Efficacy of Acetate-Amended Biostimulation for Uranium Sequestration: Combined Analysis of Sediment/Groundwater Geochemistry and Bacterial Community Structure

Jie Xu^{1, 2, 4}, Harish Veeramani⁵, Nikolla P. Qafoku⁶, Gargi Singh^{2, 3}, Maria V. Riquelme^{2, 3}, Amy Pruden^{2, 3}, Ravi K. Kukkadapu⁷, Brandy N. Gartman⁶, Michael F. Hochella, Jr.^{1, 2, 6, *}

¹Department of Geosciences, ²The Virginia Tech National Center for Earth and Environmental Nanotechnology Infrastructure, ³Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA 24061, U.S.A.; ⁴Department of Geological Sciences, University of Texas at El Paso, El Paso, TX 79968, U.S.A.; ⁵Infrastructure and Environment, School of Engineering, University of Glasgow, Glasgow G12 8QQ, Scotland; ⁶Geosciences Group, Earth Systems Science Division, Pacific Northwest National Laboratory, 902 Battelle Blvd., Richland, WA 99354, U.S.A.; ⁷Environmental Molecular Science Laboratory, Pacific Northwest National Laboratory, 902 Battelle Blvd., Richland, WA 99354, U.S.A.

* Correspondence should be sent to jxu2@utep.edu or hochella@vt.edu

Abstract

Systematic flow-through column experiments were conducted using sediments and ground water collected from different subsurface localities at the U.S. Department of Energy's Integrated Field Research Challenge site in Rifle, Colorado. The principal purpose of this study is to gain a better understanding of the interactive effects of groundwater geochemistry, sediment mineralogy, and indigenous bacterial community structures on the efficacy of

uranium removal from the groundwater with/without acetate amendment. Overall, we find that the subtle variations in the sediments' mineralogy, redox conditions, as well as contents of metal(loid) co-contaminants showed a pronounced effect on the associated bacterial population and composition, which mainly determines the system's performance with respect to uranium removal. Positive relationship was identified between the abundance of dissimilatory sulfate-reduction genes (i.e., *drsA*), markers of sulfate-reducing bacteria, and the sediments' propensity to sequester aqueous uranium. In contrast, no obvious connections were observed between the abundance of common iron-reducing bacteria, e.g., *Geobacter spp.*, and the sediments' ability to sequester uranium. In the sediments with low bacterial biomass and the absence of sulfate-reducing conditions, abiotic adsorption onto mineral surfaces such as phyllosilicates likely played a relatively major role in the attenuation of aqueous uranium; however, in these scenarios, acetate amendment induced detectable rebounds in the effluent uranium concentrations. The results of this study suggest that immobilization of uranium can be achieved under predominantly sulfate-reducing conditions, and provide insight into the integrated roles of various biogeochemical components in long-term uranium sequestration.

1. Introduction

Uranium bioremediation typically involves the injection of simple organic substrates such as ethanol or acetate into the subsurface to stimulate indigenous microbial communities that are capable of reducing and immobilizing soluble U(VI) species (Li et al., 2009; Campbell et al., 2012; Bargar et al., 2013; Qafoku et al., 2014). During U(VI) bioremediation, a wide range of organic/inorganic compounds including their intermediate species are competitively utilized as

electron donors or acceptors by physiologically diverse groups of microorganisms (Suzuki et al., 2005; Xu et al., 2010; Handley et al., 2012) and via abiotic chemical reactions (Jeon et al., 2005; Chakraborty et al., 2010; Hyun et al., 2012). Thus, the total immobilization of uranium is driven by complex series of biological and abiotic pathways.

A variety of microorganisms capable of enzymatically reducing U(VI) have been reported in the laboratory and field studies. These organisms include sulfate/sulfur-reducers, e.g., *Desulfovibrio* spp., *Desulfotomaculum* spp., and *Shewanella* spp., iron-reducers, e.g., *Geobacter* spp., denitrifiers, e.g., *Pseudomonas* spp., and spore-forming species, e.g., *Clostridium* spp., *Cellulomonas* spp., and *Anaeromyxobacter* spp. (Lovley and Phillips, 1992; Francis et al., 1994; Abdelouas et al., 1998; Ganesh et al., 1999; Pietzsch et al., 1999; Chang et al., 2001; Suzuki et al., 2003; Beyenal et al., 2004; Wall and Krumholz, 2006 ; Sharp et al., 2009; Della Vecchia et al., 2010; Handley et al., 2013; Cologgi et al., 2014). The enzymatic reduction of U(VI) by mixed microbial communities can be strongly affected by the presence of competing electron acceptors, however. For example, it had been previously shown that microbial U(VI) reduction was suppressed or even entirely inhibited when nitrate, Fe(III) oxyhydroxides and Fe(III)-containing clays also existed in the environment (Wielinga et al., 2000; Senko et al., 2002; North et al., 2004; Sani et al., 2004; Komlos et al., 2008). Microorganisms can also mediate the reduction of U(VI) indirectly via biogenic solids and soluble species. Several laboratory studies have demonstrated the reduction of U(VI) to nanoparticulate uraninite by sulfide and iron sulfide minerals (i.e., mackinawite) produced during bacterial sulfate reduction (Veeramani et al., 2011; Veeramani et al., 2013; Lee et al., 2013; Hyun et al., 2012). In addition, former work on the interactions of U(VI) with iron-reducing bacteria (i.e., *Geobacter*) indicated that the

biogenic Fe(II) could catalyze reduction of U(VI) when both species were adsorbed on clays or (oxyhydr)oxides (Liger et al., 1999; Jeon et al., 2005; Nico et al., 2009; Regenspurg et al., 2009; Luan et al., 2014). Overall, the minerals that have been reported to abiotically reduce U(VI) include framboidal pyrite (Qafoku et al., 2009; Descostes et al., 2010; Yang et al., 2014), magnetite (Missana et al., 2003; Scott et al., 2005), green rust (O'Loughlin et al., 2003), zero-valent iron (Farrell et al., 1999), and Fe(II)-bearing clays (Chakraborty et al., 2010).

Uranium can also be attenuated via a range of other processes and interactions with minerals, including surface adsorption (Arnold et al., 1998; Yusan and Akyil, 2008; Bachmaf and Merkel, 2011; Sun et al., 2013), complexation (Kelly et al., 2008; Singer et al., 2009; Stylo et al., 2013; Long et al., 2015), and co-precipitation (Bruno et al., 1995). A range of clays and oxyhydroxides including kaolinite, smectite, montmorillonite, illite, gibbsite, and goethite, were shown to be capable of immobilizing aqueous U(VI) via surface adsorption, and the actual amount depends on the specific mineral surface structure (i.e., aluminol vs. silanol sites), the solution pH, and the co-existence of trace elements (Arnold et al., 1998; Cheng et al., 2004; Chang et al., 2006; Bachmaf and Merkel, 2011). In treated mine tailings from an active uranium mill at Key Lake, Saskatchewan, Canada, uranium sequestration occurs primarily due to sorption within a complex suite of primary and secondary clay minerals, as well as the precipitation of uranium-bearing carbonate and phosphate nanoparticles (Schindler et al., 2015).

In biostimulated aquifers, the overall efficiency of uranium sequestration is a collective result of all the interactions between groundwater, sediments, and microbial populations that may have positive (i.e., adsorption, reduction and precipitation) or negative (i.e., complexation

and mass transport facilitated by groundwater flow) effects on soluble uranium species. More, changes in groundwater pH and compositions have direct control over the stability and thus, mobility of the colloidal forms of uranium in aquifers. As intricate feedback systems also exist among the different biogeochemical components, a comprehensive understanding of the immobilization/remobilization mechanisms of uranium during bioremediation remains elusive and even less is known regarding the interactive relationships between sediment mineralogy, bacterial population structure, and uranium sequestration kinetics.

To investigate the interactions of various biogeochemical components involved in uranium bioremediation processes, we have carried out systematic, controlled column experiments using field sediments that were collected from different uranium-contaminated and non-contaminated aquifers in Rifle, Colorado (USA). These sediments exhibited subtle variations in their mineralogy and geochemical compositions and presented different redox and ecological conditions. By comparison of the behavior of the various sediment systems in the presence and absence of a biostimulant (acetate), we have furthered our understanding by answering the following overarching questions: (1) what is the relationship between the sediment geochemistry and the original bacterial community composition and what are their combined effects on the removal efficiency of aqueous uranium?; (2) does biostimulation with acetate drive detectable changes in the structure and diversity of bacterial communities in the sediment and does this stimulation effect also show dependence on the sediment type?; and (3) what immobilization/remobilization mechanisms of U(VI) are likely to be involved during the biostimulation of the sediments?

