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Use of carbon stable isotopes to monitor biostimulation and electron donor fate in chromium-contaminated groundwater

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Abstract

Hexavalent chromium Cr(VI) is a common inorganic contaminant in industrial areas and represents a serious threat to human health due its toxicity. Here we report experimental results from a field-scale investigation of Cr(VI) bio-immobilization at Hanford 100H reservation, a U.S Department of Energy facility (Washington State, USA). Microbial Cr(VI) reduction was stimulated via injection of a ^{13}C-labeled sodium lactate solution into the high-permeability aquifer consisting of gravel and coarse sand sediments. Concentrations and carbon isotope ratios of metabolites, including dissolved inorganic carbon and total organic carbon, and compound-specific analysis of acetate and propionate, together with phospholipid fatty acids (biomass) have been analyzed to help provide an understanding of the predominant redox processes accompanying Cr(VI)

reduction. Results of our study indicate that the injection of an electron donor caused a sharp decrease of Cr(VI) concentration from ~32 to ~10 nM. Cr(VI) reduction was associated with a decrease in the concentration of carboxylic acids, such as lactate (~6 mM to undetectable), propionate (~9 mM to undetectable), and acetate (~6 mM to undetectable), as well as dissolved inorganic carbon (30 to 10 mM C). Carbon isotope data indicate carbon transfers from the original substrate to organic byproducts and mineralized carbon. Concentrations of metabolites and stable isotope data as well as carbon isotope mass balance calculations were used to monitor biologically mediated reduction of Cr(VI).

*Keywords***:** groundwater; carbon isotopes; chromium; biostimulation; contaminant

Introduction

Hexavalent chromium Cr(VI) is an environmental contaminant with known toxic, carcinogenic and mutagenic properties (e.g., Cieslak-Golonka, 1996; Salnikow and Zhitkovich, 2008). In industrial areas, Cr(VI) has been reported as one of the most common inorganic contaminants in soils and groundwater (U.S. EPA, 2004; Testa, 2004). Cr(VI) usually occurs as soluble chromate (CrO_4^{2-}) at pH > 6 and bichromate ($HCrO_4^-$) at pH from 0 to 6 (Rai et al., 1987; 1989; Richard and Bourg, 1991; Ball and Nordstrom, 1998). Most groundwater remediation techniques involve reduction of Cr(VI) to poorly soluble Cr(III). Both field and laboratory experiments have shown abiotic reduction of Cr(VI) induced via addition of ferrous iron (e.g., Espenson, 1970; Buerge

and Hug, 1997; Sedlak and Chan, 1997), oxide minerals, silicates and sulfides (Eary and Rai 1989; Olazabal et al., 1997; Patterson and Fendorf, 1997; Fendorf et al., 2000; Martin and Kempton, 2000), soil organic carbon (e.g., Bartlett and Kimble 1976; Banks et al., 2006; Xiao et al., 2012), and strong reductants such as dithionite (Istok et al., 1999; Taylor et al., 2000; Su and Ludwig, 2005). Moreover, biotic Cr(VI) reduction has been observed for a variety of aerobic, facultative, and anaerobic bacterial strains (e.g., Chen and Hao 1998; Schieman et al., 1998; Kim et al., 2001; Han et al., 2010; Tokunaga et al., 2003). Among the various techniques currently available, *in situ* biostimulation has been recognized as a relatively cost-effective and valuable method for the remediation of contaminated groundwater (U.S. EPA, 2013). From this perspective, microbially mediated reduction of Cr(VI) to Cr(III) has been demonstrated as a viable method for groundwater decontamination (Faybishenko et al., 2008; Truex et al., 2009; Brodie et al., 2011).

Measurements of carbon isotopic ratios have been shown to be a sensitive method for monitoring the degradation of organic contaminants and their byproducts (e.g., Dayan et al., 1999; Bill et al., 2001; Barth et al., 2002; Richnow et al., 2003; Schüth et al., 2003; VanStone et al., 2005; Mak et al., 2006; Prommer et al., 2008). Only a few studies, mainly laboratory experiments, reported Cr isotope fractionation associated with Cr(VI) reduction (Ellis et al., 2002; Sikora et al., 2008; Izbicki et al., 2008; Bassu et al. 2012; Han et al., 2012; Kitchen et al., 2012; Bassu et al., 2014). To date, the transformation and fate of organic electron donors used to stimulate Cr(VI) reduction in the field has been reported only in limited studies (Szecsody et al., 2004; Truex et al., 2009). Use of a ^{13}C-labeled electron donor added to the groundwater to stimulate biological reduction

permits tracking of its fate in the contaminated plume throughout the remediation process.

Here we report a field-scale experiment involving addition of a ^{13}C-labeled electron donor to a contaminated aquifer to stimulate Cr(VI) reduction. The $\delta^{13}C$ values of dissolved inorganic carbon, total organic carbon, organic acids, and biomass (phospholipid fatty acids) have been analyzed to help provide an understanding of the predominant redox processes accompanying hexavalent chromium reduction. The main objectives of this study was to conduct field lactate biostimulation experiments: 1) to identify and quantify carbon compounds produced as byproducts of microbial metabolism of the lactate substrate and 2) to quantify critical interrelated microbial metabolic, and geochemical mechanisms associated with *in situ* chromium reduction.

