

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/236931738>

In Situ Bioremediation of Uranium with Emulsified Vegetable Oil as the Electron Donor

ARTICLE in ENVIRONMENTAL SCIENCE & TECHNOLOGY · MAY 2013

Impact Factor: 5.33 · DOI: 10.1021/es3033555 · Source: PubMed

CITATIONS

10

READS

40

16 AUTHORS, INCLUDING:



Weimin Wu

Stanford University

152 PUBLICATIONS 3,806 CITATIONS

SEE PROFILE



Craig S Criddle

Stanford University

187 PUBLICATIONS 8,495 CITATIONS

SEE PROFILE



Scott C Brooks

Oak Ridge National Laboratory

135 PUBLICATIONS 1,993 CITATIONS

SEE PROFILE

In Situ Bioremediation of Uranium with Emulsified Vegetable Oil as the Electron Donor

David B. Watson,^{*,†,¶} Wei-Min Wu,^{*,‡,§,¶} Tonia Mehlhorn,[†] Guoping Tang,[†] Jennifer Earles,[†] Kenneth Lowe,[†] Thomas M. Gihring,[†] Gengxin Zhang,[†] Jana Phillips,[†] Maxim I. Boyanov,[¶] Brian P. Spalding,[†] Christopher Schadt,[†] Kenneth M. Kemner,[¶] Craig S. Criddle,[‡] Philip M. Jardine,[⊥] and Scott C. Brooks[†]

[†]Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831-6038, United States

[‡]Department of Civil and Environmental Engineering, Stanford University, Stanford, California 94305-4020, United States

[§]Center for Sustainable Development and Global Competitiveness, Stanford University, Stanford, California, 94305-4020, United States

[¶]Biosciences Division, Argonne National Laboratory, Argonne, Illinois, 60439, United States

[⊥]Department of Biosystems Engineering and Soil Science, University of Tennessee, Knoxville, Tennessee 37996, United States

S Supporting Information

ABSTRACT: A field test with a one-time emulsified vegetable oil (EVO) injection was conducted to assess the capacity of EVO to sustain uranium bioreduction in a high-permeability gravel layer with groundwater concentrations of (mM) U, 0.0055; Ca, 2.98; NO₃⁻, 0.11; HCO₃⁻, 5.07; and SO₄²⁻, 1.23. Comparison of bromide and EVO migration and distribution indicated that a majority of the injected EVO was retained in the subsurface from the injection wells to 50 m downgradient. Nitrate, uranium, and sulfate were sequentially removed from the groundwater within 1–2 weeks, accompanied by an increase in acetate, Mn, Fe, and methane concentrations. Due to the slow release and degradation of EVO with time, reducing conditions were sustained for approximately one year, and daily U discharge to a creek, located approximately 50 m from the injection wells, decreased by 80% within 100 days. Total U discharge was reduced by 50% over the one-year period. Reduction of U(VI) to U(IV) was confirmed by synchrotron analysis of recovered aquifer solids. Oxidants (e.g., dissolved oxygen, nitrate) flowing in from upgradient appeared to reoxidize and remobilize uranium after the EVO was exhausted as evidenced by a transient increase of U concentration above ambient values. Occasional (e.g., annual) EVO injection into a permeable Ca and bicarbonate-containing aquifer can sustain uranium bioreduction/immobilization and decrease U migration/discharge.



INTRODUCTION

In situ anaerobic bioreduction has been used for remediation of a variety of subsurface contaminants including chlorinated aliphatic hydrocarbons, perchlorate, chromate, and radionuclides.^{1,2} In situ U(VI) reduction and immobilization has been evaluated at several contaminated sites.^{3–7} The process is mediated by Fe(III)-reducing bacteria (FeRB), sulfate-reducing bacteria (SRB), and a diverse range of bacteria that convert soluble U(VI) to sparingly soluble U(IV).¹

Various electron donors such as dissolved hydrogen gas, acetate, lactate, ethanol, methanol, and glucose have been utilized to stimulate microbial reduction of U(VI) in laboratory and field tests.^{4,6,8–13} However, the utilization rate of these electron donors is relatively fast. As a result, microbial growth is likely to occur mainly near the injection well with limited penetration of the electron donor into downgradient areas. Because past studies showed more rapid reduction of U(VI)

using ethanol as the electron donor than lactate and acetate, we selected ethanol in previous field tests.⁶ U concentrations were lowered to below the U.S. EPA maximum contaminant level (MCL) for drinking water (< 0.126 μM) by weekly ethanol delivery at the U.S. Department of Energy (DOE) Oak Ridge Integrated Field Research Challenge (ORIFRC) site.^{7,14–17} During the field experiments, the injection well had to be cleaned frequently to remove biomass and to reduce clogging.^{6,7} Additionally, bioreduction reactions ceased when ethanol delivery was stopped.^{6,9} To deliver electron donor more efficiently with deeper penetration into the aquifer and maintain long-term reducing conditions, slow release electron

