Biological Uranium (VI) Reduction/immobilization Near Source Zone at the ERSP Field Research Center, Oak Ridge, TN

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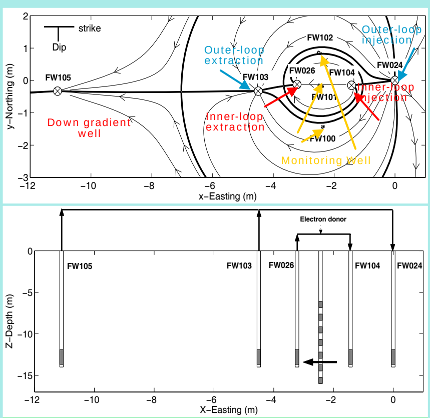
Objectives

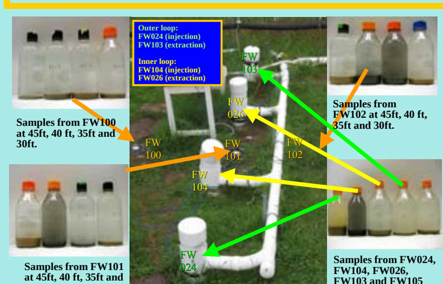
(1) Investigate the feasibility of in situ bioremediation of uranium a highly contaminated region within the subsurface of Area 3 of the DoE ERSP FRC.
(2) Hydraulic control of the target region for biostimulation.
(3) Biostimulation to create a microbial community capable of reducing residual nitrate to N_2 and mobile U(VI) to insoluble U(iV)
(4) Characterize the remediation of of U(VI) and the extent U(VI) reduction/immobilization.
(5) Investigate factors influencing the stability of remediated uranium.

Operational Stages (day 1 to1144)

(1) Development of a field facility to enable hydraulic control over groundwater flow in the subsurface.
(2) Flushing with clean water (day 9-136) to remove bulk nitrate and Al, and to increase pH close to 6.0.
(3) In-situ denitrification (day 137-184) to remove residual nitrate.
(4) In-situ U(VI) reduction/immobilization (day 185-712).
(5) Stability studies (day 713-present) to evaluate factors that influence the stability of immobilized uranium.
(6) Microbial community analysis of treatment area using clone library, MPN and microarrays.
(7) Characterization of uranium speciation in sediments using XANES & EXAFS.

Below-Ground Treatment System





Sediment samples from the treatment zone indicate the reduction status of the subsurface after biostimulation

Major Achievements and Discovery

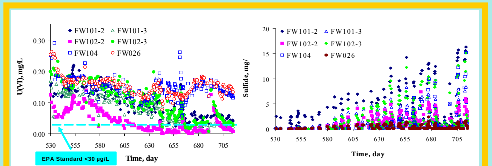
(1) Low U concentration below US EPA maximum contaminant limit (MCL) for drinking water (< 0.03 mg/L or 0.126 μM) can be achieved by in situ bioreduction. .
(2) Bioreduced/immobilized U has been stable under anaerobic conditions for 1-2 years. No anaerobic re-oxidation was observed.
(3) Dissolved oxygen reoxidizes and remobilizes bioreduced U(IV) but reduced zone has reducing capacity to protect immobilized U.
(4) U(VI) reducing bacterial community is selected and enriched in situ, including *Geobacter* spp., *Desulfovibrio* spp. etc.
(5) XANES and EXAFS analysis confirmed the presence of bioreduced U(IV) up to 60-80% in reduced zone and the change in uranium species.
(6) Hydraulic control and removal oxidants (DO and nitrate) are essential for the bioreduction/immobilization of the U contaminated site.

In situ Denitrification (day 137-184)

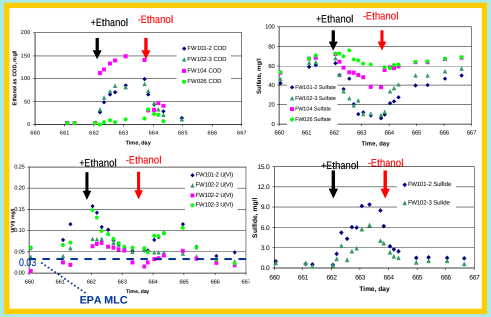
Nitrate was further reduced to below 1 mM in inner loop injection well and near zero in MLS wells by stimulation of denitrification by adding ethanol.

In situ U(VI) Reduction (day 185-712)

U(VI) was reduced stimulated microbial activities by injecting ethanol as electron donor. Uranium concentrations in monitoring wells were reduced to below 30 μg/L. During the same period, sulfate-reducing activities were also significantly increased.