2. Materials and Methods

2.1 Site Description and Sediment Sampling

The U.S. Department of Energy's Integrated Field Research Challenge (IFRC) site in Rifle, Colorado is located on a small flood plain in northwestern Colorado underlain by an aquifer comprised of 6.5m of unconsolidated sands, silts, clays and gravels deposited by the adjacent Colorado River and underlain by a relatively impermeable aquitard known as the Wasatch formation (Anderson et al., 2003; Williams et al., 2011; Qafoku et al., 2014). This site was contaminated with U, V, As, Se and other contaminants from former milling activities. Groundwater monitoring over the past decade shows varying patterns of dissolved metals behavior, with significant fluctuations in aqueous U, V, As, and Se associated with seasonal variations in water level (Qafoku et al., 2014). The sediments labeled D08, LQ 107 and CD were collected from the uranium-contaminated subsurface at Winchester, La Quinta, and Plot C experimental galleries, respectively, whereas the RABS (Rifle Aquifer Background Sediment) was collected from an area that is not affected by the subsurface U(VI) plume (See the schematic in Supplementary Materials). Average groundwater composition has also been reported previously (DOE, 1999; Anderson et al., 2003; Yabusaki et al., 2007). In general, the aquifer has elevated concentrations of SO_4 (~10 mM) and alkalinity (~10 meq/L), and is close to equilibrium with calcite. The pH is typically between 7.2 and 7.4. Dissolved Fe(II) can be quite variable across the site (Campbell et al., 2012).

The same method as that described in Qafoku et al., 2014 has been used to collect the sediments. Specifically, sediment cores were collected using a nitrogen gas borehole flush to

avoid air (oxygen) contamination, and sediment samples were immediately processed in a nitrogen-filled field-portable glove bag. Double-layered Mylar bags containing oxygen scavengers were used to pack the sediment samples before they were shipped on ice to the laboratory. Upon arrival at the laboratory, the sediment samples were stored at -80°C prior to the column experiments. It is noted that the subsurface area where the sediment samples were collected had not been impacted by previous organic carbon amendment experiments performed at the Rifle IFRC site.

2.2 Column Experiments

For each type of sediments, three columns were prepared and pumped with uranium-spiked groundwater: specifically, two were amended with 5 mM or 50 mM acetate and the other was not amended with acetate. An additional control column was packed with RABS sediment and pumped with non-uranium spiked groundwater. The 13 columns were setup and operated in parallel similar to that described in Qafoku et al. (2010). Polyvinyl-chloride columns with an internal diameter of 2.35 cm and a length of 3.6 cm were packed uniformly with sediments from the Rifle site in an anaerobic chamber (Suppl. Table S1), and millimeter-size particles of sterile pyrite were added to each column except for the uranium-negative control. These highly crystalline pyrite particles were added mainly to compare with the metal sulfides (i.e., Fe-S) indigenous to the collected sediments in terms of uranium sequestration. In later characterization of the sediments, the surfaces of these added pyrite particles were particularly examined using scanning electron microscopy and X-ray photoelectron spectroscopy. The columns were covered by porous plates (with a thickness of 0.20 cm and a pore diameter of 10 µm) at both ends to enable uniform dispersion of influent solutions and to prevent loss of

sediment to the outlet. Peristaltic pumps were used to maintain the influent at a constant flow rate of ~ 1 mL/h. The effluent of each column was collected using a Kloehe fraction collector at 3-h intervals, and stop-flow conditions (Brusseau et al., 1997; Qafoku et al., 2005; Ilton et al., 2008) were maintained to determine the effect of non-equilibrium conditions on the transport of uranium. Specifically, stop-flow events were conducted once every 3 days, after approximately 12 pore-volumes of influent passed through the column. A total of four stop-flow events were applied to each column experiment. Rifle groundwater, collected from different experimental galleries, was used as the influent solution in the column experiments. Following the third flow event, the influent solutions of all columns were also spiked with uranyl nitrate to achieve a final U(VI) concentration of $1100 \mu\text{g}\cdot\text{L}^{-1}$ (i.e., $\sim 4.62 \mu\text{M}$). During the stop-flow events and after the completion of each experiment, the columns were sealed and stored in an anaerobic chamber (COY Labs®), where the samples of reacted sediment were aseptically collected for a range of different analyses. The concentration of metals in the column effluent samples was determined using an inductively coupled plasma optical emission spectrometry (ICP-OES), and inductively coupled plasma mass spectrometry (ICP-MS).

2.3 Solid Phase Characterization

Each sediment sample was separated into different size fractions and the $< 53 \mu\text{m}$ fraction was analyzed using X-ray powder diffraction and environmental scanning electron microscopy (ESEM) coupled with energy dispersive spectroscopy (EDS) for the determination of their chemical compositions, morphology and crystal structure.

The mineral phases in each sediment sample were primarily identified using a Miniflex II XRD (Rigaku) equipped with a Cu K α source, with individual scans from 10° to 80° 2 θ (0.01° per step) and a scan speed of 0.1° per second. The morphology and composition of sediment particles in each sample was determined using a FEI Quanta 600 FEG ESEM. The ESEM samples were sputtered with gold (approximately 10 nanometers thick) immediately prior to the analysis, and final images were acquired using both secondary and back-scattered electrons. Spot-specific chemical compositions in the sediments were also determined using an EDS system attached to the ESEM. In addition, the added pyrites were examined using a scanning photoelectron spectrometer microprobe (PHI Quantera SXM, a high spatial resolution X-ray photoelectron spectrometer, or XPS) for their surface elemental compositions and oxidation states.

2.4 ^{57}Fe Mössbauer Spectroscopy

The samples for Mössbauer spectroscopy measurement were prepared inside an anoxic chamber by filtering the sediment suspension through a 0.45- μm pore-size filter (Millipore Durapore Membrane Filter). Details of the sample preparation, Mössbauer spectrometer instrumentation, and modeling of the spectra were similar to that described elsewhere (Komlos et al., 2008; Kukkadapu et al., 2010; Peretyazhko et al., 2012). Briefly, WissEl (Germany) Mössbauer electronics, and a closed-cycle cryostat SHI-850 and Sumitomo CKW-21 He compressor unit, obtained from Janis Research Company, Inc. (Wilmington, MA), were employed for the measurements. The Mössbauer data were modeled by the Recoil software (University of Ottawa, Canada) using a Voigt-based structural fitting routine (Rancourt and Ping 1991). Mössbauer analysis were obtained on RABS, DO8, and CD sediments (spectra of LQ107

were not obtained). The spectra for non-biostimulated and 50 mM acetate-amended RABS and D08 were obtained at 12 K, while those for non-biostimulated and 50mM acetate-amended CD were obtained at both RT and 12 K.

2.5 DNA Extraction, Amplification and High-throughput Sequencing

DNA was extracted using the FastDNA SPIN Kit for Soil and the FastPrep Instrument (MP Biomedical, Santa Ana, CA) according to manufacturer protocols and stored at -80 °C. Quantitative PCR (q-PCR) was applied to quantify dissimilatory sulfite reductase A (*dsrA*) genes (Kondo et al., 2007), which are specific to sulfate reduction, and bacterial 16S rRNA genes, as an indicator of the size of bacterial populations (Suzuki et al., 2000). The qPCR reaction matrix and amplification conditions are summarized in Suppl. Tables S2 and S3. All qPCR standard curves were constructed from serial dilutions of cloned positive controls ranging from 10^8 to 10^2 gene copies per μL . An optimal dilution of 1:50 was selected to minimize inhibitory effects based on comparison of PCR efficiencies across a range of dilutions for a subset of samples. All samples were analyzed in triplicate, including a negative control in each batch.

Genomic DNA extracted from the sediments was subject to pyrosequencing on a Roche 454 GS-FLX+ platform, with an average coverage of 3000 base pairs, ranging from 1913 to 7712, by the Research and Testing Laboratory (Lubbock, TX). The raw pyrosequencing data were processed using Mothur (version v.1.33.0) following the 454 standard operating procedure [64] as accessed on 05/25/14 from http://www.mothur.org/wiki/454_SOP. The graphs were plotted using the R project (version 2.11.1) and statistical analyses were conducted using “pvclust” and “lattice” software packages (version 1.2-2).

3. Results

The non-amended sediments showed dramatic difference in terms of chemical compositions, mineralogy, and indigenous bacterial communities, which were associated with the observed variations in the sediments' capacity to remove aqueous uranium. It is important to note that the effectiveness of the acetate amendment varied with the sediment type. Specifically, biostimulation enhanced the removal capacity of D08 sediments up to 95% whereas it decreased the capacity of CD sediments down to -10%. Detailed analysis of the aqueous effluent chemistry, sediment mineralogy, and indigenous bacterial population in the sediments revealed notable relationships between the various biogeochemical components during the bioremediation process.

3.1 Variation in the Effluent Chemistry of Different Sediments

Chemical compositions and pH of the background groundwater used in the breakthrough tests are shown in Table 1. The concentrations of soluble uranium and a wide range of other metal(loid)s, including calcium, iron, arsenic, manganese, barium, vanadium, molybdenum, and silicon were monitored in the effluents of different sediment systems (Fig. 1 & Suppl. Fig. S1). As identified in a previous study (Bargar et al., 2013), vanadium is a co-contaminant of concern in the groundwater at the Rifle sites, and in this study concomitant removal of uranium and vanadium likely occurred. The concentrations of iron were relatively

low or below detection limit in the effluents of all sediment columns indicating little reductive dissolution of the ferric phases.