Received: August 20, 2012

Revised: May 17, 2013

Accepted: May 21, 2013

Published: May 22, 2013

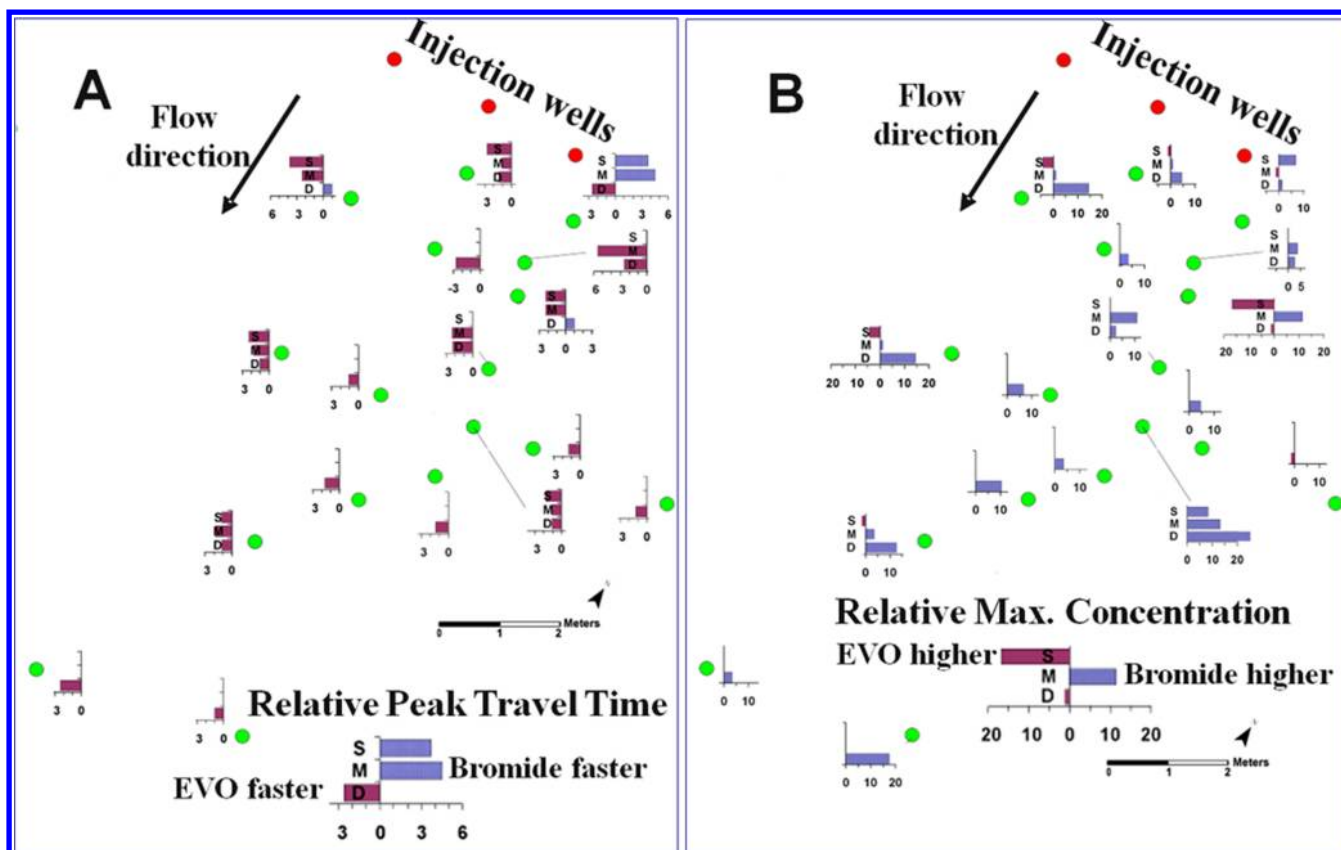


Figure 2. Comparison of bromide versus EVO migration in monitoring wells. (A) Relative peak travel time (RPTT), where the RPTT represents how much faster the EVO or Br peak concentration, respectively, arrives at a well; or $RPTT = (\text{slower peak travel time})/(\text{faster peak travel time})$. (B) Relative maximum concentration (RMC). Similarly, the RMC represents how much higher the maximum relative concentration (C_{max}/C_0) of EVO or Br measured at a well is compared to the other; or $RMC = (C_{\text{max}}/C_0)_{\text{higher}}/(C_{\text{max}}/C_0)_{\text{lower}}$. For multiple level wells, S = shallow (4.3 m bgs), M = middle (4.7 m bgs), and D = deep (5.1 m bgs). For RPTT of Br/EVO > 1.0 (purple color on the left) means that EVO travels faster than bromide and vice versa (EVO/Br > 1.0 = blue color on the right). For RMC of EVO/Br > 1.0 (purple color on the left) means that EVO relative concentration is higher than bromide and vice versa (Br/EVO > 1.0 = blue color on right).

gravel, 0–6.0 m bgs (Supporting Information (SI) Figure S1). Hydraulic conductivity was estimated to be 4.1×10^{-5} cm/s in the intact saprolite zone and 3.8×10^{-2} cm/sec in the fast flow unconsolidated zone (gravelly fill above the intact saprolite) into which the EVO was injected. The hydraulic gradient at the site is approximately 0.016 and the rate of interstitial transport determined from tracer tests is estimated to be ~ 2.2 m/day. Contaminated groundwater in the gravelly fill zone discharges continuously into Bear Creek through seeps and springs.²⁹

Groundwater composition in the upgradient monitoring well FW215 (Figure 1) prior to the EVO injection is available in SI Table S1. The salient characteristics relevant to the processes studied are U ($3.8\text{--}7.1 \mu\text{M}$), sulfate ($1.0\text{--}1.2 \text{ mM}$), nitrate ($0.2\text{--}1.5 \text{ mM}$), pH 6.64, and dissolved oxygen $<0.2 \text{ mg L}^{-1}$. The calculated aqueous U speciation is dominated by $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3$ (77.5% of U) and $\text{CaUO}_2(\text{CO}_3)_3^{2-}$ (20.3% of U). Uranium concentration on the soil–saprolite fraction of the fill material is up to $\sim 500 \text{ mg/kg}$.²⁸ The solid-phase U before EVO injection showed coordination with carbonate as grimselite ($\text{K}_4\text{Na}(\text{UO}_2)(\text{CO}_3)_3 \cdot \text{H}_2\text{O}$), liebigite ($\text{Ca}_2(\text{UO}_2)(\text{CO}_3)_3 \cdot 11(\text{H}_2\text{O})$), and ersonite ($\text{Na}_2\text{CaUO}_2(\text{CO}_3)_3 \cdot 6\text{H}_2\text{O}$).²⁹

Prior to our EVO injection study, a biostimulation test was conducted at the site from September 27, 2005 to August 29, 2006⁹ where an ethanol solution (10 mM) was injected into FW212, F213, and FW214 at a rate of 3 L/min in each well for 24 h on day one and for 1 h each day thereafter. After the

injection, the sequential removal of nitrate, sulfate, and U from the groundwater was observed. Low U concentration below EPA MCL was observed after about 108 days. After ethanol injection ceased, the U, sulfate, and nitrate concentrations rapidly rebounded to preinjection levels, similar to what was measured in January 2009 (SI Table S1).

After the ethanol biostimulation study was completed, eight multilevel sampling wells (MLS-A, B, C, D, E, F, G and H, Figure 1) with six different sampling depths were installed to supplement the existing monitoring network and to better profile groundwater characteristics. The gravelly fill zone from the top of the water table (3.7 m bgs) to the top of the intact saprolite (6.0 m bgs) was targeted for biostimulation and monitoring in this study. Upgradient wells FW215 and FW 233-2 served as control wells for groundwater monitoring. Seep 2, located about 1 m from Bear Creek and about 50 m downgradient of the injection wells, was used to monitor chemical composition of contaminated groundwater discharging to the surface water.

EVO Source. The EVO (SRS) was purchased from Terra Systems, Inc., Wilmington, DE. Its composition (% w/w) was soybean oil, 60; yeast extract, 0.3; food grade surfactant, 6.0; $(\text{NH}_4)_3\text{PO}_4$, 0.05, and remainder was water. To eliminate a rapidly utilized electron source, lactate (which is normally added to the company's stock solution) was not included in the EVO used for the injection. The chemical oxygen demand