Materials and Methods

Field Site. The field site for investigation of Cr(VI) bioimmobilization is located at the Hanford 100-H Area (Washington State), a U.S. Department of Energy former nuclear production facility. The field site is situated along the Cr(VI)-contaminated groundwater plume from the Hanford 100-D Area to the Columbia River (Fig. 1). To perform field investigations, four 18.3-m deep, 15-cm diameter boreholes were cored and completed near the existing monitoring Well 699-96-43 (monitored since 1992) in an east-northeast direction. The well layout at the Cr(VI) bioimmobilization research site is shown in Fig. 1. The Cr(VI)-contaminated unconfined aquifer is situated in the high-permeability Hanford sediments --gravel and coarse sand-- with the water table at a depth of 12.2 m. In an adjacent area, high permeability (10^{-9} – 10^{-8} m^2) was reported for the Hanford aquifer

93 materials composed of rocks, cobbles and gravels (Hammond et al. 2011). A clay layer
94 of the Ringold Formation from 14.3 m to 15.25 m depths underlies the Hanford
95 sediments. The conceptual model of one-dimensional lateral aquifer flow and Cr(VI)
96 transport between the injection and monitoring wells is supported by the location of the
97 borehole in the regional groundwater flow direction and by several conservative tracer
98 (KBr) tests (Faybishenko et al., 2008).

99 The wells were equipped with 10 cm (4-inch) PVC casings screened from the bottom
100 of the hole (except for a 0.3 m sump cap) to a depth of approximately 7.8 m. The
101 borehole annulus (above and below the water table) was packed with clean quartz sand,
102 with no grouting with an impermeable material to separate the Hanford and Ringold
103 aquifers. Groundwater samples were collected from different depths through the open
104 spacing (5 cm long) between inflatable rubber packers. These packers were attached to
105 a 5 cm diameter access (removable) pipe, which was used to install the water and
106 sediments samplers.

107 To maintain the integrity of collected water samples and prevent cross-contamination,
108 a special water sampling procedure was developed (Faybishenko et al., 2008). Water
109 samples were collected at specific depth intervals by first applying suction followed by
110 injection of argon gas to draw water samples up to the well surface. After purging the
111 water samplers with argon gas, groundwater samples were collected in 130-mL
112 sterilized serum bottles and immediately sealed with butyl rubber stoppers to prevent
113 exchange with the atmosphere. Samples were stored and shipped overnight on ice at
114 ~4℃ to Lawrence Berkeley National Laboratory, Berk eley, CA, for chemical analysis.

Injection of Sodium Lactate Solution. 210 L of groundwater was pumped out of an upgradient well prior to injection and stored in an argon-flushed barrel to prevent contamination by ambient air. Groundwater was amended with Na-lactate (6.0mM), propionate (6.8mM), acetate (5.1mM), 4.5 mM ^{13}C-labeled Na-lactate, and ~0.5 mM phosphate. Amended groundwater was injected into well 699-96-41 through a straddle packer with an open interval at a depth of 13.7- 13.9 m with a flow rate of ~1L/min using a peristaltic pump on March 19, 2010 (Fig. 1).

Cr (VI) concentrations were determined from aqueous samples filtered through a 0.22 μm pore size nylon filter. Cr(VI) of the samples was separated from Cr(III) as CrO_4^{2-} into a cation-exchange column (Alltech Maxi-Clean SPE 1.5 mL IC-H cartridge from Grace Davison Discovery Sciences, Deerfield, IL). The CrO_4^{2-} passed through the column while any Cr(III) was retained in the resin, the remaining sample was acidified to pH ~1. Samples were then diluted with 2% (v/v) ultra-high-purity nitric acid (BDH Aristar Ultra) and spiked with Ga as an internal standard. Cr(VI) concentration was then determined by an inductively coupled plasma spectrometer (ICP-MS) equipped with a dynamic reaction cell (DRC) (ICP-DRC-MS, PerkinElmer SCIEX Elan DRC II, PerkinElmer, Inc.,Waltham, MA, USA). NH_3 was used as the reaction gas to reduce interferences in the DRC. All concentrations determined using the described above methods were above the method quantitation limit (~0.01 ug/L Cr).

Carbon isotope ratio of dissolved inorganic carbon (DIC) from groundwater samples was determined in 200-μL aliquots extracted with an airtight syringe and injected into helium-flushed 5.9-mL Labco exetainer vials containing 0.2 ml of 99.5 % phosphoric acid (H_3PO_4). The CO_2 generated from the reaction of DIC with H_3PO_4 was analyzed

using a headspace autosampler (Gilson, Villiers-le-Bel, France) connected to a Tracegas preconcentrator interfaced to Micromass JA Series Isoprime isotope ratio mass spectrometer (Micromass, Manchester, UK). DIC concentrations in water samples were determined using the *m/z* 44 (CO_2) area peak. Multiple measurements of DIC laboratory standards associated with sample analysis yielded a reproducibility of ±0.52‰ (1σ; n=12) for $\delta^{13}C$ and ±0.11mM (1σ; n=12) for concentrations.