- *Uranium Removal Efficiency*

Profiles of uranium concentrations ($[U]$) in the effluents of different sediment systems (acetate-amended or non-amended) are compared in Fig. 1_A. To quantitatively describe the effects of each sediment type on uranium immobilization, we define the uranium removal efficiency (i.e., $\Delta U\%$) as the difference between the “spiked” $[U]_{in}$ in the influent and the average $[U]_e$ in the effluent (i.e. $[U]_{in} - [U]_e$) divided by $[U]_{in}$. Overall, the $\Delta U\%$ showed strong dependence on the sediment type and acetate-amendment. Under the non-amended conditions, the $\Delta U\%$ in the sediments were rather similar ranging from 0 to 10% over the duration of the 240-h experiment, whereas under the acetate-amended conditions, the $\Delta U\%$ was significantly differentiated among the sediments following a descending trend of $D08 > LQ107 \geq RABS > CD$. Specifically, the highest $\Delta U\%$ (i.e., ~ 90 to 95%) was achieved in the amended systems of D08 and the lowest $\Delta U\%$ (i.e., ~ -10 to 0%) occurred in the amended systems of CD. It is noted that the biostimulation of the sediments with acetate did not lead to enhanced $\Delta U\%$ exclusively as greater uranium concentrations were observed in the effluent of amended CD sediments relative to that in the influent.

- *Heavy Metal(loid)s*

Interesting relationships were also observed in the concentration profiles of uranium versus other elements, including barium, vanadium, calcium, arsenic, and molybdenum. The effluent $[U]$ and $[Ba]$ showed an inverse relationship in the acetate-amended systems of D08 (Fig. 1). As shown and discussed in later sections (e.g., Section 3.3), the amended D08

sediments attained sulfate-reducing conditions, indicating that the indigenous sulfate reducers had influence over both the immobilization of uranium and the mobilization of barium. In comparison, the effluent [V] seemed to be susceptible to the acetate amendment, especially in the RABs and CD sediments, where relatively high concentrations of [V] were sequestered along with the acetate amendment. Also, fluctuations were observed in the profiles of effluent [Mo] in both acetate-amended and non-amended sediments of D08 (Fig. 1E).

3.2 Characterization of Fine Sediment Mineralogy

All the sediments contained significant amounts of quartz, orthoclase, albite, anorthite, pyroxene, and phyllosilicates (e.g., muscovite and chlorite) in the examined size fraction (i.e., < 53 microns) based on XRD analyses (Suppl. Fig. S2). Some distinction among the sediments is that RABS and D08 sediments consisted of relatively high amounts of calcite, whereas LQ107 and CD contained detectable amounts of iron-bearing (oxyhydr)oxides primarily identified as goethite. Particularly, the identification of goethite was based on the peaks at 21.3 and 36.7. Another observation is that the acetate amendment resulted in detectable changes in the sediments' mineralogy (highlighted in Suppl. Fig. S2). For example, the content of calcite in the bio-amended D08 was estimated to be 10-15% higher than that in non-amended D08 using the (104) peak intensity of calcite in XRD analyses as a quantification measure (Xu et al., 2013). This could be likely due to enhanced microbial respiration and high calcium content of the groundwater. Further examination of the sediment samples using SEM-EDS showed consistent results with the XRD analyses, and most of the scanned sediment particles had high concentrations of Si and Al, followed by Fe, Mg, Ca, P, Ti, Ni, Cu, Ba, Zr, and Mn.

- *Fe-Sulfides and Other Fe-Bearing Phases*

Although not detected in the XRD patterns (primarily due to low concentrations of less than a percent or two by weight), Fe-S rich phases of different morphologies and particle sizes were frequently identified using SEM/EDS in both the acetate-amended and non-amended samples of D08 (Fig. 2). These Fe-S-rich phases ranged from micron-sized framboidal pyrite composed of nanocrystals (e.g., Fig. 2A-C), euhedral crystals (e.g., Fig. 2E), to amorphous aggregates (e.g., Fig. 2D and F), all of which are distinguishable from the added pyrite fragments. Regarding the added pyrite, XPS analyses showed that their surfaces are largely coated by fine particles rich in Al, Si, C, Mg, and Ca. High-resolution scanning of these pyrite surfaces detected little uranium regardless of the sediment type (Table S4 and Suppl. Fig. S3), suggesting that the added pyrite is not a major sink for uranium either through adsorption or reduction.

Based on the Mossbauer spectroscopic measurements (Fig. 3 and Suppl. Fig. S4), most of the Fe in the non-amended sediments of RABS, D08 and CD was associated with clays (C), framboidal pyrite, (oxyhydr)oxides (e.g., nano- goethite, Al-substituted goethite, and hematite/magnetite), and ilmenite. The observed black coloration or further darkening of RABS and D08 sediments upon acetate amendment is most probably caused by the precipitation of secondary Fe-sulfides, resulting from bacterial sulfate reduction. The Mossbauer spectra of Fe(II)-sulfide and clay Fe(III) overlap considerably, hence making it difficult to quantify the contents of Fe-sulfide in the examined sediments. It is noted that the spectra of the CD sediments demonstrated a significant difference from those of RABS or DO8 regarding iron (oxyhydr)oxide forms and contents, largely in line with the XRD analyses, which detected the

presence of ferrihydrite in CD and LQ107 only. By comparing the Mossbauer spectra of the non-amended versus 50mM acetate-amended samples, we did not find dramatic effects caused by the acetate amendment on the Fe speciation or mineral suite in RABS, D08, and CD sediments.

- *Uranium-Enriched Spots*

Several uranium-rich “hotspots” were identified in the acetate-amended sediments of D08 and RABS using SEM/EDS (e.g., Fig. S5). The areas where EDS uranium signals were observed typically contain Ca-carbonate, Fe/Cu/Zn-rich-sulfide, Fe-bearing alumino-silicate as well as Ca-rich phosphates. In the acetate-amended sediments of LQ107, uranium was found to be enriched on Fe-(oxyhydr)oxides in association with particles that were concentrated in the Fe content but depleted in O, Si, and S (e.g., Fig. 4).

3.3 Identification of Bacterial Composition and Abundance Patterns

- *Abundance of 16S rRNA and dsrA genes*

The abundance of bacterial 16S rRNA genes in the non-amended sediments of D08 and RABS were found to be three orders of magnitude higher than that in non-amended CD or LQ107 (Fig. 5A), indicating that much higher density of bacteria is indigenous to the more anoxic environment of D08 or the less contaminated niche of RABS. The amendment of the sediments with 5-mM or 50-mM acetate enhanced the total bacterial biomass (i.e., abundance of 16S rRNA genes) in RABS, D08, and LQ107 systems; however, a significant decrease was observed in 16S rRNA genes in the amended sediments of CD. Also, except for the D08 system, the biostimulation of total bacterial biomass was more effective using 5-mM acetate than using 50-mM ones. It is important that activities of bacterial sulfate reduction were identified in the

amended sediments of D08 and RABS, with the indicator gene, *drsA*, detected and quantified at different orders of magnitude (Fig. 5B). And although the amendment with higher concentrations of acetate did not lead to exclusive enhancements of total bacterial biomasses, the higher availability of acetate had a markedly positive effect on the abundance of sulfate-reducing bacteria.

- *Sediment Type versus Indigenous Bacterial Communities*

The structure and diversity of bacterial communities in the acetate-amended and non-amended sediments are displayed at phylum-, class-, and genus three levels in Figs. 6-8. The dominant phyla in the non-amended sediments were similar to one another, composed mainly of *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Acidobacteria*. The relative abundance of each phylum, however, shows apparent variations depending on the sediment type (Fig. 6). For example, the non-amended sediment of CD was dominated by *Proteobacteria* (67%), whereas the others (i.e., RABS, D08, and LQ107) contained comparable populations of *Firmicutes* (45%, 18%, and 27%, respectively) and *Proteobacteria* (21%, 34 %, and 41% respectively). It is noted that for each type of sediment, a significant fraction of the bacterial community represented by the pyrosequencing library could not be identified.