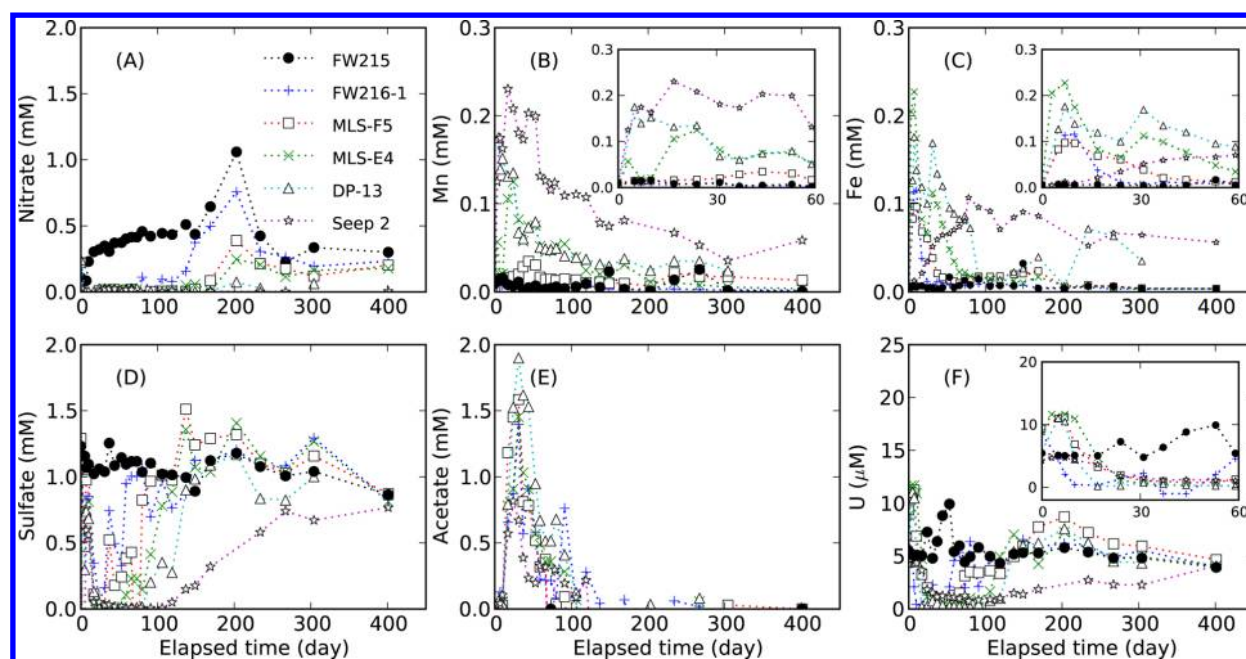


Figure 3. Groundwater geochemistry evolution in monitoring wells from the injection area toward Bear Creek after EVO injection. FW215: upgradient control well. The sequence of distance from monitoring well to injection wells is FW216-1 < MLS-F5 < MLS-E4 < DP-13 < Seep 2.

(COD) content was measured to be 1618 g/L and the specific gravity was 0.93. Gas chromatography analysis indicated the dominant long chain fatty acid (LCFA) components of the EVO were similar to α -linolenic acid (C-18:3 or $C_{18}H_{30}O_2$), linoleic acid (C-18:2 or $C_{18}H_{32}O_2$), oleic acid (C-18:1 or $C_{18}H_{34}O_2$), stearic acid (C-18:0 or $C_{18}H_{36}O_2$), and palmitic acid (C-16:0 or $C_{16}H_{32}O_2$) (Figure. S2). The EVO product was shipped in 20 L-plastic container to the site one month prior to the injection.

Tracer Test. Groundwater (3406 L) was pumped evenly from injection wells FW212, FW213, and FW214 into a 4000-L polyethylene plastic tank 8 days before the tracer test was conducted. NaBr was dissolved into the water to obtain a concentration of 450 mg L^{-1} as Br. The solution was injected evenly to FW212, FW213, and FW214 in 2 h on December 8, 2008. Groundwater samples were taken from 41 monitoring wells to monitor the Br migration for 2 weeks.

EVO Injection. On February 9, 2009, 3406 L of EVO solution (20% of SRS with 80% groundwater from the three injection wells by volume) was prepared in the 4000 L tank, and evenly injected to the three injection wells over a 2-h time frame. After injection, groundwater samples were collected by pumping and sediment samples were taken from the monitoring wells periodically using a pumping and surge block method.⁶

Groundwater samples for metal analysis (10 mL) were filtered ($0.3 \mu\text{m}$), acidified with 0.05 mL of concentrated nitric acid, and then stored at 4°C until analysis. Sediment samples for microbial community analysis were taken from monitoring wells using the surge block method.⁶ The samples for X-ray adsorption near edge spectroscopy (XANES) and extended X-ray absorption fine structure (EXAFS) analysis were taken 5–7 days before scheduled beam run time, stored in anaerobic serum bottles with nitrogen gas headspace, and maintained at 4°C until analysis. Dissolved methane in groundwater was monitored using passive samplers composed of a 1.0-mL

gastight syringe attached with a silicone tubing (length 6.35 mm and I.D 3.175 mm) following Spalding and Watson.^{30,31}

Analytical Methods. Analytical methods have been described previously.^{5–7} Anions (acetate, NO_3^- , Cl^- , and SO_4^{2-}) were analyzed with an ion chromatograph equipped with an IonPac AS-14 analytical column and an AG-14 guard column (Dionex DX-120, Sunnyvale, CA). Cations (Al, Ca, Fe, Mn, Mg, total U, K, etc.) were determined using an inductively coupled plasma mass spectrometer (ICPMS) (Perkin-Elmer ELAN 6100). The oxidation state and speciation of U in sediments were determined by XANES and EXAFS, as described previously.^{11,32} Methane and hydrogen were measured by a TCD gas chromatograph as described previously.^{30,31} The EVO or oil concentration as volatile solids (VS) in groundwater was determined by weight loss on ignition for 1 h at 550°C .^{21,22} The pH, dissolved oxygen (DO), conductivity, and temperature of the groundwater samples were measured in the field or immediately in an on-site trailer equipped with analytical instrumentation. Microbial community analysis in sediment samples utilized high-throughput 16S rRNA gene pyrosequencing and quantitative PCR described elsewhere.²⁶

RESULTS AND DISCUSSION

EVO Migration. The migration of EVO was compared with the transport of the relatively nonreactive bromide. A small fraction of Br and EVO (1.18% and 1.28% of the peak injected concentration) was detected in the closest upgradient control well FW215 8 h after the injection due to transient reversal of the hydraulic gradient caused by the injection and then disappeared within 4 and 2 days, respectively. The specific gravity of the Br solution (450 mg L^{-1}) injected was considered to be similar to groundwater. However, the specific gravity of the injected EVO suspension was around 0.99, slightly lighter than the groundwater. The arrival time of peak EVO concentration at the monitoring wells was consistently faster than bromide peak arrival time (Figure 2A). Bromide

concentration was generally higher in the deeper sampling ports, while the EVO concentration was highest in the shallower ports, indicating the possibility of density-dependent transport and EVO floating (SI Table S4). The travel time for bromide and EVO from the injection wells to Seep 2 (50 m) was approximately 2 days (SI Table S4) when there was a clear visual indication of EVO or remaining surfactant entering Bear Creek (white coloration). In the zone 13 m or less from the injection wells, the EVO concentrations reached peak concentrations 2–5 times faster than it took Br to reach its peak concentrations (Figure 2A), indicating that the EVO moved 2–5 times faster than Br. However, normalized peak EVO concentrations (or the maximum concentration detected at a well divided by the injection concentration) were 10–20 times lower than the normalized Br concentration (Figure 2B), indicating that the majority of the injected EVO was physically trapped or adsorbed in the aquifer. We attribute the faster peak EVO concentration arrival relative to Br to size-exclusion and matrix diffusion phenomena in which dissolved Br was accessible to all pores in the saturated subsurface whereas EVO, a suspension of oil droplets $\sim 1\ \mu\text{m}$ diameter, was excluded from physical entry or diffusion into smaller pores. Consequently, EVO droplets were limited to traveling in larger, faster velocity pores. The longevity of EVO in situ would be dependent on the degradation rate of EVO and degradation products, which would impact its efficiency as an electron donor source.

