groundwater used in the study was collected from the US Department of Energy's Integrated Field Research Challenge (IFRC) site located in Rifle, CO. Detailed descriptions of the geology and hydrogeology of the study site have been presented elsewhere (Anderson et al. 2003; Vronis et al. 2005; Yabusaki et al. 2007; Komlos et al. 2008a, b, c; Williams et al. 2011). Both sediment and groundwater were taken from locations unimpacted by previous amendment experiments at the Rifle IFRC site. The sediment was collected with a backhoe from a depth of 8–10 feet below the surface, placed in mason jars, and stored at 4°C without any further treatment until use. Sediment characteristics have been described fully by Komlos et al. (2007). Before loading the columns, the sediment was sieved through a 2 mm sieve to homogenize the sample and remove large clasts. Groundwater was pumped to the surface with a peristaltic pump, added to no-headspace 5-gallon plastic carboys, and stored at 4°C until use.

Column setup and operation

Ten glass columns (15 cm long, 2.6 cm in diameter, Kimble Kontes) were wet packed with 160 (± 10) g of the sediment described above. Groundwater from the Rifle site which contained ca. 10 mM sulfate and was

amended with 20 μ M U(VI), was pumped in an up-flow mode through the columns at a flow rate of 0.04 mL/min. The influent media was continuously purged with a gas mixture of carbon dioxide and nitrogen (1% CO₂, 99% N₂) to maintain pH of the influent water at circumneutral. The sediment columns were flushed with 0.2 μ m pore size-filtered groundwater for around 3 days before adding uranyl acetate to the influent to achieve a final uranium concentration of 20 μ M. The influent groundwater was pumped into the columns for 4 days until complete uranium breakthrough was observed. Biostimulation was initiated by pumping different electron donor sources (i.e., acetate, lactate, EOS®598, and HRC^{TM}) via syringe pumps (KD Scientific) into the main influent lines just before the column influent, resulting in an influent dissolved organic carbon (DOC) concentration of 200–250 mg/L. For acetate, two different concentrations (2 and 10 mM) were introduced into the columns to evaluate the effect of acetate concentration on the uranium biostimulation dynamics. For all columns, DOC was found in the effluent throughout the experiment suggesting that no column was donor limited.

EOS®598 is a commercially available organic substrate containing emulsified soybean oil (60%), food additive (10%), lactate (4%), with the balance being water. HRC^{TM} is a form of polylactate ester that when hydrolyzed, results in the release of biodegradable constituents, including lactic acid.

Columns were run in duplicate and operated in an incubator at 17°C. During the biostimulation period, effluent samples were collected to monitor dissolved species (acetate, sulfate, Fe(II), U(VI), and DOC). One column for each electron donor was sacrificed and destructively sampled at the onset of noticeable sulfate reduction (≈ 30 days of biostimulation) and the second column for each electron donor was sacrificed after 60–70 days of operation. Sediment samples were collected in an anaerobic glove box (3:97 H₂:N₂) as described by (Moon et al. 2009). Each of these sediment samples was then homogenized and analyzed for its solid phase U and Fe, as well as microbial community structure as described below.

Biogeochemical analysis

Effluent Fe(II) concentrations were measured by adding 0.5 mL of effluent solution to 0.5 mL of 1 N HCl and, after 1 h of extraction, were analyzed using

ferrozine (Lovley et al. 1987). Acetate and sulfate were analyzed using a Dionex DX500 ion chromatograph equipped with a CD25 conductivity detector and a Dionex IonPac AS14-4 mm column. Influent and effluent U(VI) concentrations were analyzed using reverse phase chromatography coupled to post column derivatization with the dye Arsenazo III (Sigma-Aldrich) as described by (Lack et al. 2002). The U(VI) detection limit using this procedure is $0.5 \text{ } \mu\text{mol L}^{-1}$. All anion and uranium samples were filtered ($0.2 \text{ } \mu\text{m}$) and stored at 4°C until analyzed. DOC in the effluent was $0.2 \text{ } \mu\text{m}$ filtered and measured using a DOC analyzer (Shimadzu DOC-5000A).