Carbon isotope ratio of dissolved organic carbon (DOC) was determined using 1.5 mL aliquots of groundwater samples, which were acidified to pH ~4.5 with a 0.5 mM HCl solution to minimize interference with carbonate carbon. 450 µL of acidified sample was transferred to a tin capsule, evaporated at room temperature and then the folded tin capsules were loaded into a zero blank autosampler connected to a ECS 4010 Elemental Analyzer (Costech Analytical Technologies Inc., Valencia, USA) coupled to an Delta V^{plus} isotope ratio mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). Repeated measurements of DOC laboratory standards had a standard deviation of ±0.5‰.

Organic acid concentrations were measured on samples filtered at 0.20 µm pore size. Ion chromatography (ICS 2000, Dionex Corp., Sunnyvale, CA) was performed with a 4 x 250 mm analytical column (IonPac AS11, Dionex) and conductivity suppressor (ASRS Ultra, Dionex), using an eluent generator (Dionex) with a potassium-hydroxide gradient. Analytical precision based on repeated standard analyses was 0.03 mM (1σ) for sample concentrations from 0.01 to 0.20 mM and 0.1 (1σ) for sample concentrations from 0.3 to 10 mM. The carbon isotope ratios of acetate and propionate were determined using a similar method as developed by Thomas et al., 2009. Briefly, 1 µL of

sample was injected into a gas chromatograph (Hewlett Packard 6890). Acetate and propionate were separated chromatographically on a Supelco Nukol fused silica capillary column (30 m x 0.25 mm x 0.25 μm,). After GC separation, both organic acids were combusted to CO_2 at 850 ℃ in a capillary quartz tube loaded with Ni, Cu, and Pt wires, water was removed, and the carbon isotope ratio was measured in a Micromass JA Series Isoprime isotope ratio mass spectrometer (Micromass, Manchester, UK). Laboratory standards associated with the injections of samples resulted in a standard deviation of 2.6‰.

Phospholipid fatty acids (PLFA) were extracted from microbial biomass. Microbial biomass was collected in the field from 400 ml of groundwater on filters (0.2-μm Anodisc, Whatman, Maidstone, England) using the Bligh-Dyer method (White, 1996). PLFA were separated from other compounds using a silicic acid column and converted to fatty acid methyl esters (FAME) via mild alkaline methylation. Resulting FAMEs were identified by GC/MS. Carbon isotope ratios of PLFA were determined using a combustion isotope ratio mass spectrometer (GC-C-IRMS) system (Thermo Fisher Scientific, Bremen, Germany). PLFA were separated chromatographically on an HP-5 fused silica capillary column (30 m x 0.25 mm, 10-μm film thickness). After GC separation, the PLFA were combusted to CO_2, water was removed, and the carbon isotope ratio was measured in the IRMS. Analytical precision was estimated with internal PLFA standards 11:0 and 19:0 spiked into each sample and their respective value yields of 29.1 ± 0.8‰ (1σ; n = 17) and 39.9 ± 6.5‰ (1σ; n = 19).

Results and Discussion

The background concentration of Cr(VI) prior to the injection on March 19, 2010, in upgradient well 699-96-43 was ~ 85 μg/L (Fig.1). A slow process of Cr(VI) natural attenuation for the period from 1993 to 2002 under background conditions was characterized by a first-order attenuation constant of 1.7×10^{-4} μg day^{-1} (Faybishenko et al., 2008). The natural attenuation was likely caused by simultaneously occurring processes of groundwater dilution and intrinsic Cr(VI) reduction under conditions of regional groundwater flow (Faybishenko et al., 2008). Prior to the injection of organic acids, the DIC concentration was ~11 mM with a $\delta^{13}C$ value of ~ -12 ‰; prior to the onset of the study, measured concentrations of carboxylic acids such as formate, acetate, propionate, pyruvate, and lactate were below instrument detection limits (0.01 mM).

After the injection in well 699-96-41 on March 19 a strong decrease in Cr(VI) concentrations (from ~32 to ~10 nM) was observed during the six following days at 13.7 and 12.9 m sampling depths (Fig. 2A; 2E). The decrease in Cr(VI) coincides with a strong decrease in the DIC concentrations of groundwater (30 to 10 mM C) and lactate concentration (from ~6 mM/L to undetectable), acetate (~5 mM/L to undetectable), propionate (~9 mM/L to undetectable) (Figs. 2B and 2F). The decrease in Cr (VI) concentrations associated with the lactate injection is larger than the natural attenuation measured under background conditions by 3 orders of magnitude. A significant change in $\delta^{13}C$ of DIC (~400‰ to 0‰) and TOC (~350‰ to ~175‰) was also observed (Figs. 2D and 2H). The $\delta^{13}C$ of the DIC sharply decreased to near background values (~ -10‰) 12 days after the injection. The maximum $\delta^{13}C$ values of acetate (400‰ to 300‰) and

propionate (350‰ to 325‰) are associated with their maximum concentrations. $\delta^{13}C$ values of acetate and propionate for concentrations below 5mM were not reported as they were below the detection limit of the GC-C-IRMS system.