Geochemical Dynamics. The aqueous solute concentrations in control well FW215 appeared relatively stable except nitrate (Figure 3), which exhibited a transient decrease in response to the small amount of EVO that reached this well at early times. Nitrate concentrations were below detection limits within 3 days following EVO injection in all of the monitoring wells (Figure 3A). Dissolved Mn concentrations increased rapidly with higher concentrations observed in downgradient wells (DP13 and Seep 2) than those near the injection wells (MLS-F5 and MLS-E4) (Figure 3B). Fe concentrations also increased but were observed to be higher near the injection wells relative to wells located further downgradient (Figure 3C), suggesting possible stronger Fe(II) sorption and/or more sulfide associated precipitation of Fe(II) than Mn(II) during downgradient migration. Decline in sulfate concentration occurred after Mn and Fe concentrations increased (Figure 3D). At the same time, Fe concentrations declined, probably, due to sulfide precipitation. Acetate, an intermediary degradation product of yeast extract and LCFAs resulting from EVO degradation, appeared in wells 5–7 days after EVO injection (Figure 3E). Uranium concentration initially increased and then rapidly declined during the first 2 weeks, and then continued declining for 60–90 days depending on well location (Figure 3F). The lowest U concentration of $0.071\ \mu\text{M}$ ($\sim 99\%$ decrease) was observed in injection well FW213 on day 17 and remained below $0.084\ \mu\text{M}$ through day 37, indicating that EVO biostimulation can achieve U in situ concentration below the U.S. EPA MCL. In most monitoring wells, U levels decreased to between 0.42 and $1.26\ \mu\text{M}$ through day 37 to 169, depending on well depth and distance from the injection wells. Wells located farther from the injection wells maintained lower U concentrations for a longer time period. In the multilevel sampling wells, lower U concentrations were maintained longer at shallower sampling depths. At Seep 2, located on the bank of Bear Creek, U reached a minimum concentration of $1.13\ \mu\text{M}$ on day 31, and remained low through day 119 and began to

increase above $1.26\ \mu\text{M}$ on day 169 (Figure 4). These observations are consistent with sequential terminal electron

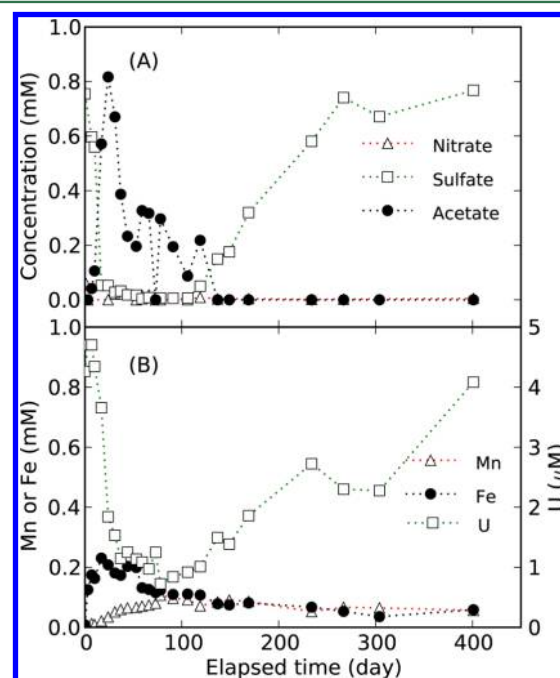


Figure 4. Groundwater geochemistry evolution in Seep 2 near Bear Creek. (A) Nitrate, sulfate, and acetate. (B) Mn, Fe, and U.

accepting processes following the thermodynamic ladder³³ for the reduction of nitrate, Mn, Fe, sulfate, and U(VI) (SI Table S2).

The initial, more rapid terminal electron acceptor reactions were likely caused by the small amount of yeast extract in EVO (0.3% as w/w), which was degraded faster than the vegetable oil components of the EVO. Acetate accumulation mainly resulted from LFCA degradation after microbial hydrolysis of the oil.²⁶ Three factors led to the eventual decreases in acetate concentration: (i) the rate of acetate consumption increased with the growth of acetate-utilizing FeRB, SRB, and methanogens, (ii) acetate was continuously washed out of the aquifer, and (iii) the depletion of EVO originally retained in the aquifer. Acetate concentrations peaked in about 40 days, and then decreased to approximately zero at about day 150. Sulfate concentration began to rebound in most monitoring wells except for Seep 2 and reached the same concentration levels of upgradient wells at approximately day 150 (Figure 3D), which was opposite the trends observed for acetate (Figure 3E). This observation indicates that either acetate was produced from oil degradation at a rate equal to the rate that acetate was consumed, or that oil depletion occurred. Thus EVO degradation lasted at least 150 days in the EVO accumulation zone, and most likely somewhat longer. However, even though sulfate concentrations rebounded to preinjection levels, the Mn and Fe concentrations remained higher than preinjection levels for 300–400 days, indicating that Fe and Mn reducing conditions were maintained in the aquifer for over a year.

Prior to EVO injection, methane in dissolved gas was detected at $\sim 0.05\%$ in several monitoring wells including background well FW215, indicating minor methanogenic activity in the subsurface. The concentration of methane increased as acetate accumulated, and reached a maximum concentration at approximately 100 d (56% and 76% in FW212

and FW213, respectively) where it consistently lagged the acetate peak concentrations (SI Figure S4). After 300 days, the methane concentration remained at about the 1% level, indicating ongoing methanogenic activity.