Fe(II) in the sediments was measured in an anaerobic glove box ($3\% \text{ H}_2$, $97\% \text{ N}_2$) by adding $0.2\text{--}0.3 \text{ g}$ of sediment to 5 mL 0.5 N HCl , extracting for 24 h (Komlos et al. 2007; Kukkadapu et al. 2006) and analyzing as described above. Total reducible Fe concentration in the sediments was determined in the same manner as the sediment associated Fe(II) concentration, except that 0.2 mL of 6.25 N hydroxylamine hydrochloride was added to the 0.5 N HCl solution at a final concentration of 0.25 N prior to the sediment addition (Komlos et al. 2007).

Microbial community analysis

Phospholipid fatty acid analysis (PLFA)

PLFA analysis was performed using previously reported procedures (White and Ringelberg 1998). Sediment samples (5 g) were extracted with a single-phase chloroform-methanol-buffer system of Bligh and Dyer (1954), as modified (D C White et al. 1979). The total lipid extract was fractionated into neutral lipids, glycolipids, and polar lipids by silicic acid column chromatography (Guckert et al. 1985). The polar lipids were transesterified to fatty acid methyl esters (FAMES) by a mild alkaline methanolysis (Guckert et al. 1985).

The FAMES were analyzed by capillary gas chromatography with flame ionization detection on a Hewlett-Packard 5890 Series 2 chromatograph with a 50 m non-polar column (0.2 mm I.D., $0.11 \text{ } \mu\text{m}$ film thickness). Preliminary peak identification was performed by comparison of retention times with known standards. Definitive identification of peaks was accomplished by gas chromatography/mass

spectroscopy of selected samples using a Hewlett-Packard 6890 series gas chromatograph interfaced to a Hewlett-Packard 5973 mass selective detector using a 20 m non-polar column (0.1 mm I.D., $0.1 \text{ } \mu\text{m}$ film thickness).

Quantitative PCR (qPCR)

DNA was extracted from sediment samples ($\approx 0.5 \text{ g}$ each) using the FastDNA spin kit for soil (BIO101, USA) and eluted in $100 \text{ } \mu\text{L}$ $1/10$ TE buffer. All qPCR was performed by Microbial Insights Inc. (Rockford, TN). Each $30 \text{ } \mu\text{L}$ TaqMan based PCR assay contained DNA template, $19 \text{ } \mu\text{L}$ TaqMan Universal PCR Master Mix (Applied Biosystems), TaqMan probe ($100\text{--}500 \text{ nM}$) and forward and reverse primers ($300\text{--}1,500 \text{ nM}$). TaqMan assays were performed on an ABI Prism 7300 Sequence Detection System (Applied Biosystems) with the following temperature program: 2 min at 50°C and 10 min at 95°C , followed by 50 cycles of 15 s at 95°C and 1 min at 58°C . The following groups of bacteria were targeted with the indicated TaqMan probe and forward/reverse primers, respectively: Eubacteria (TM1389, BACT1369/PROK1492R, (Suzuki et al. 2000); iron- and sulfate-reducing bacteria (GBC2, 361F/685R, (Stults et al. 2001)); dissimilatory sulfite reductase gene (1F/5R), (Karr et al. 2005); and Geobacteraceae (GBC2, 561F/825R, (Stults et al. 2001)). Each $30 \text{ } \mu\text{L}$ SYBR green PCR assay contained DNA template, $19 \text{ } \mu\text{L}$ PfuBuffer (Stratagene), 0.4 mM MgCl_2 , 0.2 mM of each dNTP (Roche Applied Science), SYBR green ($1:30000$ dilution, Molecular Probes), 1 U PfuTurbo HotStart DNA polymerase (Stratagene), DMSO ($0\text{--}0.5 \text{ } \mu\text{L}$), and forward and reverse primers ($500\text{--}2,500 \text{ nM}$). SYBR green assays were performed using an ABI Prism 7000 Sequence Detection System (Applied Biosystems) with temperature cycles varied based on primer set. Calibrations were obtained using a serial dilution of positive control DNA. The Sequence Detector program subtracted background signal for each sample during cycles 3 through 15. The fluorescence threshold was computed as $10 \times$ the standard deviation of the background signal and the original concentration of DNA in each sample was determined by comparing the C_t sample values with the calibration data. Gene copy numbers were calculated assuming $9.13 \times 10^{14} \text{ bp/} \mu\text{g DNA}$.