Of all the non-amended sediments, only D08 contained ~ 7% of *Chloroflexi* while LQ107 had ~ 5% of *Nitrospira*. Within the *Firmicutes* phylum, comparable percentages of *Bacilli* and *Clostridia* were found in non-amended RABS and D08 (Fig. 6); by contrast, CD and LQ107 were either dominated by *Bacilli* or *Clostridia*. A closer examination of the *Clostridia* class (Fig. 8) showed that relatively high percentages of *Desulfosporosinus* and *Desulfotomaculum* were present in the non-amended sediments of LQ107 (i.e., 53% and 8.7%, respectively) and D08

(i.e., 29.6% and 5.0%, respectively). Species in both genera have been previously demonstrated as spore-forming metal- and sulfate-reducers although the evidence of elevated bacterial sulfate-reduction (i.e., the *dsrA* gene) was only detected in the D08 sediments. At genus levels, the δ -*Proteobacteria* population in non-amended LQ107 was dominated by *Geobacter* whereas that in RABS was dominated by *Desulfuromonas* and other genera of *Desulfuromonadaceae*. In comparison, the δ -*Proteobacteria* in non-amended D08 was composed of a small fraction of *Anaeromyxobacter* (i.e., 6%), as well as comparable abundances of *Geobacter* (31%) and *Desulfuromonadaceae* (46%).

- *Effects of Acetate-Amendment on Bacterial Community Structures*

Amendment of the sediments with acetate resulted in significant changes in indigenous bacterial communities (as shown in Fig. 6-8). In general, the amended sediments contained higher percentages of *Actinobacteria*, *Chloroflexi*, *Cyanobacteria*, *Clostridia*, and δ -*Proteobacteria*, and were relatively suppressed in α -/ β -*Proteobacteria* and *Nitrospira*. This change indicated that the sediments were switching to more anaerobic conditions along with the acetate-amendment and also suggested that more diverse metabolic pathways were enabled. Among the *Clostridia* detected in RABS, the initial dominant population of *Clostridium_Sensu Stricto* was gradually exceeded by *Desulfosporosinus* along with the acetate amendment; in comparison, the *Clostridia* communities in the LQ107 and D08 sediments were constantly dominated by *Desulfosporosinus* with or without the amendment. It was also noted that biostimulation of D08 sediments resulted in a broader array of detected *Clostridia*. For example, a spectrum of H₂-producing and SO₄⁻/SO₃⁻/S₂O₃⁻-reducing genera including *Anaerosporebater*, *Anaerovorax*, *Dehalobacter*, *Desulfitibacter*, *Desulfonispora*, *Gracillibacter*,

and *Clostridium*_III were identified in the biostimulated D08. In addition, the δ -Proteobacteria community in the amended sediments of LQ107 and RABS (5mM-acetate only) was significantly enhanced in *Geobacter* spp. whereas the *Geobacter* population in the amended sediment of D08 was slightly suppressed by the emergence of more diverse sulfate-reducers, composed mainly of *Desulfobacter*, *Desulforhopalus*, and *Desulfovibrio* spp. Strict anaerobes like *Anaeromyxobacter* also started to occur in significant abundance in the amended sediments of D08 and LQ107 (Fig. 8). Species belonging to the genus of *Anaeromyxobacter* were formerly demonstrated capable of respiring diverse electron acceptors including nitrate, nitrite, ferric iron (III), manganese oxides, arsenate and uranium (VI) (Treude et al., 2003; Wu et al., 2006; Kudo et al., 2013). Lastly, the acetate amendment of the sediments also led to enhanced communities of *Azotobacter* in RABS and *Colwellia* in LQ107 within the γ -Proteobacteria class.

4. Discussion

Through the combined geochemical and microbiological analyses, we find that the subtle variations in the sediments' mineralogy, redox conditions, as well as contents of metal(loid) co-contaminants showed a marked effect on the associated bacterial population and composition, which mainly determines the system's performance regarding uranium removal. Whether the acetate-amendment could help the sediments to develop sulfate-reducing conditions is indicated as a key factor for effective uranium sequestration.

4.1 Bacteria-Dominated Sequestration of Aqueous Uranium

- *Roles of Sulfate-, Iron- & Nitrate-Reducers*

The efficiency of uranium removal is closely related to the abundance and diversity of bacterial communities in the sediments. In particular, a clear association was observed between the abundance of sulfate-reducing bacteria and the percent removal of aqueous uranium. As shown in the pyrosequencing results, the bacterial population capable of sulfate-reduction (in the biostimulated D08) consisted of both Gram-negative and Gram-positive bacteria, such as *Desulfobacter*, *Desulforhopalus*, *Desulfovibrio*, and *Desulfotomaculum*, mostly distributed within the δ -*Proteobacteria* and *Clostridia* classes. With the exception of *Desulfotomaculum*, however, many of the identified sulfate-reducing genera are not known or previously demonstrated to couple acetate oxidation with sulfate reduction. Results of this study suggest that either (some of) these sulfate-reducing genera are capable of utilizing acetate directly as electron donors, or are capable of mixotrophic behavior by using acetate as a carbon source and other compounds (e.g., H₂) as electron donors. In the acetate-amended sediments of D08, we detected concurrent enhancement of *Anaerovorax* and *Clostridium*_III as well as *Cyanobacteria* and *Chlorobi*, all of which include the majority of known H₂-producing bacteria. This observation suggests that the latter sulfate-reduction pathway by mixotrophy was highly likely in the biostimulated sediments. Also, a relatively large portion of spore-forming sulfate-reducing bacteria (e.g., *Desulfotomaculum* spp.) that are capable of reducing a range of metals including uranium (Junier et al., 2009) was identified within the *Clostridia* class in the amended or non-amended sediments of D08 and LQ107. Their activities in terms of uranium reduction, however, were apparently restricted in the LQ107 sediments based on the q-PCR results. This observation is consistent with the fact that higher contents of heavy metals and Fe-bearing minerals exist in

LQ107, which likely activated other metabolic pathways in these highly versatile microorganisms.

By contrast, no apparent connections were recognized between the bioremediation efficiency ($\Delta U\%$) and the abundance of known nitrate-reducing (e.g., *Pseudomonas*) or iron-reducing (i.e., *Geobacter* and *Desulfuromonas*) bacteria. As illustrated in Fig. 6-8, the CD sediments, either acetate-amended or non-amended, contained a relatively high abundance of nitrate-reducing bacteria while the LQ107 sediments contained significant abundances of both nitrate- and Fe-reducing bacteria. In the D08 sediments, a small fraction of denitrifiers might also exist in the Chloroflexi phylum, which has been previously identified at uranium-contaminated sites and was demonstrated capable of utilizing acetate as both carbon source and electron donors (Akob et al., 2011; Osaka et al., 2006). Correspondingly, the sediments with relatively dominant nitrate- and iron-reducing bacteria alone (i.e., CD and LQ107) sequestered aqueous uranium to a much lesser extent (-10% to 15%) compared to those that contained elevated sulfate-reducers as well (i.e., D08 and RABS). The lack of association between the abundance of nitrate/iron-reducers with uranium removal efficiency revealed a preference for the indigenous bacterial communities in utilizing nitrate and Fe(III) (oxyhydr)oxides than uranium as electron acceptors. Such observation is largely in line with previous interpretations that nitrate-reduction is much more thermodynamically favorable for anaerobes than U(VI)-reduction (Handley et al., 2012), although controversy remains regarding the reduction potentials of Fe(III)/Fe(II) versus U(VI)/U(reduced). This controversy partly originates from the complexity and diversity associated with bioavailable iron(III) forms (Bethke et al., 2001). Since the acetate-amended sediments of LQ107 in our study have been shown to

contain significant amounts of poorly-crystalline Fe-(oxyhydr)oxides that are bioavailable, the poor uranium removal efficiency observed in the LQ107 samples inferred that the bacterial reduction of poorly-crystalline Fe-(oxyhydr)oxides might be more favored thermodynamically than U(VI) respiration.

Moreover, much higher fluctuations in the effluent uranium concentrations were observed for the sediments that consisted of relatively larger iron-reducing populations (e.g., RABS+5mM Ac, D08, LQ+50mM Ac in Fig. 1_A) than those dominated by either less anoxic, nitrate-reducing conditions (i.e., CD, CD+50mM Ac in Fig. 1_A) or more anoxic, sulfate-reducing conditions (i.e., D08+5/50mM Ac in Fig. 1_A). This finding suggests that uranium reduction is not completely inhibited in a dominantly iron-reducing environment, but might be readily reversed leading to its remobilization. Similar processes has been proposed previously by showing that reduced uranium could be readily re-oxidized by iron(III) oxides even under sulfate-reducing conditions (Sani et al., 2004).