Microbial Community Evolution. Detailed microbial community dynamics over time in groundwater of select monitoring wells during the EVO injection test was reported by Gihring et al.²⁶ In this study we compared the previous results reported for groundwater samples to the characterization results of a smaller number of sediment surge samples (SI Table S5). The bacterial sequences identified in sediment samples were similar to those found in groundwater after EVO injection.²⁶ The microbial community structure changed significantly in all monitoring wells with less impact in upgradient well FW215 after the EVO injection. Sequences belonging to members of Family *Veillonellaceae* were abundant in 16S rRNA gene libraries and increased in relative abundance in most wells after EVO injection (SI Table S5). Based on our previous work and other literature on these organisms, we believe these are likely performing the initial hydrolysis of vegetable oil (triglycerides) to produce LCFA since they are generally recognized as fermentative bacteria and many have lipase activity (e.g., *Anaerovibrio lipolyticus*³⁴). Some members of this family may also be involved in yeast extract degradation which was present in 0.3% of EVO used. Members of the *Ruminococcaceae* family, known for fermentation of complex substrates, also increased in 16S rRNA gene sequence relative abundance in some wells and may have been important in the degradation of EVO. The relative abundance of sequences classified as members of *Desulfobacteraceae* increased after EVO injection with the majority of the sequences closely related to *Desulforegula conservatrix* which grows by incomplete LCFA-oxidation coupled with sulfate reduction, and produces acetate as a major end product.³⁵ Given the preponderance of *Desulforegula*-like organisms after EVO injection, members of this group likely mediated LCFA oxidation to produce acetate. We have not detected them in abundance previously at the ORIFRC site when ethanol was used for U(VI) bioreduction.^{7,14,16,17} Gene sequences classified as members of *Rhodocyclaceae*, *Neisseriaceae*, and *Comamonadaceae* also increased relative abundance in some wells. These taxa have been previously associated with subsurface nitrate reduction and likely mediated denitrification following EVO injection. Sequences related to *Desulfobulbaceae* and *Geobacteraceae* increased in relative abundance after EVO amendment and were likely important in sulfate and iron(III) reduction, respectively. These taxa are also known for U(VI) reduction.³⁶ Increases in the relative abundance of these known U(VI)-reducing microorganisms have also been observed with ethanol as electron donor in sediments¹⁴ and groundwater^{7,16,17} at this site.

U(VI) Reduction to U(IV). Uranium valence state in aquifer solids retrieved from the control well remained as oxidized hexavalent (U(VI)) and did not change throughout the test period as determined by XANES analysis (Table 1). The predominance of U(VI) was also observed in all samples from monitoring wells prior to EVO injection. After EVO injection, a decrease in solid-phase U(VI) and increase in U(IV) were observed in samples taken between day 36 and 137 from one selected injection and four monitoring wells. This confirmed that U(VI) in the sediments was reduced to U(IV) and the decrease in aqueous U concentrations after EVO injection was also due to U(VI) reduction to U(IV) as observed with

Table 1. Percentage of U(VI) (%) in Total U(VI) + U(IV) in Sediment Samples Taken before and after EVO Injection Based on XANES Analysis^a

well name	use	days since EVO injection				
		−23	36	53	137	234
FW215	control	98	98	96	100	98
FW213	injection	Nd	Nd	12	0	8
FW216	monitoring	100	Nd	Nd	38	96
FW234	monitoring	100	62	Nd	Nd	85
GP01	monitoring	98	28	Nd	70	96
GP03	monitoring	96	28	Nd	38	90

^aEVO injection on February 9, 2009 (day 1). The analytical error of XANES for U(IV) or U(VI) is about $\pm 10\%$. Nd = not determined. −23 day = 23 days prior to EVO injection.

previous ethanol bioreduction experiments.^{6,7,17} The U(VI) content increased to >85% in monitoring well samples taken on day 234 (Table 1) as EVO was depleted, indicating reoxidation of bioreduced U(IV) to U(VI) by incoming nitrate¹⁷ and possible adsorption of U(VI) from upgradient groundwater flowing in. This is consistent with the increase of aqueous U concentrations observed during the same time frame (Figure 3F). In the injection well FW213, U(VI) content remained low on day 234. This was likely due to a greater amount of residual EVO near the injection well that maintained anaerobic conditions for a longer time period. Based on EXAFS analysis, the reduced U(IV) was not uraninite as observed in previous pure culture studies³⁷ but had the form of a Fe–U(IV) complex, similar to those observed previously in microcosm and field studies.^{11,32} If the Fe–U(IV) complex is reoxidized by O₂ and/or nitrate, the iron(III) formed may provide adsorption sites to sequester U(VI) that is released off the solid phase and/or that is flowing in from upgradient.

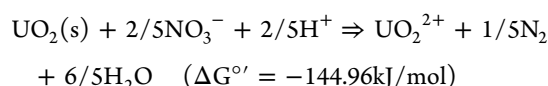
As described above, U concentrations below the U.S. EPA MCL were observed only in the injection wells and a few monitoring wells during a short period (days 17–30). In most monitoring wells, the U concentration was reduced to a level of 0.42–1.26 μM (a 92–77% concentration decrease). This concentration is higher than those observed during previous bioreduction tests in Area 3 of the ORIFRC with ethanol as the electron donor.^{6,7,17} Energetically, the reduction of U(VI) as $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3^0$ to U(IV) as UO_2 (Area 2 condition) is much less favorable than $\text{UO}_2(\text{CO}_3)_3^{4-}$ to UO_2 (Area 3 conditions) based on a standard reduction potential (E°) of -0.204 V versus $+0.061\text{ V}$ (SI Table S2). The higher U concentrations after EVO injection in this study relative to Area 3 ethanol studies was likely caused by higher Ca (2.8–3.3 mM versus 0.6–1.0 mM) and HCO_3^- concentration (5.0–5.5 mM versus 1.2–2.0 mM), and thus a higher fraction of $\text{Ca}_2\text{UO}_2(\text{CO}_3)_3^0$ (>80.2% versus <50% of total U) in this experiment.^{6,7,17} Additionally, penetration of U- and nitrate-containing groundwater from surrounding areas could also lead to higher U concentrations and negatively impact U(VI) reduction since the influx of contaminated upgradient groundwater was not controlled in this study as it was during experiments at the Area 3 site and Area 2 treated was much larger than in the Area 3 study.^{5–7}

U(IV) Oxidation by Nitrate. Aqueous U rebounded in monitoring wells as sulfate concentrations increased and acetate disappeared (Figure 3D and E). Uranium concentrations temporarily increased to above those of upgradient well FW215, as nitrate concentrations appeared in the monitoring

wells except for Seep 2 and finally declined to the U levels found in control well FW215 (Figure 3A and F). The temporary higher than background U concentrations were likely due to oxidation of bioreduced U(IV) to U(VI), with associated mobilization of uranium off the solid phase and the addition of incoming U(VI) from upgradient.

As the EVO was depleted, the rebound of U(VI) occurred initially in upgradient monitoring wells and proceeded to downgradient wells (Figure 3F). By day 300, U concentration in Seep 2 remained below that of the control well. This suggests that the reducing and sequestering capacity created by EVO injection was consumed first in upgradient regions as nitrate- and O₂-containing groundwater migrated into upgradient areas of the treatment zone and then toward downgradient regions as the EVO was consumed. The region near Bear Creek was not exposed to nitrate for over 300 days, and the U concentration in Seep 2 remained well below that of upgradient control FW215 until the end of the test period.

It is important to note that nitrate alone does not directly oxidize U(IV) at an appreciable rate.³⁸ Microorganisms can mediate its enzymatic oxidation and facilitate biotic oxidation as follows:



This has been reported for pure cultures of FeRB *Geobacter metallicreducens*,³⁹ *Anaeromyxobacter dehalogenans* 2CP-C,⁴⁰ and Fe(II)-oxidizing *Thiobacillus denitrificans*.⁴¹ All of these microorganisms have been found at the ORIFC site.^{14–16,42,43} Uranium(IV) reoxidation in the presence of nitrate via microbial activity has been observed in sediment column tests⁴⁴ and confirmed in field tests at the Oak Ridge site.¹⁷

Uranium Discharge to Surface Water. At Seep 2, the furthest downgradient monitoring location near Bear Creek, U concentrations increased briefly at day 5 after EVO injection and then decreased to a minimum value of about 0.7 μM on day 70 (Figure 4). The initial increase was likely due to the upgradient release of adsorbed U(VI) from ferric hydroxides that were reduced and dissolved. Uranium concentrations rebounded gradually coincident with increased sulfate concentrations after day 100. However, U concentrations remained 50% below the preinjection concentrations until day 300 when sulfate concentration was high but still below that of the control well (Figure 3D).