Clone libraries

DNA was extracted using the FastDNA spin kit (BIO 101 Inc., Vista, Calif.) and then cleaned up using the Wizard DNA clean-up system (Promega, Madison, Wis.). The 16S rRNA genes were amplified by PCR using primers 8F and 519R as previously described (Holmes et al. 2007). PCR products were then purified with a Gel Extraction Kit (Qiagen, Valencia, CA, USA), and clone libraries were constructed with a TOPO TA cloning kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. For each clone library, 48 clones were sequenced with the M13F primer at the University of Massachusetts Sequencing Facility. Completed gene sequences were aligned to the greengenes database and classified using the NCBI database and BLAST search matches for percent identity (DeSantis et al. 2006).

Results

Over the course of the experiment, organic carbon was removed in almost all columns, corresponding with increases in Fe(II) production, U(VI) retention, and sulfate reduction (Figs. 1, 2). The only case in which there were some discrepancies was the column run with acetate at 2 mM for only 23 days, but the graphs do suggest the beginning of U(VI) removal and they show Fe(II) production. There was good correlation between the geochemical parameters (DOC consumed, Fe(II) produced, sulfate consumed, and U(VI) retained) with Pearson's R-values typically ranging from 0.81 to 0.94. The only pair with a lower correlation were Fe(II) versus sulfate ($r = 0.65$), which was expected due to Fe(II) precipitation with sulfide as sulfate is reduced, a common occurrence with these sediments (Komlos et al. 2008a).

The extent of U(VI) removal varied with different electron donors (Fig. 1). Less U(VI) was retained and less Fe(II) was measured in the effluent from the columns which were amended with either 2 or 10 mM acetate. Acetate amended columns only retained 5–7 μM of the original 20 μM of U(VI). When EOS or HRC was provided as the electron donor, effluent U(VI) levels remained steady until about day 25–30 when they dropped dramatically to undetectable levels and remained low for the duration of the experiments. U(VI) was removed much sooner with lactate

additions, but did not reach the low levels achieved with EOS or HRC.

PLFA was used to determine bacterial biomass in the columns (Table 1). The oil in the EOS amendment confounded the PLFA results, and therefore there is no reliable biomass data for that treatment. In the other treatments (HRC, lactate, and acetate), the PLFA biomass estimates correlate extremely well with DOC consumed ($r = 0.97$), U(VI) retained ($r = 0.96$), and sulfate consumed ($r = 0.98$) and reasonably well with Fe(II) produced ($r = 0.76$; Fig. 3).

The PLFA profiles suggested that each of the various electron donor treatments yielded different and diverse communities. Monounsaturated fatty acids, typically associated with Gram-negative bacteria, dominated the PLFA profiles of all treatments, but the distribution of these acids varied with electron donor added. Both the HRC- and lactate-amended columns had higher amounts of terminally branched saturated PLFA's, indicative of Gram-positive spore-forming bacteria. The highest amounts of mid-chain branched PLFA's associated with Actinobacteria were found in the acetate-amended columns. The acetate-amended columns also had much higher abundances of the fatty acid 16:1x7c, which is closely associated with *Geobacter* species. The lactate and HRC columns also had fatty acid 16:1x7c, but only half as much as the acetate-amended columns, and the EOS-amended columns had almost none. The oil in the EOS made it difficult to compare PLFA profiles in the EOS-amended columns with other treatments, but the community in these columns also appeared to be dominated by monounsaturated fatty acids, with a smaller amount of terminally branched saturated fatty acids. There were very small amounts of polyunsaturated and branched monounsaturated PLFAs in some of the treatments with no apparent patterns.