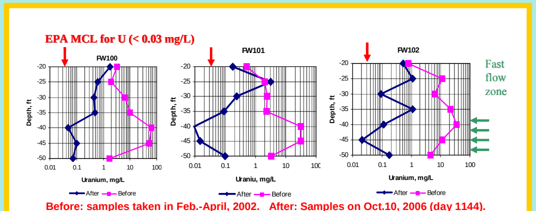


The results below from day 660-667 is an example how U(VI) was reduced during biostimulation. The U(VI) reduction was associated with sulfate reduction . When ethanol was injected an uranium concentration below EPA MLC was achieved. In absence of added electron donor uranium levels increased because recirculated groundwater containing uranium and reduced bioreduction rate.



U Concentrations in Monitoring Wells before and after Three Year Bioremediation

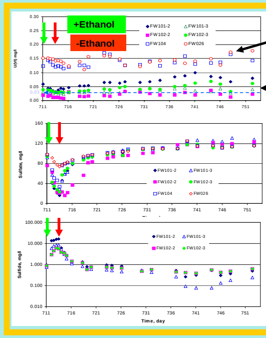
U concentrations in MLS wells have been decreased significantly. Below US EPA MLC has been achieved in fast flow zone.



Before: samples taken in Feb.-April, 2002. After: Samples on Oct.10, 2006 (day 1144).

Stability of Immobilized Uranium in situ

The test was performed by maintaining recirculation between wells without addition of ethanol for 41 days. The results indicated that immobilized uranium was stable in situ under anaerobic condition.



U(VI) concentrations in the injection and extraction wells were stable.

U(VI) concentrations in the MLS wells slowly increased, but never reached the concentration at the injection and extraction wells..

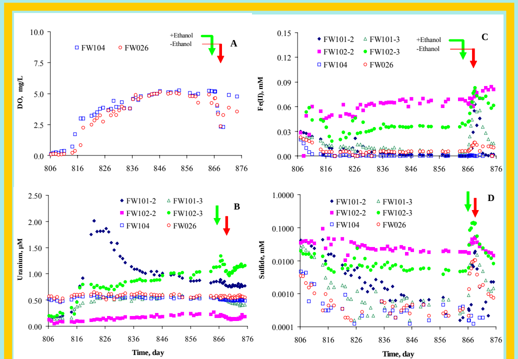
Sulfate concentrations in injection and extraction wells increased and became stable. Sulfate concentrations in MLS wells increased to the level of injection and extraction wells, indicating low SRB activity.

Sulfide concentrations at the MLS wells decreased but remained at significant levels throughout the 41 day starvation period.

This indicates that anaerobic condition persisted without continued ethanol addition .

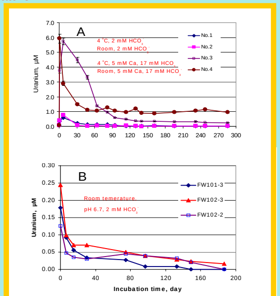
DO Re-oxidizes Immobilized Uranium

The test was performed by introducing dissolved oxygen (DO) to the reduced area for 70 days. DO caused remobilization of uranium but the extent of remobilization differed for different wells. FeRB and SRB activities were restored as soon as ethanol was added and U(VI)-reduction activity also resumed.



Bioreduced U is Stable under Anaerobic Conditions

A. Sample from FW104 stored at 4 ºC and room temperature. **B**. Samples from MLS wells under room temperature.



Microbial Community Analysis

The 16S rRNA gene clone libraries show *Geobacter* spp. and *Desulfovibrio* spp. are predominant species related to U(VI) reduction in sediments (**A**) and groundwater (**B**) samples. Microarray analysis shows function genes related to SRBs (**C**).

A

Family	Dominant Genus	Relative abundance (% of total clones) 104	101-2	101-3	102-2	102-3	26
Desulfovibrionaceae	*Desulfovibrio*	7	15	5	4	12	5
Geobacteraceae	*Geobacter*	2	1	1	11	1	1
Rhodocyclaceae	*Ferribacterium*	12	6	38	10	17	18
Hydrogenophilaceae	*Thiobacillus*	5	27	0	1	4	5
Acidobacteraceae	*Geothrix*	12	7	10	4	10	16
Oxalobacteraceae	*Duganella*	9	10	2	2	11	2
Xhantomonadaceae	*Rhodanobacter*	6	2	0	5	5	0
Commanonadaceae	*Acidovorax*	2	1	2	1	2	6
Sphingomonadaceae	*Sphingomonas*	6	0	1	2	2	1
other families		39	31	41	60	36	46