The daily mass of U discharge to Bear Creek decreased by 70–80% by day 30 (Figure 5). This was maintained for 100 days until sulfate started to increase, then gradually decreased to around 40% by day 300, and returned to preinjection levels by day 400 as the EVO was depleted. The cumulative decrease in U discharged to Bear Creek was 60% on day 100 and reached a maximum of 62% on day 150. During the course of 1 year, there was still a reduction of more than 50% of the U discharging to Bear Creek. This analysis suggests that sulfate concentrations in downgradient monitoring wells may be used as an indicator parameter to achieve optimal bioreduction conditions needed to reduce U discharge with a minimum of EVO injections.

Implications. In this study, a single 2-h EVO injection into a shallow unconfined high-permeability aquifer at the ORIFRC formed a biologically active subsurface environment for in situ reduction of U(VI) to U(IV) and consequently decreased U discharge to surface water for over a year. EVO was distributed

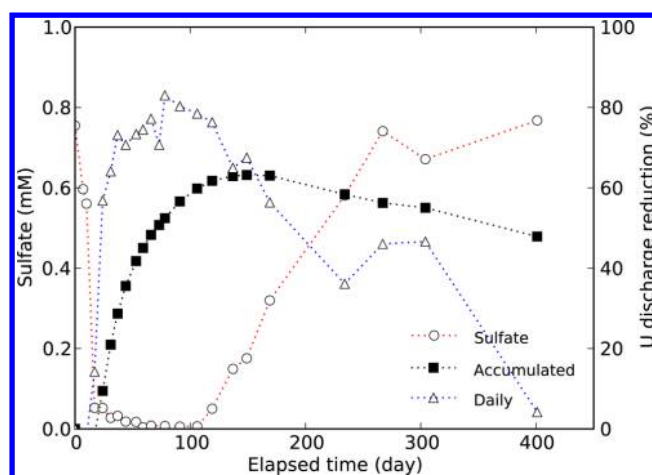


Figure 5. Reduction in uranium discharge to Bear Creek versus sulfate concentration in well Seep 2 over 400 days.

and retained in the subsurface by a combination of mechanisms such as sorption and physical pore size exclusion and EVO trapping, suggesting that EVO bioreduction is more sustainable in situ than soluble substrates such as ethanol.⁴⁵ EVO stimulated microbial populations including LCFA-degrading SRB and most known metal and U(VI) reducing bacteria and, similar to ethanol, the sequential reduction of nitrate, Mn(IV), Fe(III), and sulfate, and transient accumulation of acetate were observed. Under reducing conditions, U concentrations in groundwater near the injection wells decreased to below the U.S. EPA MCL. The EVO injection decreased the U concentration discharging to surface water by 80% within 3 months and reduced the cumulative total U discharge by 50% over a 1-year time period even after the EVO was depleted upgradient. U concentration rebound was observed together with the rebound of sulfate concentrations. Our field test results indicated that EVO can stimulate and sustain U(VI) bioreduction and immobilization in a relatively Ca and carbonate rich (3.0–3.5 mM and 5.5–6.6 mM, respectively), high-permeability aquifer with seasonal or annual injections to effectively decrease U discharge to surface water receptors. Because the aquifer tested has much higher flow rates by at least 1 order of magnitude than most contaminated sites, EVO injections could be done on an even less frequent basis at a site with a lower rate of EVO utilization due to a slower influx of contaminants and oxidants. This study confirms that EVO may be suitable for sustained in situ bioreduction of other metals and nitrate.

■ ASSOCIATED CONTENT

⑤ Supporting Information

Aquifer stratigraphy, groundwater composition of control well, relevant geochemical reactions, data source of Gibbs free energy, maximum concentrations and arrival time of Br and EVO in monitoring wells, relative abundance of 16S rRNA gene sequences, EVO composition, and methane versus acetate concentrations. This is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*Tel.: +1-865-241-4749; fax: +1-865-576-8646; e-mail: watsondb@ornl.gov(D.W.). Tel.: +1 650 724 5310; fax: +1 650 723 7258; e-mail: wei-min.wu@stanford.edu (W.-M.W.).

Author Contributions

[†]D.W. and W.-M.W. contributed equally to this work.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank Ms. Xiangping Yin for analytical and laboratory assistance and Dr. Fang Zhang for data analysis. This research was funded by the U.S. DOE, Office of Science, Office of Biological and Environmental Research (BER), Subsurface Biogeochemical Research Program. Oak Ridge National Laboratory is managed by UT-Battelle, LLC, for the U.S. DOE under contract DE-AC05-18 00OR22725. Argonne National Laboratory (ANL) contributions were supported in part by the ANL Scientific Focus Area project, which is part of the SBR Program of the Office of BER, U.S. DOE under contracts DE-AC05-76RLO and DE-AC02-06CH11357, respectively. Use of the Advanced Photon Source (APS) was supported by the DOE-SC Office of Basic Energy Sciences, under contract DE-AC02-06CH11357. MRCAT/EnviroCAT operations are supported by DOE and the MRCAT/EnviroCAT member institutions.

