Reoxidation of Bioreduced Uranium by Dissolved Oxygen and Nitrate and Restoration of Reduced Conditions at ORIFC site, Oak Ridge, TN

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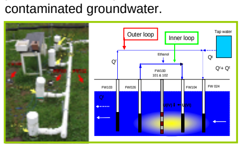
ABSTRACT

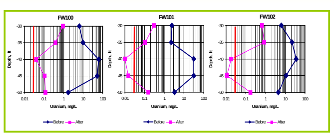
The research objective is to study enhanced contaminant stability strategies for source control. The work was performed in bioreduced zone, Area 3, DOE ERSD IFC site, Oak Ridge, TN. *In situ* bioremediation of a highly uranium-contaminated aquifer has been conducted by controlling groundwater flow and stimulating microbial growth through weekly injection of ethanol. Aqueous U(VI) fell below the US EPA drinking water limit (<30 µg/L). The bioreduced U(IV) was stable under anaerobic conditions. The impact of DO and nitrate on stability of bioreduced U(IV) in the subsurface was examined *in situ*. Introduction of DO (4.0-5.5 mg/L) through injection of oxygenated water to subsurface caused reoxidation and remobilization of uranium. After DO was introduced, sulfide and Fe (II) concentrations gradually decreased to near zero in the area close to injection wells with increased U concentration. The extent of remobilization of U was observed to be higher in wells that were closer to the injection well. After exposure to DO for more than 60 days, resumption of ethanol injection restored Fe(III), sulfate and U(VI) reduction within 1.5 days. As ethanol injection continued U concentration fell below 30 µg/L again. When controlling of groundwater flow was suspended, nitrate-contaminated groundwater infiltrated to the reduced area and caused the rebound of U concentration. The penetration of outside contaminated groundwater was confirmed by tracer tests with bromide. Nitrate (>2 mM) was then injected to the bioreduced subsurface together with bromide tracer. This caused observation of nitrite and ammonia with decrease of sulfide and Fe (II) to zero. Reoxidation and remobilization of uranium was observed. Resumption of ethanol injection after nitrate exposure for 6 days showed sequential restored reactions of denitrification, Fe (III)-, sulfate-, and U (VI) reduction. U (VI) reduction occurred as sulfide started accumulation. The reoxidized subsurface was restored to anaerobic conditions with U below 30 µg/L through ethanol injection. Bacteria known to reduce U (VI), including *Desulfovibrio* spp., *Desulfosporosinus* spp., *Anaeromyxobacter* spp., and *Geobacter* spp. were found. *Geothrix* spp. was a dominant Fe(III)-reducer. Fe(II)-oxidizing *Thiobacillus* was also present in groundwater and sediments. The XANES analysis of uranium speciation confirmed the changes in U(VI)/U(IV) ratio before and after nitrate reoxidation tests.

Site Description and Test Strategies

Test System is composed of an outer loop and an inner loop. The outer loop protected inner loop from the invasion of contaminated groundwater.



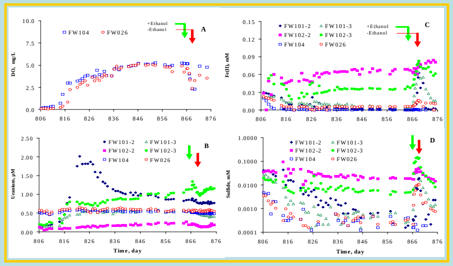
Bioreduction/immobilization of U *in situ*



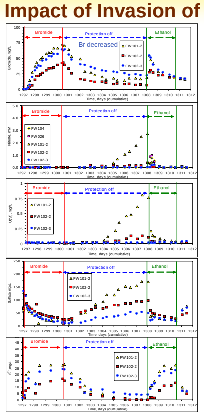
Bioreduction of uranium below US EPA MCL was achieved by weekly injection of ethanol for one more year. This was associated with Fe(III)- and sulfate-reduction.

DO Re-oxidation Test

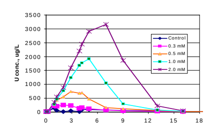
This test was performed by introducing dissolved oxygen (DO) into the reduced area for 70 days. U(VI) remobilization of uranium occurred but the extent of remobilization differed for different wells. FeRB and SRB activities resumed as soon as ethanol was added. U(VI)-reduction activity also resumed.



Impact of Invasion of Contaminated Groundwater

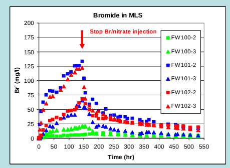


This test was performed by suspension of protection of inner loop from invasion of outside contaminated groundwater. Initially, bromide was injected to the test zone and then the protection was turn-off. Disappearance of bromide and increase in nitrate concentration indicated the invasion of outside groundwater. The invasion caused U(VI) rebound in some monitoring wells.