B

Day	Monitoring well FW-101-2	Injection well FW-104
166	Unc. bacterium clone 300I-F12 (26%) *Herbaspirillum* sp. isolate G8A1 (39%)	Unc. bacterium clone 300I-F12 (23%) *Herbaspirillum* sp. isolate G8A1 (27%) Unc. soil bacterium clone D04 (11%)
535	*Acidovorax delafieldii* isolate N7-18 (10%) *Acidovorax delafieldii* isolate N7-18 (7%) Unc. δ-proteobacterium clone 177T36 (6%) Unc. *Actinobacteriaceae* clone Hrh678 (6%) *Dechlorosoma* sp. C6 (5%)	Unc. Sludge bacterium H22 (15%) Unc. bacterium clone 300I-F12 (7%) Unc. Bacterium clone 015B-B03 (7%) *Acaligenes defragans* strain:PD-19 (6%) *Dechlorosoma* sp. C6 (10%)
641	Unc. bacterium clone TTMF87 (16%) Unc. δ-proteobacterium clone 177T36 (14%) Unc. *Desulfovibrionacaceae* bacterium (7%) *Desulfovibrio magneticus* (6%) Unc. δ-proteobacterium clone 036T7 (9%) Unc. *Geobacter* sp. clone KB-1 1 (7%) Unc. *Phyllobacterium* sp. clone Ph (6%) *Rhizobium* sp. SDW058 (6%)	Unc. δ-proteobacterium clone 177T36 (17%) Unc. *Desulfovibrionacaceae* bacterium (4%) *Desulfovibrio magneticus* (2%) Unc. *Actinobacteriaceae* clone Hrh678 (7%) Unc. δ-proteobacterium clone 036T7 (6%) Unc. *Geobacter* sp. clone KB-1 1 (4%)

C

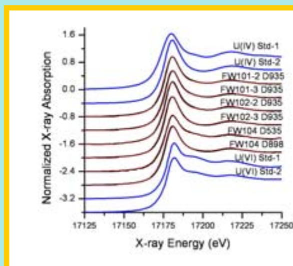
Dominant SRBs in Monitoring Well 102-2 During U Reduction Phase

Dominant Genes	Processing Days 163	166	170	184	248	255	269	278	298	323
58:20142091 uncultured bacterium	P				P		P		P	
FW300347A lab clone	P	P	P	P	P	P	P	P	P	
29:12667654 uncultured sulfate-reducing bacterium					P	P	P	P		
60:14090293 Desulfomicrobium norvegicum					P	P	P	P	P	P
54:14389177 uncultured sulfate-reducing bacterium					P	P	P		P	
20502009 uncultured sulfate-reducing bacterium						P	P		P	
14389259 uncultured sulfate-reducing bacterium						P	P		P	
14389189 uncultured sulfate-reducing bacterium		P			P	P	P	P	P	P
49:14389143 uncultured sulfate-reducing bacterium	P				P	P	P	P	P	P
48:TPB16241B lab clone					P	P			P	P
FW003077A lab clone						P			P	P
13561054 Desulfosarcina variabilis					P	P			P	
20501981 uncultured sulfate-reducing bacterium					P	P			P	
14669527 uncultured bacterium					P	P	P		P	
2576394 Allochromatium vinosum					P	P	P		P	
40:14276806 Desulfotomaculum kuznetsovii					P	P	P		P	
FW003269B lab clone	P				P	P	P	P	P	P
TPB16070B lab clone	P				P	P	P	P	P	
16:14090305 Desulfovibrio burkinensis						P		P		P
67:12667582 uncultured sulfate-reducing bacterium	P	P			P	P	P	P		P
1:15055582 Thermodesulfovibrio yellowstonii									P	P
14:18034292 Desulfovibrio piger									P	P
FW005228A lab clone					P	P	P			P
41:15080893 Desulfotomaculum thermoacetoxidans					P	P				P
66:14389159 uncultured sulfate-reducing bacterium					P	P	P		P	P
40:13560085 Desulfotomaculum kuznetsovii	P					P	P		P	P
40253034 uncultured sulfate-reducing bacterium	P					P			P	
13898427 uncultured phenanthrene mineralizing bacterium		P				P			P	
32:FW300167B lab clone		P				P				
14389151 uncultured sulfate-reducing bacterium		P				P	P		P	
57:5006483 Alvinella pompejana symbiont 7.6		P			P	P	P	P	P	P
49:14389137 uncultured sulfate-reducing bacterium	P	P			P	P	P	P		
TPB16070A lab clone		P	P	P	P	P	P	P	P	P
FW015084B lab clone		P						P	P	

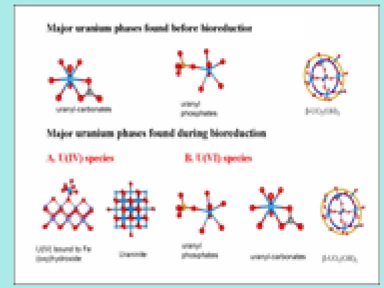
1. P in the table stands for "Gene presents";
2. Dominant sulfate-reducing genes increased during the process;
3. A large portion of dominant genes were found to be lab clones from FRC site

Confirmation of U(VI) Reduction in Sediments by XANES and EXAFS Analysis

XANES analysis of sediment samples taken on day 935 and other days shows U(IV) was found in injection, monitoring and extraction wells up to 60-80% of total U. This confirmed microbial U(VI) reduction *in situ*.



EXAFS analysis of sediment samples before and during bioreduction shows uranium phase change. U(IV) formed was mainly bound to Fe.



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