REFERENCES

- (1) Hazen, T.; Tabak, H. Developments in bioremediation of soils and sediments polluted with metals and radionuclides: 2. Field research on bioremediation of metals and radionuclides. *Rev. Environ. Sci. Biotechnol.* **2005**, *4* (3), 157–183.
- (2) Rittmann, B. E.; McCarty, P. L. *Environmental Biotechnology: Principles and Applications*; McGraw-Hill: New York, 2001.
- (3) Anderson, R. T. DOE genomics: Applications to in situ subsurface bioremediation. *Remediat. J.* **2006**, *17* (1), 23–38.
- (4) Istok, J. D.; Senko, J. M.; Krumholz, L. R.; Watson, D.; Bogle, M. A.; Peacock, A.; Chang, Y. J.; White, D. C. In situ bioreduction of technetium and uranium in a nitrate-contaminated aquifer. *Environ. Sci. Technol.* **2003**, *38* (2), 468–475.
- (5) Wu, W.-M.; Carley, J.; Fienen, M.; Mehlhorn, T.; Lowe, K.; Nyman, J.; Luo, J.; Gentile, M. E.; Rajan, R.; Wagner, D.; Hickey, R. F.; Gu, B.; Watson, D.; Cirpka, O. A.; Kitanidis, P. K.; Jardine, P. M.; Criddle, C. S. Pilot-scale in situ bioremediation of uranium in a highly contaminated aquifer. 1. Conditioning of a treatment zone. *Environ. Sci. Technol.* **2006**, *40* (12), 3978–3985.
- (6) Wu, W.-M.; Carley, J.; Gentry, T.; Ginder-Vogel, M. A.; Fienen, M.; Mehlhorn, T.; Yan, H.; Carroll, S.; Pace, M. N.; Nyman, J.; Luo, J.; Gentile, M. E.; Fields, M. W.; Hickey, R. F.; Gu, B.; Watson, D.; Cirpka, O. A.; Zhou, J.; Fendorf, S.; Kitanidis, P. K.; Jardine, P. M.; Criddle, C. S. Pilot-scale in situ bioremediation of uranium in a highly contaminated aquifer. 2. Reduction of U(VI) and geochemical control of U(VI) bioavailability. *Environ. Sci. Technol.* **2006**, *40* (12), 3986–3995.
- (7) Wu, W.-M.; Carley, J.; Luo, J.; Ginder-Vogel, M. A.; Cardenas, E.; Leigh, M. B.; Hwang, C.; Kelly, S. D.; Ruan, C.; Wu, L.; Van Nostrand, J.; Gentry, T.; Lowe, K.; Carroll, S.; Luo, W.; Fields, M. W.; Gu, B.; Watson, D.; Kemner, K. M.; Marsh, T.; Tiedje, J.; Zhou, J.; Fendorf, S.; Kitanidis, P. K.; Jardine, P. M.; Criddle, C. S. In situ bioreduction of uranium (VI) to submicromolar levels and reoxidation by dissolved oxygen. *Environ. Sci. Technol.* **2007**, *41* (16), 5716–5723.
- (8) Anderson, R. T.; Vrionis, H. A.; Ortiz-Bernad, I.; Resch, C. T.; Long, P. E.; Dayvault, R.; Karp, K.; Marutzky, S.; Metzler, D. R.; Peacock, A.; White, D. C.; Lowe, M.; Lovley, D. R. Stimulating the in

situ activity of *Geobacter* species to remove uranium from the groundwater of a uranium-contaminated aquifer. *Appl. Environ. Microbiol.* **2003**, *69* (10), 5884–5891.

(9) Fang, Y.; Scheibe, T. D.; Roden, E. E.; Kamolpornwijit, W.; Brooks, S. C. In *3D Field-Scale Reactive Transport Modeling of in Situ Immobilization of Uranium in Structured Porous Media via Biostimulation*; CMWR-XVI: Copenhagen, Denmark, 2006.

(10) Gu, B.; Wu, W.-M.; Ginder-Vogel, M. A.; Yan, H.; Fields, M. W.; Zhou, J.; Fendorf, S.; Criddle, C. S.; Jardine, P. M. Bioreduction of uranium in a contaminated soil column. *Environ. Sci. Technol.* **2005**, *39* (13), 4841–4847.

(11) Kelly, S. D.; Wu, W.-M.; Yang, F.; Criddle, C. S.; Marsh, T. L.; O'Loughlin, E. J.; Ravel, B.; Watson, D.; Jardine, P. M.; Kemner, K. M. Uranium transformations in static microcosms. *Environ. Sci. Technol.* **2009**, *44* (1), 236–242.

(12) Luo, W.; Wu, W.-M.; Yan, T.; Criddle, C.; Jardine, P.; Zhou, J.; Gu, B. Influence of bicarbonate, sulfate, and electron donors on biological reduction of uranium and microbial community composition. *Appl. Microbiol. Biotechnol.* **2007**, *77* (3), 713–721.

(13) Madden, A. S.; Palumbo, A. V.; Ravel, B.; Vishnivetskaya, T. A.; Phelps, T. J.; Schadt, C. W.; Brandt, C. C. Donor-dependent extent of uranium reduction for bioremediation of contaminated sediment microcosms. *J. Environ. Qual.* **2009**, *38* (1), 53–60.

(14) Cardenas, E.; Wu, W.-M.; Leigh, M. B.; Carley, J.; Carroll, S.; Gentry, T.; Luo, J.; Watson, D.; Gu, B.; Ginder-Vogel, M.; Kitanidis, P. K.; Jardine, P. M.; Zhou, J.; Criddle, C. S.; Marsh, T. L.; Tiedje, J. M. Microbial communities in contaminated sediments, associated with bioremediation of uranium to submicromolar levels. *Appl. Environ. Microbiol.* **2008**, *74* (12), 3718–3729.

(15) Hwang, C.; Wu, W.; Gentry, T. J.; Carley, J.; Corbin, G. A.; Carroll, S. L.; Watson, D. B.; Jardine, P. M.; Zhou, J.; Criddle, C. S.; Fields, M. W. Bacterial community succession during in situ uranium bioremediation: Spatial similarities along controlled flow paths. *ISME J.* **2008**, *3* (1), 47–64.

(16) Van Nostrand, J. D.; Wu, W.-M.; Wu, L.; Deng, Y.; Carley, J.; Carroll, S.; He, Z.; Gu, B.; Luo, J.; Criddle, C. S.; Watson, D. B.; Jardine, P. M.; Marsh, T. L.; Tiedje, J. M.; Hazen, T. C.; Zhou, J. GeoChip-based analysis of functional microbial communities during the reoxidation of a bioreduced uranium-contaminated aquifer. *Environ. Microbiol.* **2009**, *11* (10), 2611–2626.

(17) Wu, W.-M.; Carley, J.; Green, S. J.; Luo, J.; Kelly, S. D.; Nostrand, J. V.; Lowe, K.; Mehlhorn, T.; Carroll, S.; Boonchayanant, B.; Löffler, F. E.; Watson, D.; Kemner, K. M.; Zhou, J.; Kitanidis, P. K.; Kostka, J. E.; Jardine, P. M.; Criddle, C. S. Effects of Nitrate on the Stability of Uranium in a Bioreduced Region of the Subsurface. *Environ. Sci. Technol.* **2010**, *44* (13), 5104–5111.

(18) Faybishenko, B.; Hazen, T. C.; Long, P. E.; Brodie, E. L.; Conrad, M. E.; Hubbard, S. S.; Christensen, J. N.; Joyner, D.; Borglin, S. E.; Chakraborty, R.; Williams, K. H.; Peterson, J. E.; Chen, J.; Brown, S. T.; Tokunaga, T. K.; Wan, J.; Firestone, M.; Newcomer, D. R.; Resch, C. T.; Cantrell, K. J.; Willett, A.; Koenigsberg, S. In situ long-term reductive bioimmobilization of Cr(VI) in groundwater using hydrogen release compound. *Environ. Sci. Technol.* **2008**, *42* (22), 8478–8485.

(19) Yang, Y.; McCarty, P. L. Comparison between donor substrates for biologically enhanced tetrachloroethene DNAPL dissolution. *Environ. Sci. Technol.* **2002**, *36* (15), 3400–3404.

(20) Coulibaly, K. M.; Borden, R. C. Impact of edible oil injection on the permeability of aquifer sands. *J. Contaminant Hydrol.* **2004**, *71* (1–4), 219–237.