- *Geochemical Controls of the Biostimulation Effects*

Given the variations in redox conditions and mineralogy among the four types of sediments, and in concentrations of trace elements in their groundwater, it is not surprising to find dissimilar bacterial communities associated with these systems. In general, much higher abundance and diversity of bacterial communities were observed in the anoxic sediments than the oxic ones. For example, the acetate-amended sediments of RABS, D08 and LQ107 as well as the non-amended sediments of D08 contained much higher abundance of bacterial communities than those of CD (either amended or non-amended). The relatively high levels of

cytotoxic metal(loid)s, such as strontium, vanadium, chromium and arsenic, co-existing in some of the sediments also showed adverse impact on the abundance of the overall bacterial population. Interestingly, an inverse relationship was observed between the contents of small iron(III)-bearing mineral particles, i.e., goethite (estimated by the intensity of the peaks at 21.3° and 36.7° in the XRD patterns, shown in Fig. S2) and the diversity of bacterial communities at the genus level (indicated by the Shannon-Wiener indices provided in Table 2). These Fe-(oxyhydr)oxides could accommodate high levels of toxic species like arsenic, chromium, and copper through surface adsorption, and thus, might have a limiting effect on the growth of bacteria that are intolerant to heavy metals. Here we also note that substantial gaps may exist in our knowledge of the bacterial communities in terrestrial subsurface settings. For example, the *Desulfosporosinus* genus has been formerly shown to consist of both sulfate- and metal-reducing species (e.g., arsenic) in the laboratory and field studies (Senko et al., 2009; Pester et al., 2010; Sitte et al., 2013). The combined q-PCR and pyrosequencing results of our study, however, revealed that the relatively high abundance of *Desulfosporosinus* in the amended sediments of LQ107 behaved as metal-reducers only because ultra-low amounts of the *dsrA* gene were detected in the LQ107 samples.

Another important observation in this study is that the acetate amendment of the sediments was no less effective in terms of uranium removal using the 5-mM acetate than the 50-mM one, indicating that the acetate concentration used was not a limiting factor for microbial uranium reduction. Based on the bacterial diversity analyses at the phylum, class, and genus three levels (Table 2), it can be inferred that the amendment of a single abundant carbon source (like using the 50-mM acetate) enhanced the bacterial diversity but did not necessarily

catalyze higher bioremediation efficiencies. This is because biostimulation via acetate-amendment is a non-selective process that benefits both target and non-target microorganisms for uranium sequestration. When deeper anoxic conditions were achieved in the acetate-amended sediments (i.e., D08 + 50mM Ac), there was also likely accumulation of reduced Fe, Mn, and S species in the microenvironment, and therefore, alternative metabolic pathways including Fe- and sulfide-oxidation could be activated. For example, in the 50-mM acetate-amended D08, the population of iron-oxidizers in the genus of *Lysobacter* was significantly enhanced compared to that in other D08 sediments, and so was the *Chlorobi* class that includes a variety of phototrophic anoxygenic sulfide-oxidizers (e.g., green sulfur bacteria).

The close relationship identified between the uranium removal efficiency and the abundance of sulfate-reducing bacterial gene markers supports the conclusion that sulfate reduction can be effective for immobilizing aqueous uranium. This conclusion is consistent with those of former field-scale investigations carried out at multiple uranium-contaminated sites (Cardenas et al., 2008; N'Guessan et al., 2008; Williams et al., 2013; Lezama-Pacheco et al., 2015; Mouser et al., 2015). Although aqueous U(VI) was shown to form $\text{Ca-UO}_2\text{-CO}_3$ complexes in the slightly alkaline groundwater used in this study (e.g., Williams et al., 2011), the high uranium-removal efficiency (up to 95%) observed in the amended D08 sediments indicated that the presence of such aqueous ternary complexes had little effect on the uranium bioavailability.

4.2 Non-Biological Pathways

Abiotic processes also acted in concert with biotic processes to effectively remove aqueous uranium. We presume that interactions of soluble uranium species with mineral

surfaces, especially those of fine-sized and Fe-bearing minerals, also contributed to the overall sequestration. This assertion is mainly supported by our observations that (1) the bio-stimulated versus non-stimulated sediments of LQ107 showed similar capacity in uranium sequestration and (2) the biostimulated sediments of CD sequestered less uranium than the non-stimulated ones. In both scenarios, the biostimulation had a minimal or adverse effects on the sequestration of aqueous uranium. A viable explanation is that LQ107 and CD sediments contained more fractions of fine and poorly-crystalline iron-bearing minerals such as ferrihydrite and clays (e.g., chlorite) compared to RABs and D08, and these minerals provided good traps for aqueous uranium via surface adsorption. When the associated biomass was enhanced via the acetate amendment however, these adsorbents for uranium were likely most susceptible to dissolution and surface modification, which contributed to the remobilization of the adsorbed uranium. The biostimulated LQ107 system did not show significant rebound in effluent uranium because of the relatively high abundance of iron-reducing bacteria boosted in these sediment, which could generate soluble Fe(II) species and contribute positively the overall uranium sequestration. By comparison, the RABS sediments, which contained relatively high amount of carbonate minerals and where the total bacterial markers were high but sulfate-reducing markers were low, represents another scenario for us to evaluate the role of abiotic processes in uranium sequestration. More specifically, we first found a decrease in the calcite content when acetate was introduced to the RABS sediments, but when the biostimulation intensity was enhanced (i.e., 50 mM vs. 5 mM), the calcite content increased (based on XRD analyses). This variation in the carbonate mineral content may have a dominant effect on uranium sequestration as uranyl has a high affinity for Ca-carbonate precipitates.

Meanwhile, the relatively high bacterial biomass in RABS sediments likely provided extra traps for uranium through surface sorption.

The diverse forms of U(VI)-reduction end-products, which depend on a range of geochemical conditions, including solution pH, ionic composition and strength, and initial U (VI) concentrations (Suzuki et al., 2005; Kelly et al., 2008; Bernier-Latmani et al., 2010; Wang et al., 2013; Alessi et al., 2014) make them difficult to characterize. Probing the end-products of uranium immobilization was further confounded by the relatively low environmentally-relevant uranium concentrations used in this study (Williams et al., 2011). In comparison, most previous studies employed high initial concentrations of uranium ranging from 100 to 1200 μM . Also, although the end-products of U(VI) reduction were formerly assumed to be the sparingly soluble uraninite, a number of recent experimental and field studies have reported the formation of non-uraninite species such as adsorbed monomeric U(IV) complexes (Bernier-Latmani et al., 2010; Veeramani et al., 2011; Bargar et al., 2011; Stylo et al., 2013).

4.3 Implications

The findings of this study contribute to a growing body of evidence that highlights the importance of promoting and maintaining sulfate-reducing conditions for effective sequestration of U(VI). The positive connection between the uranium removal efficiency and the abundance of sulfate-reducing bacterial markers complements previous findings that U(VI) can be reduced abiotically by biogenic bisulfides and iron sulfides via bacterial sulfate reduction or enzymatically in pure or mixed cultures. Importantly, this study presents a systematic analysis of the various biogeochemical components involved during bioremediation of uranium-

contaminated sites and reveals the interactive relationships between bacterial communities, sediment mineralogy, and groundwater geochemistry.

The re-oxidation and mobilization of uranium are seminal challenges governing the success of remediation at uranium contaminated sites. Given the relatively low concentrations of aqueous U(VI) at the Rifle sites ($\sim 1 - 2\mu\text{M}$), it is feasible to achieve comparable levels of sulfate-reducing activity via acetate amendment of sediments in order to maintain the uranium concentrations below the regulated level by the U.S. EPA drinking water standard. Variables such as the duration of biostimulation, choice and concentration of electron donors, depend on multiple intrinsic factors such as groundwater pH, redox conditions, sediment mineralogy, and the presence of co-contaminants. Future work should also focus on developing techniques that will help determine the speciation of uranium at environmentally relevant (low) concentrations in heterogeneous subsurface environments.

Acknowledgements

Funding for this project was provided by DOE-BER Grant DE-SC0006825 and DOE-BES Grant DE-FG02-06ER15786, and facilities were made available through the Virginia Tech's Institute for Critical Technology and Applied Sciences (ICTAS). Mössbauer spectroscopy analysis was performed under proposal 47291 at the Environmental Molecular Sciences Laboratory (EMSL) using EMSL, a DOE Office of Science National User Facility sponsored by the Office of Biological and Environmental Research and located at Pacific Northwest National Laboratory under proposal 47291.

References

1. Alessi, D.S., Lezama-Pacheco, J.S., Janot, N., Suvorova, E.I., Cerrato, J.M., Giammar, D.E., Davis, J.A., Fox, P.M., Williams, K.H., Long, P.E., Handley, K.M., Bernier-Latmani, R., Bargar, J.R., 2014. Speciation and reactivity of uranium products formed during *in situ* bioremediation in a shallow alluvial aquifer. *Environ Sci & Technol*, 48, 12842-12850.
2. Abdelouas, A., Lu, Y., Lutze, W., Nuttall, H.E., 1998. Reduction of U(VI) to U(IV) by indigenous bacteria in contaminated ground water. *J Contam Hydrol*, 35, 217-233.
3. Akob, D.M., Kerkhof, L., Kuesel, K., 2011. Linking specific heterotrophic bacterial populations to bioreduction of uranium and nitrate in contaminated subsurface sediments by using stable isotope probing. *Appl Environ Microbiol*, 77, 8197-8200.
4. Anderson, R.T., Vrionis, H.A., Oriz-Bernad, I., Resch, C.T., Long, P.E., Dayvault, R., Karp, K., Marutzky, S., Metzler, D.R., Peacock, A., White, D.C., Low, M., Lovley, D.R., 2003. Stimulating the *in situ* activity of *Geobacter* species to remove uranium from the groundwater of a uranium-contaminated aquifer. *Appl Environ Microbiol*, 69, 5884-5891.
5. Arnold, T., Zorn, T., Berhard, G., Nitsche, H., 1998. Sorption of uranium(VI) onto phyllite. *Chem Geol*, 151, 129-141.
6. Bachmaf, S., Merkel, B.J., 2011. Sorption of uranium(VI) at the clay mineral–water interface. *Environ Earth Sci*, 63, 925–934.
7. Bargar, JR., Williams, K.H., Campbell, K.M., Long, P.E., Stubbs, J.E., Suvorova, E.I., Lezama-Pacheco, J.S., Alessi, D.S., Stylo, M., Webb, S.M., Davis, J.A., Giammar, D.E., Blue, L.Y., Bernier-Latmani, R., 2013. Uranium redox transition pathways in acetate-amended sediments. *Proc Natl Acad Sci USA*, 110, 4506-4511.