In the downgradient well 699-96-44, located 2.5 meters from the injection well, a decline in Cr(VI) concentrations was observed 18 days (4/05) after the injection, which coincides with an increase in concentrations of acetate (from undetectable to 0.1 mM) and propionate (from undetectable to 0.12 mM) (Fig. 3B). Lactate was only detected at trace concentrations (< 0.02 mM), indicating a nearly complete consumption of the injected lactate. An increase in DIC concentrations from ~10 mM to ~15 mM is associated with higher DIC $\delta^{13}C$ values (-12‰ to 100‰) shown in Figs. 3C and 3D).

Whereas the trends of concentration and isotopic value depicted in Figs. 2 and 3 are likely affected by the groundwater flow and chemical transport processes, several lines of evidence suggest that the detected compounds are microbial metabolites derived from the injected electron donor species. In particular,

1) Lactate in the injection well was nearly completely depleted four days following the injection. Moreover, in the downgradient well 699-96-44, only trace concentrations of lactate were observed due to relatively rapid lactate consumption. The diminution of organic acid $\delta^{13}C$ values during the week following the injection are compatible with the consumption of electron donors associated with microbial metabolism rather than attenuation by dispersion or dilution.

2) The high $\delta^{13}C$ values of TOC, acetate, and propionate reflect the original value of the ^{13}C-labeled lactate.

3) The high $\delta^{13}C$ values of DIC indicate carbon transfer from original ^{13}C-lactate to final mineralization through metabolic pathway.

Hence, $\delta^{13}C$ values and metabolites concentrations strongly suggest a transfer of carbon from labeled ^{13}C lactate to propionate, acetate, and DIC. The acetate and propionate and DIC suggest that the lactate was metabolized through fermentative processes (e.g., Seeliger et al., 2002). The physiology and stoichiometry of lactate fermentation to propionate to acetate has been widely discussed in the literature (e.g., Schink, 1997; Wolfe, 2005; Beller et al., 2013). The overall reaction lactate fermentation to acetate and propionate can be described as:

$$3\,C_3H_6O_3 \rightarrow\ C_2H_4O_2\ +\ 2\,C_3H_6O_2\ +\ CO_2 +\ H_2O \qquad (1)$$

The linear relationship between propionate and acetate concentrations is shown in Fig. 4A, 4C and 4E. A molar carbon ratio of ~ 2.5 between propionate and acetate (equivalent to a stoichiometric ratio of ~1.67) was observed for the higher concentrations. The observed stoichiometric ratio of propionate to acetate is similar to, but somewhat lower than, the ratio of 2:1 observed for the bacterium *Pelosinus* sp. strain HCF1, which was dominant in and isolated from lactate- and Cr(VI)-amended Hanford 100H aquifer experimental systems (Beller et al. 2013). In the injection well, acetate concentrations decreased linearly with Cr(VI) concentration after the consumption of lactate four days after the injection (Fig. 4B; 4D), suggesting that propionate and acetate acted as electron donors for Cr(VI) reduction after the fermentation of the lactate.

The carbon transfer between the lactate substrate and its byproducts with a carbon isotope mass balance can be estimated from

$$\frac{d\,\delta^{13}C^{\mathrm{lact}}}{dt} =\ a\,\delta^{13}C^{\mathrm{prop}} +\ b\,\delta^{13}C^{\mathrm{acet}} +\ c\,\delta^{13}C^{\mathrm{CO2}} \qquad (2)$$

where $\delta^{13}C^{lact}$, $\delta^{13}C^{acet}$, $\delta^{13}C^{prop}$, $\delta^{13}C^{CO2}$ are, respectively, the carbon isotope composition of lactate, acetate, propionate, and bacterial respiration DIC expressed in conventional delta-notation in per mil. The coefficients a, b, and c are the mole fractions of each fermentation byproduct.

The carbon isotopic composition of the lactate ($\delta^{13}C^{lact}$) in groundwater is related to the DOC according to:

$$\frac{d\,\delta^{13}\mathrm{C}^{\mathrm{DOC}}}{dt} = e\,\delta^{13}\mathrm{C}^{\mathrm{lact}} + f\,\delta^{13}\mathrm{C}^{\mathrm{prop}} + g\,\delta^{13}\mathrm{C}^{\mathrm{acet}} + h\,\delta^{13}\mathrm{C}^{\mathrm{om}} \quad (3)$$

where $\delta^{13}C^{DOC}$ is the carbon isotope composition of dissolved organic carbon; $\delta^{13}C^{om}$ represents the dissolved organic molecules other than lactate and fermentative byproducts which can include low molecular weight compounds (i.e., carbohydrates, amino acids and sugars) and high molecular weight organic substances (i.e., proteins, phospholipid acids, lignin, and humic acids) (Artinger et al. 2000; Peter et al., 2012; Regan et al., 2017; Shen et al., 2015), and coefficients, e, f, g and h are similarly defined as in equation (2) as mole fractions.