Analysis of 16S rRNA gene sequences also revealed distinct differences between the acetate-amended columns and the other treatments (Table 2). As was observed in the PLFA profiles, the highest proportion of Actinobacteria was found in acetate-amended columns. These columns also had a much higher proportion of Proteobacteria, including the Beta-proteobacteria *Dechloromonas* and the Delta-proteobacteria *Geobacter* and its close relative *Pelobacter*. For the other three treatments, the communities were mainly dominated by Firmicutes. The most common Firmicutes were *Clostridium*, found primarily

Fig. 1 Changes in dissolved species concentration over time in days for different electron donors. Electron donors (Acetate, EOS, HRC, and lactate) are separated into rows and examined elements (Fe(II), U(VI), sulfate, and DTOC) are separated into columns. Within each row, there are columns that were run for different lengths of time. The number of days each specific column was run is noted in the upper right corner of the first graph of each row. Throughout the figure, the solid circles are

the shorter time frames, 23–32 days, and the open circles/triangles are the longer time frames, 48–73 days. In the first row, there is one extra line of solid triangles for the acetate column run for 73 days. The bottom row contains the singular acetate column that was run with only 2 mM acetate and it was only run for 23 days. The effluent Fe(II), U(VI), and sulfate are the concentration that was measured on each day. The DTOC measured the influent TOC minus the effluent TOC on each day

in the later timepoints for EOS and HRC, *Propionispora*, largely associated with the EOS and HRC amendments, and *Sporomusa*, which was important in the lactate-amended columns.

Discussion

The results demonstrate that the electron donor that is added to the subsurface sediments can greatly impact

Fig. 2 Cumulative change in dissolved species concentration over time in days for different electron donors. The symbols in this graph are set up exactly the same as Fig. 1, with solid circles for shorter time frames and open circles for longer time frames. For Fe(II), U(VI), and sulfate, these graphs show summation

over time of the absolute value of influent minus effluent to produce the cumulative retained amounts. For the DTOC, the graphs simply show summation over time of influent minus the effluent, thus producing some negative values when TOC was released by the columns

the effectiveness of uranium removal and that this is associated with different microbial community compositions. These findings suggest that a wider range of electron donors should be evaluated for bioremediation of uranium-contaminated groundwater.

Both dissimilatory metal-reduction and sulfate reduction were important processes in these sediments at various points in time, as evidenced by the tight correlation between Fe(II) production and sulfate, U(VI), and DOC removal, and consistent with

Table 1 PLFA profiles and biomass from columns

	Acetate 2 mM 23 days	Acetate 10 mM 23 days	Acetate 10 mM 48 days	Acetate 10 mM 73 days	EOS 24 days	EOS 61 days	HRC 32 days	HRC 60 days	Lactate 25 days	Lactate 73 days
Biomass pmol PLFA/gram sediment	844.0	642.9	2090.2	1570.3	12755.2	21011.4	2419.3	5564.3	2192.8	9042.7
Fraction of total fatty acids (%)										
Normal saturated	23.5	25.8	23.7	22.5	48.2	81.0	25.5	22.6	19.9	20.3
Mid-chain branched	6.0	6.7	2.5	4.4	nd	nd	1.8	1.2	1.6	0.8
Terminally branched saturated	5.1	6.3	5.4	10.2	2.5	0.8	9.7	13.2	6.4	12.0
Branched monounsaturated	1.8	1.8	1.0	1.5	nd	nd	1.7	1.5	0.8	1.7
Monounsaturated	60.8	57.1	66.0	60.3	49.3	18.3	60.5	60.8	69.9	64.8
16:1w7c	26.6	24.8	32.8	30.0	4.0	2.3	15.0	17.5	16.9	15.3
Geobacter										
Polyunsaturated	2.7	2.2	1.5	1.0	nd	nd	0.9	0.6	1.3	0.3