(21) Borden, R. C. Effective distribution of emulsified edible oil for enhanced anaerobic bioremediation. *J. Contaminant Hydrol.* **2007**, *94* (1–2), 1–12.

(22) Borden, R. C. Concurrent bioremediation of perchlorate and 1,1,1-trichloroethane in an emulsified oil barrier. *J. Contaminant Hydrol.* **2007**, *94* (1–2), 13–33.

(23) Hunter, W. J. Use of vegetable oil in a pilot-scale denitrifying barrier. *J. Contam. Hydrol.* **2001**, *53* (1–2), 119–131.

- (24) Zhang, F.; Wu, W.-M.; Parker, J. C.; Mehlhorn, T.; Kelly, S. D.; Kemner, K. M.; Zhang, G.; Schadt, C.; Brooks, S. C.; Criddle, C. S.; Watson, D. B.; Jardine, P. M. Kinetic analysis and modeling of oleate and ethanol stimulated uranium (VI) bio-reduction in contaminated sediments under sulfate reduction conditions. *J. Hazard. Mater.* **2010**, *183* (1–3), 482–489.
- (25) Tang, G.; Wu, W.-M.; Watson, D. B.; Parker, J. C.; Schadt, C.; Shi, X.; Brooks, S. C. U(VI) bioreduction with emulsified vegetable oil as the electron donor—Microcosm tests and model development. *Environ. Sci. Technol.* **2013**, *47*, 3209–3217.
- (26) Gihring, T. M.; Zhang, G.; Brandt, C. C.; Brooks, S. C.; Campbell, J. H.; Carroll, S.; Criddle, C. S.; Green, S. J.; Jardine, P.; Kostka, J. E.; Lowe, K.; Mehlhorn, T. L.; Overholt, W.; Watson, D. B.; Yang, Z.; Wu, W.-M.; Schadt, C. W. A limited microbial consortium is responsible for extended bioreduction of uranium in a contaminated aquifer. *Appl. Environ. Microbiol.* **2011**, *77* (17), 5955–5965.
- (27) Watson, D. B.; Kostka, J. E.; Jardine, P. M. *The Oak Ridge Field Research Center Conceptual Model*; Oak Ridge National Laboratory: Oak Ridge, TN, 2004.
- (28) Moon, J.-W.; Roh, Y.; Phelps, T. J.; Phillips, D. H.; Watson, D. B.; Kim, Y.-J.; Brooks, S. C. Physicochemical and mineralogical characterization of soil–saprolite cores from a field research site, Tennessee. *J. Environ. Qual.* **2006**, *35* (5), 1731–1741.
- (29) Phillips, D. H.; Watson, D. B.; Kelly, S. D.; Ravel, B.; Kemner, K. M. Deposition of uranium precipitates in dolomitic gravel fill. *Environ. Sci. Technol.* **2008**, *42* (19), 7104–7110.
- (30) Spalding, B. P.; Brooks, S. C.; Watson, D. B. Hydrogel-Encapsulated Soil: A tool to measure contaminant attenuation in situ. *Environ. Sci. Technol.* **2010**, *44* (8), 3047–3051.
- (31) Spalding, B. P.; Watson, D. B. Passive sampling and analyses of common dissolved fixed gases in groundwater. *Environ. Sci. Technol.* **2008**, *42* (10), 3766–3772.
- (32) Kelly, S. D.; Kemner, K. M.; Carley, J.; Criddle, C.; Jardine, P. M.; Marsh, T. L.; Phillips, D.; Watson, D.; Wu, W.-M. Speciation of uranium in sediments before and after in situ biostimulation. *Environ. Sci. Technol.* **2008**, *42* (5), 1558–1564.
- (33) Bethke, C. M.; Sanford, R. A.; Kirk, M. F.; Jin, Q.; Flynn, T. M. The thermodynamic ladder in geomicrobiology. *Am. J. Sci.* **2011**, *311* (3), 183–210.
- (34) Henderson, C. A study of the lipase produced by *Anaerovibrio lipolytica*, a rumen bacterium. *J. Gen. Microbiol.* **1971**, *65* (1), 81–89.
- (35) Rees, G. N.; Patel, B. K. *Desulforegula conservatrix* gen. nov., sp. nov., a long-chain fatty acid-oxidizing, sulfate-reducing bacterium isolated from sediments of a freshwater lake. *Int. J. Syst. Evol. Microbiol.* **2001**, *51* (5), 1911–6.
- (36) Wall, J. D.; Krumholz, L. R. Uranium reduction. *Annu. Rev. Microbiol.* **2006**, *60* (1), 149–166.
- (37) Suzuki, Y.; Kelly, S. D.; Kemner, K. M.; Banfield, J. F. Radionuclide contamination: Nanometre-size products of uranium bioreduction. *Nature* **2002**, *419* (6903), 134–134.
- (38) Senko, J. M.; Mohamed, Y.; Dewers, T. A.; Krumholz, L. R. Role for Fe(III) minerals in nitrate-dependent microbial U(IV) oxidation. *Environ. Sci. Technol.* **2005**, *39* (8), 2529–2536.
- (39) Finneran, K. T.; Housewright, M. E.; Lovley, D. R. Multiple influences of nitrate on uranium solubility during bioremediation of uranium-contaminated subsurface sediments. *Environ. Microbiol.* **2002**, *4* (9), 510–516.
- (40) Wu, Q.; Sanford, R. A.; Löffler, F. E. Uranium(VI) reduction by *Anaeromyxobacter dehalogenans* strain 2CP-C. *Appl. Environ. Microbiol.* **2006**, *72* (5), 3608–3614.
- (41) Beller, H. R. Anaerobic, nitrate-dependent oxidation of U(IV) oxide minerals by the chemolithoautotrophic bacterium *Thiobacillus denitrificans*. *Appl. Environ. Microbiol.* **2005**, *71* (4), 2170–2174.
- (42) Amos, B. K.; Sung, Y.; Fletcher, K. E.; Gentry, T. J.; Wu, W.-M.; Criddle, C. S.; Zhou, J.; Löffler, F. E. Detection and quantification of *Geobacter lovleyi* strain SZ: Implications for bioremediation at tetrachloroethene- and uranium-impacted sites. *Appl. Environ. Microbiol.* **2007**, *73* (21), 6898–6904.
- (43) North, N. N.; Dollhopf, S. L.; Petrie, L.; Istok, J. D.; Balkwill, D. L.; Kostka, J. E. Change in bacterial community structure during in situ biostimulation of subsurface sediment cocontaminated with uranium and nitrate. *Appl. Environ. Microbiol.* **2004**, *70* (8), 4911–4920.
- (44) Moon, H. S.; Komlos, J.; Jaffé, P. R. Uranium reoxidation in previously bioreduced sediment by dissolved oxygen and nitrate. *Environ. Sci. Technol.* **2007**, *41* (13), 4587–4592.
- (45) Tang, G.; Watson, D. B.; Wu, W.-M.; Schadt, C. W.; Parker, J. C.; Brooks, S. C. U(VI) bioreduction with emulsified vegetable oil—Model application to a field test. *Environ. Sci. Technol.* **2013**, *47*, 3218–3225.