The expressions (2) and (3) are valid only with the following caveats: (i) the carbon substrate is bioavailable, (ii) substrate, bacterial strains, and byproducts are homogenously distributed, and (iii) physical processes such as dissolution, advection, diffusion, dilution, sorption, and volatilization can be neglected.

The validity of the mass balance Eq. (2) was tested using the observational data obtained at two different depths (13.7 m and 12.9 m) of the injection well for the four days following the injection, after which the mass balance was invalid due to the nearly complete consumption of lactate (Figs. 2 and 3). Considering the lack of constrains on the mass transport of substrates and products and on the system homogeneity, the

linear relationship between the measured $\delta^{13}C$ values of DIC and the estimated $\delta^{13}C$ values of lactate indicates a reasonable relationship for carbon transfer from labeled ^{13}C lactate to the organic and mineralized carbon (Fig. 5A). The relationship between the $\delta^{13}C$ values of DOC and estimated lactate indicates that DOC is mainly composed of products of ^{13}C-labeled lactate (Fig. 5B).

Fifteen PLFAs were present in most of the groundwater samples. The PLFA composition indicated that the suspended microbial community was consistent during the injection experiment, although the relative abundances of microbes changed. The average $\delta^{13}C$ values of background PLFA sampled before the injection was 26.0 ± 4.2‰. We observed an enrichment in $\delta^{13}C$ in PLFA chain length from 14 to 17 carbons in the days following the injection (Fig. 6). Maximum $\delta^{13}C$ values were associated with branched PLFA *anteiso*-15:0 (or a-15:0) (~325‰ and ~300‰) and with 16:1ω7c (~180‰ and 125‰) (Figs 6 A, B). PLFA a-15:0 is characteristic of Gram-positive bacteria and their high $\delta^{13}C$ suggests a key role in lactate degradation. Of interest is the increase of $\delta^{13}C$ value in the i17:1w7c lipid (Fig. 6B), which is a characteristic of sulfate-reducing bacteria. This suggests that lactate was used as an organic carbon source and favorable conditions for growth of sulfate-reducing bacteria. More enrichment in 16:1w7c and 18:1w9c was observed in the downstream well, shown in Fig. 6C, which is a characteristic of Gram-negative bacteria. From 38 to 65% of PLFA mole fraction did not show enrichment, indicating that there were bacterial communities present which did not consume the carbon from the labeled lactate. The ^{13}C enrichment of some PLFA indicates a carbon transfer from labeled lactate to a portion of the microbial community.

Conclusions

^{13}C-labeled lactate was used to monitor the fate and transformation of electron donors associated with biologically mediated reduction of Cr(VI) at the Hanford 100H field site. Simultaneously with Cr(VI) reduction, a strong decrease in δ^{13}C values and concentrations of lactate, propionate, acetate TOC, and DIC indicated microbial activity during lactate amendment. The high δ^{13}C values of metabolic products indicated carbon transfers from the original ^{13}C-labeled lactate substrate to metabolites and CO_2 through a fermentative process rather than attenuation by dispersion or dilution. Gram-positive bacteria characterized by branched PLFA a-15:0 and 16:1ω7c were associated with high δ^{13}C values suggesting their key role in lactate transformation. Overall, we have demonstrated that amendment of an ^{13}C-labeled electron donor added to groundwater systems could be a useful method for identifying dominant pathways of carbon metabolism in the subsurface during biologically mediated reduction of Cr(VI). Our approach may be transferred to other contaminated sites by a variety of metal and organic contaminants. In contaminated sites, using ^{13}C-labeled electron donors coupled with the reduction of metal or of organic contaminants can be a viable method in estimating the efficiency of biostimulation and the fate of organic electron donors.

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Figure captions

Fig. 1. (A) Test site location showing the chromium plumes at Hanford, Washington state (WA). (B) Well layout at the Cr-test site at Hanford 100H. Well 699-96-41 was used for injection and for monitoring before and after the injection. Wells 699-96-44 was used as a monitoring well.

Fig. 2. Injection well 699-96-41, concentrations and isotopic evolution versus time, the gray bars indicate the injection day. At 13.7 m depth: (A) Cr (VI) concentration over time; (B) organic acid concentration; (C) dissolved inorganic carbon (DIC) and total organic acids expressed as mM carbon; (D) $\delta^{13}C$ values of organic acids and DIC. At 12.9 m depth: (E) variation of Cr (VI) concentration; (F) organic acid concentration; (G) DIC and total organic acids expressed as mM carbon; (H) $\delta^{13}C$ values of organic acids and DIC.

Fig. 3. Downgradient monitoring well 699-96-44, concentrations and isotopic evolution versus time, the gray bars indicate the injection day. At 13.1 m depth: (A) Cr (VI) concentration over time; (B) organic acid concentration; (C) dissolved inorganic carbon (DIC) and total organic acids expressed as mM carbon; (D) $\delta^{13}C$ values of DIC.