8. Barron, A.R., Wurzbürger, N., Bellenger, J.P., 2009. Molybdenum limitation of asymbiotic nitrogen fixation in tropical forest soils. *Nat Geos*, 2, 42-45.
9. Bellenger, J.P., Wichard, T., Xu, Y., 2011. Essential metals for nitrogen fixation in a free-living N₂-fixing bacterium: chelation, homeostasis and high use efficiency. *Environ Microbiol*, 13, 1395-1411.
10. Bernier-Latmani, R., Veeramani, H., Vecchia, E.D., Junier, P., Lezama-Pacheco, J.S., Suvorova, E.I., Sharp, J.O., Wigginton, N.S., Bargar, J.R., 2010. Non-uraninite products of microbial U(VI) reduction. *Environ Sci & Technol*, 44, 9456-9462.
11. Bethke, C.M., Sanford, R.A., Kirk, M.K., Jin, Q., Flynn, T.M., 2011. The thermodynamic ladder in geomicrobiology. *Am J Sci*, 311, 183-210.
12. Beyenal, H., Sani, R.K., Peyton, B.M., Dohnalkova, A.C., Amonette, J.E., Lewandowski, Z., 2004. Uranium immobilization by sulfate-reducing biofilms. *Environ Sci & Technol*, 38, 2067-2074.
13. Boland, D.D., Collins, R.N., Glover, C.J., Payne, T.E., Waite, T.D., 2014. Reduction of U(VI) by Fe(II) during the Fe(II)-accelerated transformation of ferrihydrite. *Environ Sci & Technol*, 48, 9086-9093.
14. Bruno, J., DePablo, J., Duro, L. 1995. Experimental study and modeling of the U (VI)-Fe(OH)₃ surface precipitation/coprecipitation equilibria. *Geochim Cosmochim Acta*, 59, 4113-4123.
15. Brusseau, M.L., Hu, Q.H., Srivastava, R., 1997. Using flow interruption to identify factors causing nonideal contaminant transport. *J Contam Hydrol*, 24, 205-219.
16. Campbell, K.M., Kukkadapu, R.K., Qafoku, N.P., Peacock, A.D., Leshar, E., Williams, K.H., Bargar, J.R., Wilkins, M.J., Figueroa, L., Ranville, J., Davis, J.A., Long, P.E., 2012. Geochemical,

- mineralogical and microbiological characteristics of sediment from a naturally reduced zone in a uranium-contaminated aquifer. *Appl Geochem*, 27, 1499-1511.
17. Cardenas, E., et al. 2008. Microbial communities in contaminated sediments, associated with bioremediation of uranium to submicromolar levels. *Appl Environ Microbiol*, 74, 3718–3729.
18. Chakraborty, S., Farve, F., Banerjee, D., Scheinost, A.C., Mullet, M., Ehrhardt, J., Brendle, J., Vidal, L., Charlet, L., 2010. U(VI) sorption and reduction by Fe(II) sorbed on montmorillonite. *Environ. Sci. Technol.*, 44, 3779-3785.
19. Chang, Y., Peacock, A.D., Long, P.E., Stephen, J.R., McKinley, J.P., Macnaughton, S.J., Hussain, A.K., Saxton, A.M., White, D.C., 2001. Diversity and characterization of sulfate-reducing bacteria in groundwater at a uranium mill tailings site. *Appl Environ Microbiol*, 67, 3149-3160.
20. Chang, H.S., Korshin, G.V., Wang, Z.M., Zachara, J.M., 2006. Adsorption of uranyl on gibbsite: A time-resolved laser-induced fluorescence spectroscopy study. *Environ Sci & Technol*, 40, 1244-1249.
21. Cheng, T., Barnett, M.O., Roden, E.E., Zhuang, J.L., 2004. Effects of phosphate on uranium(VI) adsorption to goethite-coated sand. *Environ Sci & Technol*, 38, 6059-6065.
22. Cologgi, D.L., Speers, A.M., Bullard, B.A., Kelly, S.D., Reguera, G., 2014. Enhanced uranium immobilization and reduction by *Geobacter sulfurreducens* biofilms. *Appl Environ Microbiol*, 80, 6638-6646.

23. Dalla-Vecchia E., Veeramani H., Suvorova E.I., Wigginton N.S., Bargar J.R. and Bernier-Latmani R., 2010. U(VI) reduction by spores of *Clostridium acetobutylicum*. *Research in Microbiology*, 161, 765-771.
24. Descostes, M., Schlegel, M.L., Eglizaud, N., Descamps, F., Miserque, F., Simoni, E. 2010. Uptake of uranium and trace elements in pyrite (FeS₂) suspensions. *Geochim Cosmochim Acta*, 74, 1551–1562.
25. Farrell J., Bostick, W.D., Jerebak, R.J., Fiedor, J.N. 1999. Uranium removal from groundwater using zero valence iron media. *Ground Water*, 37, 618-625.
26. Francis, A.J., Dodge, C.J., Lu, F., Halada, G.P., Clayton, C.R. 1994. XPS and XANES studies of uranium reduction by *Clostridium* sp. *Environ Sci & Technol*, 28, 636-639.
27. Ganesh, R., Robinson, K.G., Chu, L., Kucsmas, D., Reed, G.D., 1999. Reductive precipitation of uranium by *Desulfovibrio desulfuricans*: Evaluation of cocontaminant effects and selective removal. *Wat Res*, 33, 3447-3458.
28. Handley, K.M., Wrighton, K.C., Piceno, Y.M., Andersen, G.L., DeSantis, T.Z., Williams, K.H., Wilkins, M.J., N'Guessan, A.L., Peacock, A., Bargar, J.R., Long, P.E., Banfield, J.F., 2012. High-density PhyloChip profiling of stimulated aquifer microbial communities reveals a complex response to acetate amendment. *FEMS Microbiol Ecol* 81, 188-204.
29. Handley, K.M., VerBerkmoes, N.C., Steefel, C.I., Williams, K.H., Sharon, I., Miller, C.S., Frischkorn, K.R., Chourey, K., Thomas, B.C., Shah, M.B., Long, P.E., Hettich, R.L., Banfield, J.F., 2013. Biostimulation induces syntrophic interactions that impact C, S and N cycling in a sediment microbial community. *ISME J*, 7, 800-816.

30. Hyun, S.P., Davis, J.A., Sun, K., Hayes, K.F., 2012. Uranium reduction by iron monosulfide mackinawite. *Environ Sci & Technol*, 46, 3369-3376.
31. Ilton, E.S., Qafoku, N.P., Liu, C. Moore, D.A., Zachara, J.M., 2008. Advective removal of intraparticle uranium from contaminated vadose zone sediments, Hanford, US. *Environ Sci & Technol*, 42, 1565-1571.
32. Jeon B.H., Dempsey, B.A., Burgos, W.D., Barnett, M.O., Roden, E.E., 2005. Chemical reduction of U(VI) by Fe(II) at the solid-water interface using natural and synthetic Fe(III) oxides. *Environ Sci & Technol*, 39, 5642-5649.
33. Junier, P. et al. 2009. Metal reduction by spores of *desulfotomaculum reducens*. *Environ Microbiol*, 11, 3007-3017.
34. Kelly, S.D., Kemner, K.M., Carley, J., Criddle, C., Jardine, P.M., Watson, D.W., Wu., W.M., 2008. Speciation of uranium in sediments before and after *in situ* biostimulation. *Environ Sci & Technol*, 42, 1558-1564.
35. Komlos, J., Peacock, A., Kukkadapu, R.K., Jaffe, P.R., 2008. Long-term dynamics of uranium reduction/reoxidation under low sulfate conditions. *Geochim Cosmochim Ac*, 72, 3603-3615.
36. Kondo, R., Purdy, K.J., Silva, S., 2007. Spatial dynamics of sulphate-reducing bacterial compositions in sediment along a salinity gradient in a UK estuary. *Microbes Environ*, 22, 1-19.
37. Kudo, K., Yamaguchi N., Makino T., Ohtsuka T., Kimura K., Dong D.T., Amachi S. 2013. Release of arsenic from soil by a novel dissimilatory arsenate-reducing bacterium, *Anaeromyxobacter* sp. strain PSR-1. *Appl Environ Microbiol*, 79, 4635–42.

