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Integrated Field-Scale Subsurface Research Challenge
Oak Ridge Field Research Center
Environmental Remediation Sciences

Laboratory and *In-Situ* Biostimulation of Uranium Reduction and Immobilization using Long Chain Fatty Acids-containing Organics as Sustained Release Electron Donors at the Oak Ridge IFC site

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INTRODUCTION

Bioreduction of U(VI) to U(IV) with ethanol as an electron donor was tested in Area 2 and 3 at IFRC site, Oak Ridge, TN during the last four years. Low U concentration (below US EPA MCL of 0.03 mg/L) can be achieved by frequent injection of the electron donor. To reduce the costs and improve the sustainability for remediation and site maintenance, we are exploring the effectiveness of slowly degrading substrates such as Ca-oleate and a commercial emulsified vegetable oil (SRS) as alternative electron donor sources.

OBJECTIVES OF THE STUDY

- ❑Selection of most appropriate electron donor using microcosm tests.
- ❑Characterization of bio-reduction process and microbial communities involved.
- ❑Characterization of retention of slow-release substrate in sediments.
- ❑Hydraulic characterization of test site using bromide tracer, and
- ❑Field test of in situ biostimulation by injection of electron donor.

SITE AND BACKGROUND OF THE TESTS

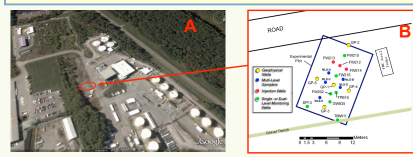


Figure 1. (A) ORIFRC site aerial view. (B) Map of Area 2; FW212, FW 213 and FW214 are injection wells. The remaining wells are monitoring wells.

Geochemical properties of groundwater in Area 2 are: pH 6.6-6.9; nitrate, 0.3-0.6 mM; sulfate, 1.0-1.2 mM; uranium, 1.0-1.3 mg/L; Ca^{2+}, 2.2-2.8 mM; alkalinity, 5.8-6.8 mM. The high groundwater calcium concentrations are less favorable for U(VI) reduction.

The test area has a hydraulic conductivity of 0.1 cm/sec, gradient of 0.02 and thickness of 1.20 m and width of 5.2 m

SLOW RELEASE ELECTRON DONOR SOURCE

Oleate and emulsified vegetable oil (SRS) were selected as a slow release electron donor sources for microcosm test for comparison with ethanol. Oleic acid is a long-chain unsaturated fatty acid ($C_{18}H_{34}O_2$) with low water solubility and a density of 0.895-0.947 g/cm^3. Under anaerobic conditions, oleate is degraded via β-oxidation with hydrogen as an intermediate. SRS, commercial product, contains 60% of vegetable oil, 6% food-grade surfactant and 0.3% yeast extract and has density 0.93 and 1.74 gCOD/g. Vegetable oil is hydrolyzed to long-chain VFA including oleate and then degraded.

The degradation of VFA is performed by syntrophic acetogens and sulfate-reducing bacteria (SRB) with sulfate or bicarbonate as electron acceptors. Hydrogen and acetate produced are also used as electron donors, and Fe(III) and U(VI) are thus bioreduced during the process.

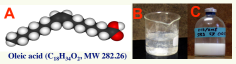


Figure 2. (A). Oleic acid structure. (B) Ca-oleate precipitates in Area 2 groundwater. (C) SRS (5%) diluted in water showing milky-like emulsion.

MICROCOSM TEST AND COMMUNITY ANALYSIS

Microcosm tests were conduced under ambient temperature condition with sediment (650 mg U/kg) and groundwater (sulfate, 1.0 mM, pH 6.9, calcium 2.2 mM) from Area 2. SRS and oleate were used as electron donor sources in comparison with ethanol. The initial sulfate concentrations were 1.0 and 5.0 mM for SRS and ethanol while 1.0, 5.0 and 9.0 mM for oleate microcosms to test the impact of sulfate.

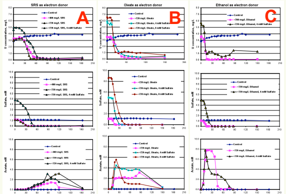


Table 1. Percentage of U(VI) and U(IV) as determined by linear combination fitting of XANES spectra. Uncertainty is ± 10 % (after 3 month bioreduction)

Sample	U(VI):U(IV) %
Control	85:15
ethanol	44:56
ethanol + sulfate (4 mM)	35:65
oleate	65:35
oleate + sulfate (4 mM)	39:61
oleate + sulfate (8 mM)	35:65
SRS	44:56
SRS + sulfate (4 mM)	41:59

Fig. 3. Changes of uranium, sulfate and acetate concentrations in microcosms with (A) SRS, (B) oleate and (C) ethanol as electron donor. Note: amendment of sulfate caused release U(VI) from sediment to aqueous phase.

All three electron donor supported U(VI) reduction with similar total reduced U(VI)/U(IV) (Table 1). The sequence of rate for sulfate reduction and acetate production was: ethanol > oleate > SRS. Therefore, SRS was selected as the electron donor for field test.

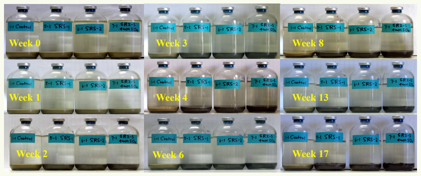


Fig. 4. Microcosms with SRS as electron donor. Biodegradation (color change) started after two week incubation. High sulfate concentration resulted in more FeS.