Fig. 4. Variation of molar carbon ratio of propionate and acetate and variation of Cr(VI) and acetate concentrations. (A) and (B) well 699-96-41 at 13.7 m depth; (C) (D) 699-96-41 at 12.9 m depth; (E) and (F) downstream well 699-96-44 at 13.1 m depth.

Fig. 5. (A) Calculated $\delta^{13}C$ values of lactate using the isotopic mass balance (2). (B) Relationship between $\delta^{13}C$ values of measured DOC and calculated lactate.

Fig. 6. $\delta^{13}C$ values of PLFA biomarkers. PLFA biomarker profiles show enrichment in ^{13}C after the injection of ^{13}C labeled lactate: (A) well 699-96-41 at 13.7 m depth; (B) well

699-96-41 at 12.9 m depth; (C) downstream well 699-96-44 at 13.1 m depth. 11:0 and 19:0 are internal reference materials.

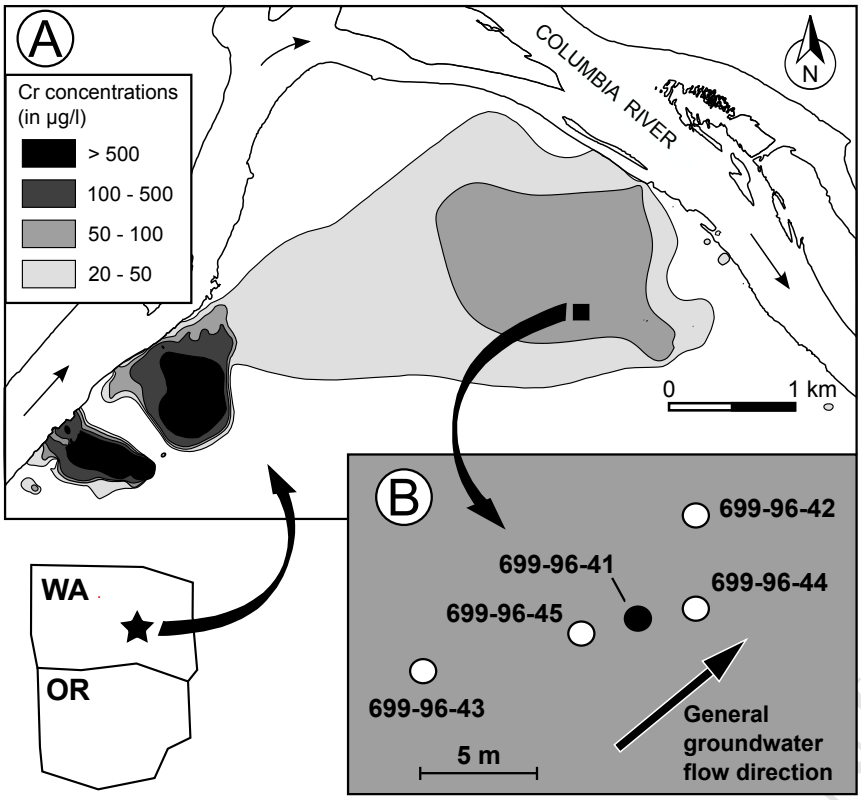

Fig. 1

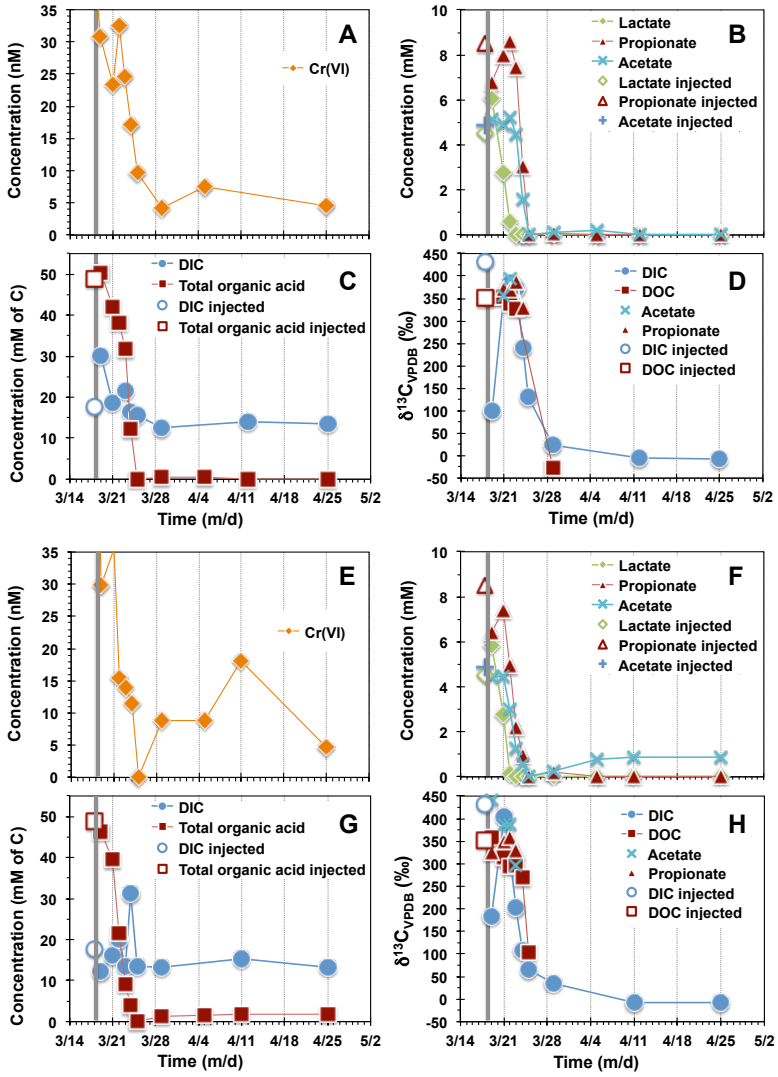
A
Cr(VI)
Concentration (nM)
B
Lactate
Propionate
Acetate
Lactate injected
Propionate injected
Acetate injected
Concentration (mM)
C
DIC
Total organic acid
DIC injected
Total organic acid injected
Concentration (mM of C)
D
DIC
DOC
Acetate
Propionate
DIC injected
DOC injected
δ13CVPDB (‰)
Time (m/d)
3/14
3/21
3/28
4/4
4/11
4/18
4/25
5/2
E
F
G
H