previous lab and field studies using sediments from this site (Anderson et al. 2003; Komlos et al. 2008a; Williams et al. 2011). Surprisingly, acetate, which has been found to be a good amendment for short-term uranium removal at the field site (Anderson et al. 2003; Vrionis et al. 2005; Guessan et al. 2008), was the least effective of the amendments tested for enabling the sustained removal of U(VI) from influent solutions. In the field, uranium removal is associated with a dramatic increase in the growth of *Geobacter* species (Holmes et al. 2007; Vrionis et al. 2005), as well as when their activity is sustained by providing acetate at non-limiting concentrations (Williams et al. 2011). In contrast, in the column studies acetate additions did promote some increase in the proportion of *Geobacter*, but they remained as minor constituents of the community. This was associated with a lower accumulation of Fe(II) than with the other amendments, suggesting that acetate was not effective in stimulating dissimilatory metal reduction in the sediments used in this study.

Acetate amendments stimulated uranium removal much better in previous similar column experiments (Komlos et al. 2008a; b); however there is substantial heterogeneity in the sediments at the Rifle site (Vrionis et al. 2005), and it is speculated that differences in the mineralogy or initial microbial community were

responsible for the differences in response to acetate. Specifically, the increased proportion of beta-proteobacteria, a diverse and opportunistic group (Brümmer et al. 2000; Rubin et al. 2007; Araya et al. 2003), may have interacted with the *Geobacter* in these columns. In particular, *Rhodoferrax*, which were found in higher proportions in the acetate-amended columns, have been known to limit *Geobacter* growth in areas with high ammonium (Mouser et al. 2009; Zhuang et al. 2011). The extent to which the sediments in this study may have differed in specific geochemical properties from those used in previous studies was not assessed (e.g. sediment associated nitrogen and phosphorus concentrations).

U(VI) retention was most effective in the columns amended with EOS or HRC, as U(VI) was reduced to undetectable levels with these electron donors. There was a substantial lag prior to uranium removal with these amendments, but once uranium removal was initiated, these amendments were very effective. The observed lag may be related to the fact that EOS and HRC are complex mixtures of multiple organics, some of which may be broken down by a series of microorganisms (Haas et al. 2001; Long et al. 2006). There were substantial differences in the composition of the microbial community between the EOS- or HRC-amended sediments and those amended with

Fig. 3 Biomass measured by PLFA versus a TOC consumed (blue) and Fe(II) produced (red) and b U(VI) removed (blue) and sulfate consumed (red). Values from the acetate columns are shown by solid squares, from the HRC columns are solid diamonds, and the lactate columns are open circles. There is a clear, direct relationship between each variable and biomass when looking at the three columns combined

acetate or lactate, including a diverse collection of Firmicutes and the sulfate-reducing bacteria *Desulfo-tomaculum*, and *Desulfosporosinus*, all of which contain species known to reduce U(VI) (Junier et al. 2010; Suzuki et al. 2004; Wall and Krumholz 2006). In these columns especially, sulfate-reducers may have played an important role as the onset of U(VI) retention corresponded with the onset of sulfate-reduction. Uranium reducing bacteria can be phylogenetically and physiologically diverse (Wall and Krumholz 2006) and at low (1 μ M or less) concentrations of U(VI), even a very minor component of the

microbial community could conceivably be responsible for most of the uranium removal.

Lactate additions supported better U(VI) removal than acetate, but significant amounts of U(VI) remained in the effluent, however, the cumulative retention of U(VI) over time intervals of ca. 60 days (Fig. 2) was still reasonably comparable to that observed for the EOS- or HRC-amended sediments. Previous studies suggested that lactate was somewhat less effective than acetate (Finneran et al. 2002). The community composition of the lactate-amended sediments was different than that of the acetate-amended sediments, with *Sporomusa*, a lactate-using acetogen species (Moller et al. 1984), as the most abundant phylogenetic clade. This is in contrast to our expectations that lactate could stimulate uranium-reducing strains of *Desulfovibrio* (Lovley and Phillips 1992). *Sporomusa*'s ability to reduce U(VI) is unknown. One possible mechanism for uranium reduction in the lactate-amended columns was through organic acid release by *Sporomusa*, which stimulated uranium-reducing *Geobacter*, whose presence was supported by the PLFA results. Similar mechanisms have been reported using other electron donors (Edwards et al. 2007; Madden et al. 2008).