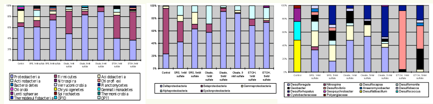
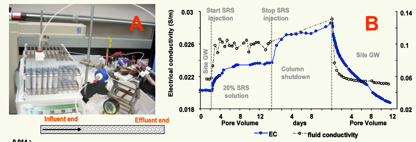


Fig.5. Influence of electron donor source and sulfate concentrations on (left) bacterial phyla, (middle) *Proteobacteria* and (right) δ-*Proteobacteria*. Significantly different communities were observed. SRS resulted in the most diversified community. *Desulfuregula* was predominantly SRB with oleate and SRS. *Desulfovibrio* dominated with ethanol. Geobacter and Anaeromyxobacter were also found.

COLUMN EXPERIMENTS FOR SRS ADSORPTION



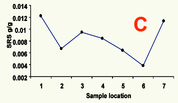


Figure 6. (A) Column test device for SRS adsorption. (B) Electrical conductivity response to SRS injection, degradation (stop SRS injection period) and washing out with groundwater. (C) SRS adsorption in sediment along the column.

Column test with sediment from ORIFC area 2 indicated that electrical conductivity increased during SRS injection and increased further as SRS degradation started. Thus, application to field experiments appeared plausible. About 10 mg SRS/g sediment absorption was measured.

TRACER TEST AND SITE PREPARATION

A nonreactive tracer test was performed on December 8, 2008. Bromide solution (450 mg/L, 900 gal) prepared using groundwater from Area 2 was injected evenly to FW212, FW213 and FW214 in one hour (Fig. 1b). Samples were taken from 50 monitoring wells for two weeks to determine hydraulic connection between these injection wells and monitoring wells. The results of tracer test helped with the understanding flow paths (Figure 8B) and the design for *in situ* SRS biostimulation test.

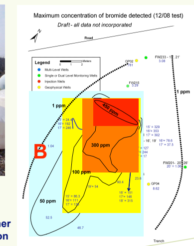


Fig.8 (A)Br solution tanks and surface electrical device on the site. (B) Maximum bromide concentration observed during tracer test. Higher bromide concentration suggest better connection with injection wells.

Prior to the injection of SRS, ground water samples were taken from more than 50 wells for background geochemistry survey, microbial samples of groundwater and sediment were collected from selected wells. Site hydrology was characterized also by surface electrical methods (Fig. 8A).

FIELD BIOSTIMULATION FOR U(VI) REDUCTION

Field test was initiated on Feb. 9, 2009. SRS was diluted to 20% solution and 900 gallons of solution was injected into a transect of three wells over a 1.5 hours time period. Bioreduction of nitrate was observed within one week, sulfate reduction occurred within two weeks, and U concentrations decreased after 2-3 weeks. After 5 weeks, sediment samples were taken from several downgradient monitoring wells and analyzed via XAS. The analyses confirmed U(VI) reduction to U(IV). Filtered groundwater and surged sediment samples have been taken over the full time-course for microbial population monitoring.

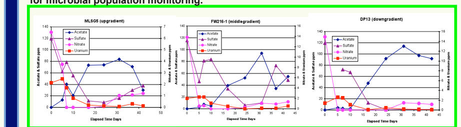


Fig.10. Geochemical changes in up-, middle and down-gradient monitoring wells after 1.5 months of the SRS injection. Reduction of nitrate, sulfate and U(VI) and accumulation of acetate were observed.

No.	Sample ID	U,mg/kg	U6%	U4%
1	FWB-124 core 17-20 section #1 (before)	nd	90	10
2	FW202 3/17/09 (after)	nd	85	15
3	FW215 1/16/09 Control, before)	nd	95	[illegible]
4	FW215 3/17/09 (Control, after)	519.4	95	[illegible]
5	FW216 1/27/09 (before)	112.4	100	[illegible]
6	FW234 1/16/09 (before)	nd	100	[illegible]
7	FW234 from 3/12/09 (after)	441.9	60	[illegible]
8	FWB-234 core March 2009(after)	nd	55	[illegible]
9	GP01 1/16/09 (before)	nd	90	10
10	GP01 3/17/2009 (after)	96.4	15	85
11	GP03 1/16/09 (before)	nd	85	15
12	GP03 3/17/09 (after)	152.3	15	85

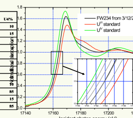


Figure11. XANES analysis confirmed U(VI) reduction to U(IV) which occurred in monitoring wells receiving SRS after 40 days of SRS injection. No U(IV) was observed in control well FW215.

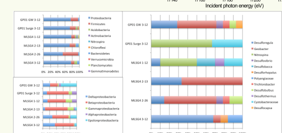


Figure11. Initial 16S bacterial community analysis in sediment and groundwater communities over first month of stimulations. *Desulforegula* (a Long Chain FA fermenting SRB) and *Geobacter* (acetate utilizing FeRB) increase in abundance over the time period examined

SUMMARY

- Slow release electron donor, oleate and emulsified vegetable oil (SRS) support U(VI) reduction in ORIFRC sediment at significantly slower rates relative to ethanol. The reaction rate of SRS was slowest and was sustained longer than oleate.
- Microbial organisms related U(VI) reduction were stimulated by electron donor sources. Microbial community developed depends on electron donor source and sulfate concentration. Highly diversified community was found with SRS.
- Column test showed electrical conductivity increase related to SRS injection as well as degradation. The sediments of ORIFRC Area 2 were shown to have high affinity for SRS.
- The hydrology of test area has been characterized using bromide tracer and surface electrical methods. The background of geochemistry and microbiology has been investigated.
- SRS has been injected to the subsurface. Reduction of nitrate, Fe(III), sulfate and U(VI) was observed sequentially. U(VI) reduction to U(IV) was confirmed by XANES analysis.

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