Fig. 2

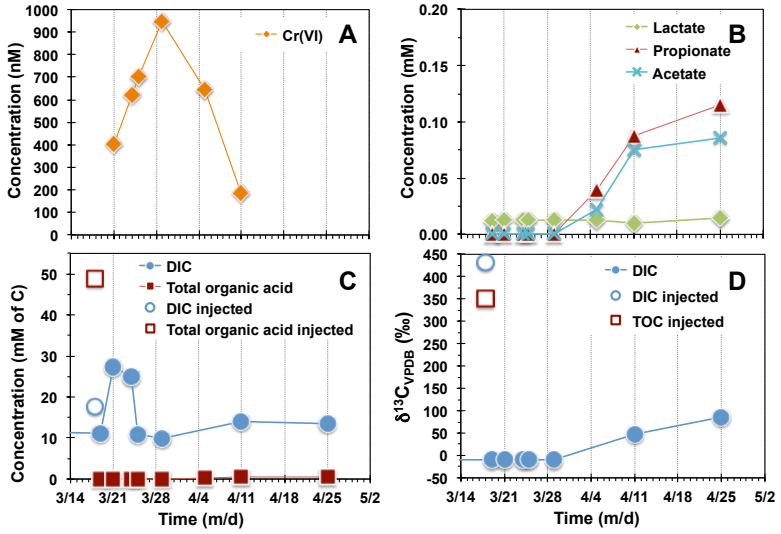
A
Cr(VI)
Concentration (nM)
1000
900
800
700
600
500
400
300
200
100
0
B
Lactate
Propionate
Acetate
Concentration (mM)
0.20
0.15
0.10
0.05
0.00
C
DIC
Total organic acid
DIC injected
Total organic acid injected
Concentration (mM of C)
50
40
30
20
10
0
3/14
3/21
3/28
4/4
4/11
4/18
4/25
5/2
Time (m/d)
D
DIC
DIC injected
TOC injected
δ13CVPDB (‰)
450
400
350
300
250
200
150
100
50
0
-50
3/14
3/21
3/28
4/4
4/11
4/18
4/25
5/2
Time (m/d)

Fig. 3

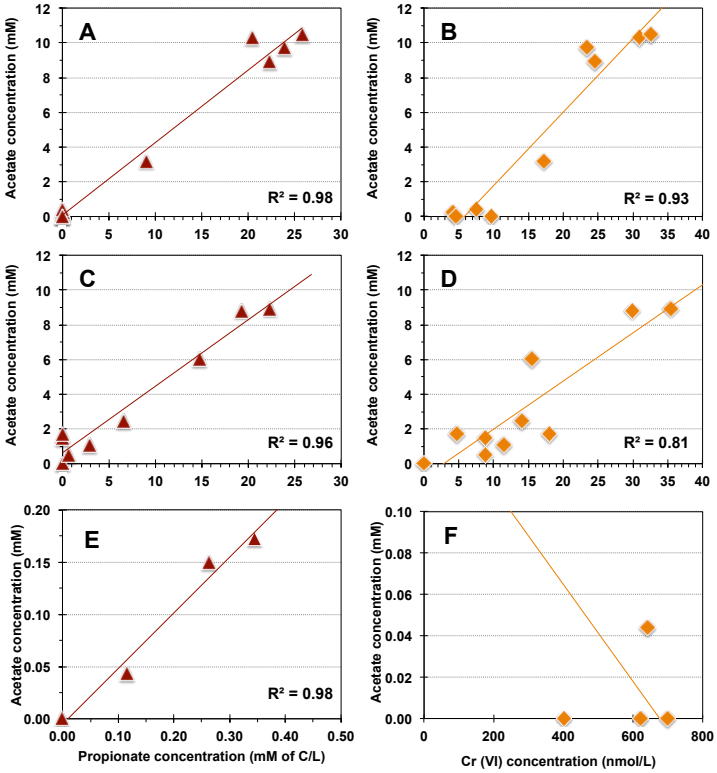
A
B
C
D
E
F
Acetate concentration (mM)
R² = 0.98
R² = 0.93
R² = 0.96
R² = 0.81
R² = 0.98
Propionate concentration (mM of C/L)
Cr (VI) concentration (nmol/L)