38. Kukkadapu, R.K., Qafoku, N., Arey, B.W., Resch, C.T., Long, P.E., 2010. Effect of extent of natural subsurface bioreduction on Fe-mineralogy of subsurface sediments. *Journal of Physics: Conference Series*, 217, 012047.
39. Li L., Steefel, C., Williams, K., Wilkins M.J., Hubbard, S. 2009. Mineral transformation and biomass accumulation associated with uranium bioremediation at Rifle, Colorado. *Environ Sci & Technol*, 43, 5429-5435.
40. Liger, E., Charlet, L., Van Cappellen, P., 1999. Surface catalysis of uranium (VI) reduction by iron(II). *Geochim Cosmochim Ac*, 63, 2939-2955.
41. Long, P.E., Williams, K.H., Davis, J.A., Fox, P.M., Wilkins, M.J., Yabusaki, S.B., Fang, Y., Waichler, S.R., Berman, E., Gupta, M., Chandler, D.P., Murray, C., Peacock, A., Giloteaux, L., Handley, K., Lovley, D.R., Banfield, J.F., 2015. Biocarbonate impact on U(VI) bioreduction in a shallow alluvial aquifer. *Geochim Cosmochim Ac* 150, 106-124.
42. Lovley, D.R., Philips, E.J.P. 1992. Bioremediation of uranium contamination with enzymatic uranium reduction. *Environ Sci & Technol*, 26, 2228-2236.
43. Missana, T., Maffiotte, C., Garcia-Gutierrez, M., 2003. Surface reaction kinetics between nanocrystalline magnetite and uranyl. *J Colloid Interface Sci*, 261, 145-160.
44. Mouser P.J., N'Guessan, A.L., Qafoku, N.P., Sinha, M., et al., 2015. "Influence of carbon and microbial community priming on the attenuation of uranium in a contaminated floodplain aquifer." *Ground Water*, 53, 600-613
45. N'Guessan, A.L., Vrionis, H.A., Resch, C.T., et al., 2008. Sustained removal of uranium from contaminated groundwater following stimulation of dissimilatory metal reduction. *Environ Sci & Technol*, 42, 2999-3004.

46. Nico, P.S., Stewart, B.D., Fendorf, S., 2009. Incorporation of oxidized uranium into Fe hydroxides during Fe(II) catalyzed remineralization. *Environ Sci & Technol*, 43, 7391-7396.
47. North, N., Doohopf, S.L., Petrie, L., Istok, J.D., Balkwill, D.L., Kostka, J.E., 2004. Change in bacterial community structure during in situ biostimulation of subsurface sediment co-contaminated with uranium and nitrate. *Appl Environ Microbiol*, 70, 4911-4920.
48. O'Loughlin, E.J., Kelly, S.D., Cook, R.E., Csencsits, R., Kemner, K.M., 2003. Reduction of U(VI) by mixed Fe(II)/Fe(III) hydroxide (green rust): Formation of UO₂ nanoparticles. *Environ Sci & Technol*, 37, 721-727.
49. Osaka T., et al. 2006. Identification of acetate- or methanol-assimilating bacteria under nitrate-reducing conditions by stable-isotope probing. *Microb Ecol*, 52, 253–266.
50. Peretyazhko, T.S., Zachara, J.M., Kukkadapu, R.K., Heald, S.M., et al., 2012. Pertechetate (TcO₄⁻) reduction by reactive ferrous iron forms in naturally anoxic, redox transition zone sediments from the Hanford Site, USA. *Geochim Cosmochim Acta*, 92, 48-66.
51. Pester, M., Bittner, N., Deevong, P., et al., 2010. A 'rare biosphere' microorganism contributes to sulfate reduction in a peatland. *ISME J*, 4, 1591-1602.
52. Pietzsch, K., Hard, B.C., Babel, W. 1999. A *Desulfovibrio* sp. capable of growing by reducing U(VI). *J Basic Microbiol* 39, 365-372.
53. Qafoku, N.P., Zachara, J.M., Liu, C., Gassman, P.L., Qafoku, O.S., Smith, S.C., 2005. Kinetic desorption and sorption of U(VI) during reactive transport in a contaminated Hanford sediment. *Environ Sci & Technol*, 39, 3157-3165.

54. Qafoku, N.P., Kukkadapu, R.K., McKinley, J.P., Arey, B.W., Kelly, S.D., Wang, C., Resch, C.T., Long, P.E., 2009. Uranium in framboidal pyrite from a naturally bio-reduced alluvial sediment. *Environ Sci & Technol*, 43, 8528-8534.
55. Qafoku, N.P., Dresel, D.E., Ilton, E.S., McKinley, J.P., Resch, C.T., 2010. Chromium transport in an acidic waste contaminated subsurface medium: The role of reduction. *Chemosphere*, 81, 1492-1500.
56. Qafoku, N.P., Gartman, B.N., Kukkadapu, R.K., Arey, B.W., Williams, K.H., Mouser, P.L., Heald, S.M., Bargar, J.R., Janot, N., Yabusaki, S., Long, P.E., 2014. Geochemical and mineralogical investigation of uranium in multiple-element contaminated, organic-rich subsurface sediment. *Appl Geochem*, 42, 77-85.
57. Regenspurg, S., Schild, D., Schaefer, T. et al., 2009. Removal of uranium(VI) from the aqueous phase by iron(II) minerals in presence of bicarbonate. *Appl Geochem*, 24, 1617-1625.
58. Sani, R.K., Peyton, B.M., Amonetter, J.E., Geesey, G.G., 2004. Reduction of uranium(VI) under sulfate-reducing conditions in the presence of Fe(III)-hydroxides. *Geochim Cosmochim Acta*, 68, 2639-2648.
59. Schindler, M., Legrand, C.A., Hochella, M.F., 2015. Alteration, adsorption and nucleation processes on clay–water interfaces: Mechanisms for the retention of uranium by altered clay surfaces on the nanometer scale. *Geochim Cosmochim Acta*, 153, 15-36.
60. Schloss, P.D., Gevers, D., Westcott, S.L., 2011. Reducing the effects of PCR amplification and sequencing artifacts on 16S rRNA gene-based studies. *PLoS ONE*, 6, e27310.

61. Schloss, P.D., et al., 2009. Introducing mothur: Open-source, platform-independent, community-supported software for describing and comparing microbial communities. *Appl Environ Microbiol*, 75, 7537-41.
62. Scott, T.B., Allen, G.C., Heard, P.J., Randell, M.G., 2005. Reduction of U(VI) to U(IV) on the surface of magnetite. *Geochim Cosmochim Acta*, 69, 5639-5646.
63. Senko, J.M., Istok, J.D., Suflita, J.M., Krumholz, L.R., 2002. In-situ evidence for uranium immobilization and remobilization. *Environ Sci & Technol*, 36, 1491-1496.
64. Senko, J.M., Zhang, G., McDonough, J.T., et al., 2009. Metal reduction at low pH by a *Desulfosporosinus* species: Implications for the biological treatment of acidic mine drainage. *Geomicrobiol J*, 26, 71-82.
65. Sharp, J.O., Schofield, E.J., Veeramani, H., Suvorova, E.I., Kennedy, D.W., Marshall, M.J., Metha, A., Bargar, J.R., Bernier-Latmani, R. 2009. Structural similarities between biogenic uraninites produced by phylogenetically and metabolically diverse bacteria. *Environ Sci & Technol*, 43, 8295-8301.
66. Singer, D.M., Maher, K., Brown, G.E., 2009. Uranyl–chlorite sorption/desorption: Evaluation of different U(VI) sequestration processes. *Geochim Cosmochim Acta*, 73, 5989-6007.
67. Sitte, J., Pollok, K., Langenhorst, F., et al., 2013. Nanocrystalline nickel and cobalt sulfides formed by a heavy metal-tolerant, sulfate-reducing enrichment culture. *Geomicrobiol J*, 30, 36-47.
68. Stylo, M., Alessi, D.S., Shao, P.P., Lezama-Pacheco, J.S., Bargar, J.R., Bernier-Latmani, R., 2013. Biogeochemical controls on the product of microbial U(VI) reduction. *Environ Sci & Technol*, 43, 12351-12358.