Although the microbial communities for all the electrons donors were highly diverse and variable among the amendments, in general, the columns that contained more biomass overall had the highest U(VI) retention, and cumulative U(VI) retained was correlated to biomass across the acetate, HRC, and lactate columns and across the various incubation lengths (Fig. 3). There is a possibility that the increased biomass caused increased U(VI) sorption to the sediments and uranium speciation was not evaluated in these studies. However, previous column studies under similar conditions (Komlos et al. 2008c) demonstrated that microbial U(VI) reduction to U(IV) was the major mechanism of U retention. Therefore, we expect that even if some sorption occurred that it was not as important as microbial reduction.

Choosing the right electron donor for uranium-bioremediation in the field is important in trying to balance things like attainability, cost, and effectiveness. Previously, many attempts to choose the most effective agent have been focused on trying to stimulate a particular species or community of bacteria that are known to be effective uranium-reducers, even

Table 2 Dominant species/families found in columns (percent of community)

Phylogeny fraction of total clones (%)	Average percent identity	Acetate 2 mM 23 days	Acetate 10 mM 23 days	Acetate 10 mM 48 days	Acetate 10 mM 73 days	EOS 24 days	EOS 61 days	HRC 32 days	HRC 60 days	Lactate 25 days	Lactate 73 days
Actinobacteria	91	1.0	1.8	3.2	3.6	nd	nd	0.6	nd	3.5	1.5
Alpha- nroteobacteria	92	4.8	12.3	3.2	2.7	1.3	1.8	0.0	2.5	1.2	5.1
Beta- proteobacteria	94	21.2	22.8	12.7	5.5	2.0	0.6	3.2	4.1	nd	nd
Dechloromonas	97	15.4	14.0	4.8	2.7	nd	0.6	1.3	2.5	nd	nd
Gamma- nroteobacteria	92	7.7	4.4	15.9	7.3	3.4	2.9	1.3	nd	5.8	nd
Delta- proteobacteria	88	9.6	7.9	4.8	13.6	0.7	7.0	1.9	0.8	1.2	9.6
Geobacter	87	5.8	4.4	nd	7.3	nd	0.6	nd	0.8	nd	nd
Pelobacter	87	3.8	3.5	nd	0.9	nd	nd	nd	nd	nd	nd
Desulfovibrio	90	nd	nd	nd	0.6	nd	nd	4.4	nd	nd	nd
Firmicutes	91	1.0	nd	6.3	12.7	42.3	36.8	41.3	41.8	38.4	32.4
Clostridium	91	nd	nd	1.6	4.5	0.7	9.9	7.1	23.0	1.2	1.5
Propionispora	93	nd	nd	nd	nd	11.4	10.5	17.4	2.5	1.2	0.7
Sporomusa	92	nd	nd	nd	nd	27.5	4.1	3.2	2.5	26.7	23.5
Desulfosporosinus	93	nd	3.2	nd	nd	3.9	1.6	1.5	4.7	nd	Nd
Desulfotomaculum	89	6.4	nd	nd	0.6	nd	nd	1.5	nd	nd	nd

though in some cases the agent is only effective for a short period of time (Finneran et al. 2002; Anderson et al. 2003). This study demonstrates that more complex organic amendments which have been successfully used for the bioremediation of other contaminants (Hazen et al. 2005; Haas et al. 2001; Borden 2007; Long and Borden 2006; Lindow et al. 2005) produce diverse bacterial communities, and that overall production of microbial biomass may be an equally important predictor of effectiveness in U(VI) retention as the stimulation of specific bacterial species. This opens up a number of new possibilities when choosing options for uranium bioremediation, in turn leading to more effective, sustainable, and cost-effect methods of cleaning up remaining waste sites.

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