Fig. 4

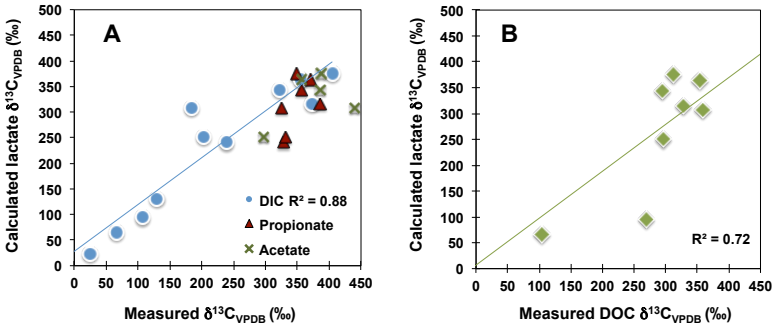
A
B
Calculated lactate δ13CVPDB (‰)
Measured δ13CVPDB (‰)
Measured DOC δ13CVPDB (‰)
DIC R² = 0.88
Propionate
Acetate
R² = 0.72
0
50
100
150
200
250
300
350
400
450
500

Fig. 5

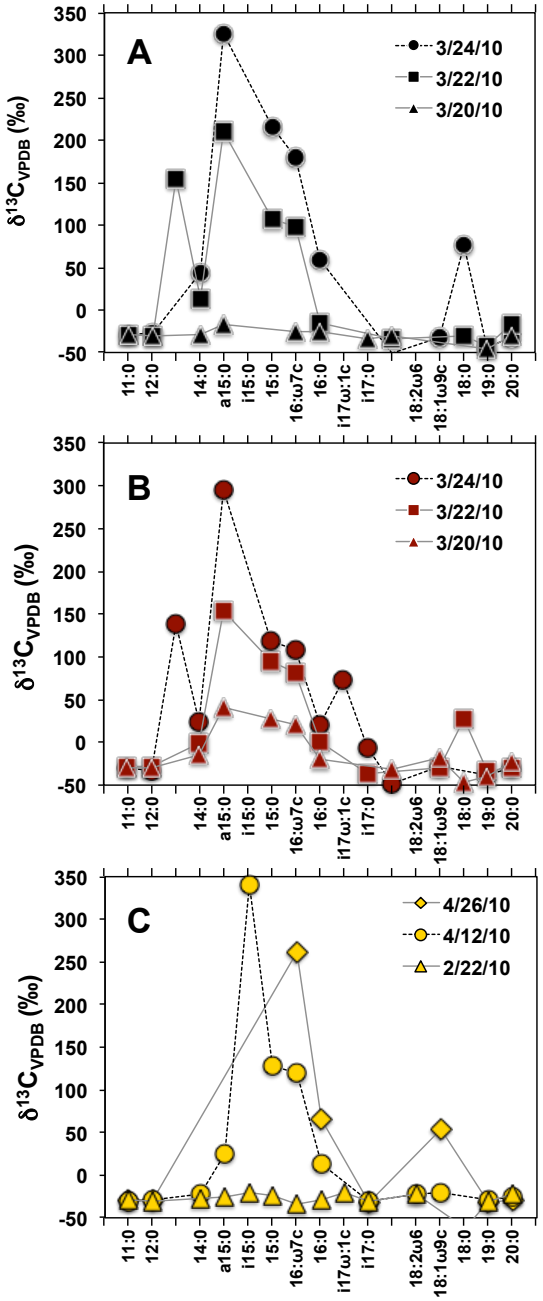
A
3/24/10
3/22/10
3/20/10
B
3/24/10
3/22/10
3/20/10
C
4/26/10
4/12/10
2/22/10
δ13CVPDB (‰)
350
300
250
200
150
100
50
0
-50
11:0
12:0
14:0
a15:0
i15:0
15:0
16:ω7c
16:0
i17ω:1c
i17:0
18:2ω6
18:1ω9c
18:0
19:0
20:0

Fig. 6

HIGHLIGHTS

- In situ microbial Cr(VI) reduction was stimulated via injection into groundwater of sodium lactate solution
- Transformation and fate of organic electron donors were associated with Cr(VI) reduction
- Carbon isotope ratios of metabolites, including DIC, TOC, acetate and propionate, and PLFA (biomass) were measured to estimate the efficiency of biostimulation
- Carbon isotope ratios show the microbial metabolites derived from the injected electron donor
- Carbon isotope ratios of PLFA demonstrates the transfer of carbon from ^{13}C-labelled lactate to a portion of the microbial community