69. Sun, Y., Li, J., Wang, X., 2014. The retention of uranium and europium onto sepiolite investigated by macroscopic, spectroscopic and modeling techniques. *Geochim Cosmochim Acta*, 140, 621-634.
70. Suzuki, M.T, Taylor, L.T., DeLong, E.F., 2000. Quantitative analysis of small subunit rRNA genes in mixed microbiak populations via 5'-nuclease assays. *Appl Environ Microbiol*, 66, 4605-4614.
71. Suzuki, Y., Kelly, S.D., Kemmer, K.M., Banfield, J.F., 2003. Microbial populations stimulated for hexavalent uranium reduction in uranium mine sediment. *Appl Environ Microbiol*, 69, 1337-1346.
72. Suzuki, Y., Kelly, S.D., Kemmer, K.M., Banfield, J.F., 2005. Direct microbial reduction and subsequent preservation of uranium in natural near-surface sediment. *Appl Environ Microbiol*, 71, 1790-1797.
73. Treude N, Rosencrantz D, Liesack W, Schnell S. 2003. Strain FAc12, a dissimilatory iron-reducing member of the Anaeromyxobacter subgroup of Myxococcales. *FEMS Microbiol Ecol*, 44, 261-9.
74. Veeramani, H. et al. 2011. Products of abiotic U(VI) reduction by biogenic magnetite and vivianite. *Geochim Cosmochim Acta*, 75, 2512-2528.
75. Veeramani, H., Scheinost, A.C., Monsegue, N., Qafoku, N.P., Kukkadapu, R., Newville, M., Lanzirrotti, A., Pruden, A., Murayama, M., Hochella, M.F., 2013. Abiotic reductive immobilization of U(VI) by biogenic mackinawite. *Environ Sci & Technol*, 47, 2361-2369.
76. Wall J.D., Krumholz, L.R. 2006. Uranium reduction. *Annual Review of Microbiology*, 60, 149-166.

77. Wang, Y., Frutschi, M., Suvorova, E., Phrommavanh, V., Descostes, M., Osman, A., Geipel, G., Bernier-Latmani, R., 2013. Mobile uranium(VI)-bearing colloids in a mining-impacted wetland. *Nature Comm*, 4, 2942.
78. Wersin, P., Hochella, M.F., Persson, P., Redden, G., Leckie, J.O., Harris, D.W., 1994. Interaction between aqueous uranium (VI) and sulfide minerals: Spectroscopy evidence for sorption and reduction. *Geochim Cosmochim Acta*, 58, 2829-2843.
79. Wielinga, B., Bostick, B., Hansel, C.M., Rosenzweig, F., Fendorf, S., 2000. Inhibition of bacterially promoted uranium reduction: Ferric (hydr)oxides as competitive electron acceptors. *Environ Sci & Technol*, 34, 2190-2195.
80. Williams, K.H., Long, P.E., Davis, J.A., Wilkins, M.J., N'Guessan, A.L., Steefel, C.I., Yang, L., et al., 2011. Acetate availability and its influence on sustainable bioremediation of uranium-contaminated groundwater. *Geomicrobiol J*, 28, 519-539.
81. William, K.H., Bargar, J.R., Lloyd, J.R., et al., 2013. Bioremediation of uranium-contaminated groundwater: a systems approach to subsurface biogeochemistry. *Curr Opin Biotechnol*, 24, 489-497.
82. Wu, Q., Sanford, R.A., Löffler, F.E., 2006. Uranium(VI) reduction by *Anaeromyxobacter dehalogenans* strain 2CP-C. *Appl Environ Microbiol*, 72, 3608-14.
83. Xu J, Yan C, Zhang F, Konishi H, Xu, H, Teng H (2013). Testing the cation-hydration effect on the crystallization of Ca–Mg–CO₃ systems. *Proc Natl Acad Sci USA*, 110, 17750-17755.
84. Xu, M., Wu, W., Wu, L., He, L., Van Nostrand, J.D., Deng, Y., Luo, J., Carley, J., Ginder-Vogel, M., Gentry, T.J., Gu, B., Watson, D., Jardine, P.M., Marsh, T.L., Tiejie, J.M., Hazen, T., Criddle,

- C.S., Zhou, J., 2010. Responses of microbial community functional structures to pilot-scale uranium in situ bioremediation. *ISME J*, 4, 1060-1070.
85. Yang, Z., Kang, M., Ma, B., Xie, J., Chen, F., Charlet, L., 2014. Inhibition of U(VI) reduction by synthetic and natural pyrite. *Environ Sci & Technol*, 48, 10716-10724.
86. Yabusaki, S.B., Fang, Y., Long, P.E., Resch, C.T., Peacock, A.D., Komlos, J., Jaffe, P.R., Morrison, S.J., Dayvault, R.D., White, D.C., Anderson, R.T., 2007. Uranium removal from groundwater via in situ biostimulation: field-scale modeling of transport and biological processes. *J Contam Hydrol*, 93, 216–235.
87. Yusan, S., Akyil, S. 2008. Sorption of uranium(VI) from aqueous solutions by akaganeite. *J Hazard Mater*, 160, 388–395.

Table

Table 1. Major chemical compositions for the background solutions used in the column experiments. The background solutions were groundwater collected from the various sediment galleries.

Elemental Composition	Ave. concentration (µg·L ⁻¹)			
	RABS	CD	D08	LQ107
Boron (B)	543	-	-	-
Calcium (Ca)	47400	105000	74200	90800
Magnesium (Mg)	106000	109000	96500	87100
Manganese (Mn)	12300	-	-	-
Potassium (K)	11000	13200	8230	10200
Silicon (Si)	13600	11900	13400	12300
Sodium (Na)	259000	231000	199000	164000
Strontium (Sr)	2150	2790	2320	2240
Sulfur (S)	277000	286000	236000	202000
Vanadium (V)	2390	2640	-	471
Uranium (U)	190	161	186	178
pH	7.65	7.89	8.21	8.42

Note: iron was under the detection limits in all background solutions.

Table 2. Shannon Wiener indices that indicate bacterial diversity for different sediment samples at phylum-, class-, and genus levels, respectively.

Sediment Type	Treatment	<i>Phylum</i>	<i>Class</i>	<i>Genus</i> <i>Clostridia</i>	<i>Genus</i> <i>δ-Proteobacter</i>	<i>Genus</i> <i>γ-Proteobacter</i>
RABS	0 Ac	1.40	2.00	1.15	1.09	0.74
RABS	5mM Ac	1.37	1.94	1.45	0.87	1.04
RABS	50mM Ac	1.50	2.05	1.31	1.07	1.07
CD	0 Ac	1.18	2.08	-	-	1.45
CD	50 mM Ac	1.38	1.99	0.13	0.62	0.67
D08	0 Ac	1.62	2.24	1.12	1.50	1.30
D08	5mM Ac	1.59	2.14	1.15	1.75	0.88
D08	50mM Ac	1.72	2.32	1.66	1.57	1.13
LQ107	0 Ac	1.50	2.18	1.00	0.64	0.52
LQ107	5mM Ac	1.36	2.09	0.83	0.29	0.73
LQ107	50mM Ac	1.47	2.16	0.99	0.30	0.88

Figure 1 Compiled time profiles of effluent elemental compositions for the acetate-amended (blue and red symbols) and non-biostimulated (black symbols) sediment. In each row, the results regarding one element were compared among the RABS, CD, D08, and LQ107 systems (presented from left to right, respectively).

Figure 2 Scanning electron micrographs of iron-sulfide phases (indicated by white arrows) in the < 53 μm fractions of D08 sediments. (a-c) Framboidal pyrite with/without distinctive sub-crystals in biostimulated and non-biostimulated D08 samples; (d) amorphous Fe-S aggregates in biostimulated D08 samples; and (e-f) solid phases with recognizable euhehedral shapes in biostimulated D08 samples.

Figure 3 Mossbauer spectroscopy analysis of the acetated-amended and non-amended sediments of CD.

Figure 5 Real-time PCR analysis of total 16S rRNA and *dsrA* genes extracted from acetate-amended and non-amended sediments. The *dsrA* genes in the CD and LQ107 samples were either not detected or many orders of magnitude lower.

Figure 6 Phylum-level population structures of indigenous bacteria in the acetate-amended and non-amended sediments of RABS, CD, D08, and LQ107. The calculated “relative abundances of different bacterial phyla” in each sample was scaled in proportion to the total 16S rRNA numbers detected in that specific sample so that the “absolute” abundances of indigenous bacterial classes can be compared among different sediment samples.

Figure 7 Class-level population structures of indigenous bacteria in the acetate-amended and non-amended sediments of RABS, CD, D08, and LQ107. The calculated “relative abundances of different bacterial classes” in each sample was scaled in proportion to the total 16S rRNA numbers detected in that specific sample so that the “absolute” abundances of indigenous bacterial classes can be compared among different sediment samples. The Clostridia, δ -Proteobacteria and γ -Proteobacteria classes in each sample have been highlighted in dark frames.

(A) Genus-Level Bacterial Structure (Delta-Proteobacteria)

(B) Genus-Level Bacterial Structure (Gamma-Proteobacteria)

Figure 8 Genus-level population structures in the δ -Proteobacteria, γ -Proteobacteria, and Clostridia among the various acetate-amended and non-amended sediments. Similarly, the calculated “relative abundances of different genera” in each sample was scaled in proportion to the 16S rRNA numbers of delta-Proteobacteria detected in that specific sample so that the “absolute” abundances of indigenous bacterial classes can be compared among different sediment samples.