Microcosm test confirmed that nitrate caused the rebound of U(VI). The test was performed using reduced sediment sample from FW104 (U content 10.2m g/kg) at pH 6.3. Initial nitrate concentrations: 0, 0.3, 0.5, 1.0 and 2.0 mM.Test was performed in 150 mL serum bottles ambient (21-24 °C). Each bottle contained 130 mL sediments and groundwater. Ethanol (5 mM) was added to reduce nitrate and then re-oxidized U(VI).

Nitrate Re-oxidation Test



Trace and Nitrate Injection. This test was performed by injection of nitrate together with bromide to inner loop to oxidize the reduced zone. Bromide entered the fast flow area (FW101-2 and FW102-3) quickly. All other wells also responsed to the bromide injection

Based on the ratio of Br/nitrate injected, nitrate was uptaken by microorganisms. Nitrite and ammonia were observed as products.

Geochemical Response and Bioreaction. Nitrate occurred in MLS after injection and decreased to zero after nitrate injection stopped and ethanol was injected (A). Nitrite and ammonia were produced during nitrate injection (B & C). U(VI) rebounded during nitrate injection indicating reoxidation (D). Ethanol injection (E) restored the activity of sulfate reduction (F & G) and Fe(III) reduction (H).

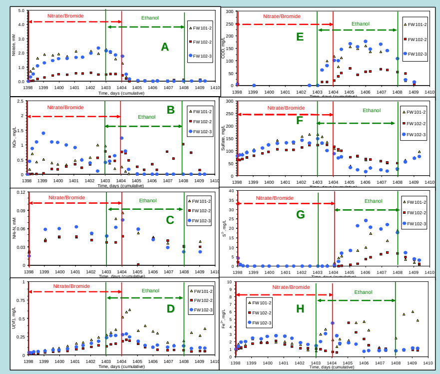


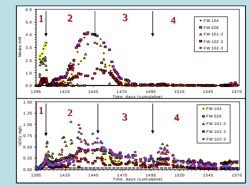
Table U valent change before and after nitrate-reoxidation

MLS	U(VI):U(IV) Ratio		
Date	12/6/2006	9/21/2007	12/18/2007
Status	Reduced	Oxidized	Restored
FW101-2	13:87	100:0	33:67
FW102-2	20:80	90:10	36:64
FW102-3	13:87	50:50	15:85

The re-oxidized sediments with brown-yellow color and the reduced sediments with dark color.

Restoration of Reduced Condition

This test was performed to allow the reduced zone exposure to nitrate for two more months and then restore reduced condition by weekly ethanol injection.



1.Day 1397-1404: Nitrate was injected to the inner loop together with bromide. This caused U remobilization.

2. Day 1404-1451: Ethanol was injected for 4 days and U decreased. Subsequently, nitrate contaminated water invaded to the reduced zone and caused U re-oxidation.

3. Day 1452-1496: Restored well system protection to prevent the invasion. Nitrate decreased gradually. No ethanol injection was conducted during this period.

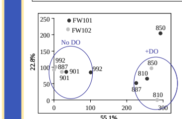
4. Day 1496-1570: Ethanol was injected weekly to reduce U(VI). Microbial activities of SRB, FeRB and U(VI) reduction were stimulated. The U concentrations in MLS finally fell below EPA MCL.

The reoxidation and restoration of reduced U were confirmed by XANES analysis

Microbial communities

Family	Dominant Genus	Relative abundance (% of total clones)					
		104	101-2	101-3	102-2	102-3	026
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
Cystobacteraceae	*Anaeromyxobacter*	1	4	2	3	3	1
Peptococcaceae	*Desulfosporosinus*	2	1	5	2	1	0
other families		36	26	34	55	32	45

Microbial Community Analysis of the sediment samples before the DO re-oxidation tests indicated that U(VI) reducing *Desulfovibrio*, *Desulfosporosinus*, *Anaeromyxobacter* and *Geobacter* were found. *Geothrix* spp. was a dominant Fe(III)-reducers. Fe(II)-oxidizing *Thiobacillus* was present in the sediments and also detected in groundwater after DO reoxidation. This microorganism may play a role in oxidation of FeS and U(IV). *Geobacter* may also oxidize U(IV) to U(VI) in the presence of nitrate. Nitrite produced by SRB and other bacteria also oxidize U(IV) and Fe(II).



Detrended Correspondence Analysis (DCA) results of array data from the MLS before and after DO reoxidation. There are two main clusters (circled) – one of wells during the reoxidation period (right) and the other after oxygen control is reestablished (left). This suggests that the communities were significantly altered by the reoxidation period. Well FW101-2 received more DO than well FW102-3, which would explain why the two wells do not cluster together on d 850 and 887. While recovery from the stress of oxygen exposure occurred (indicated by increased gene numbers and decreased U), recovered communities differed from the original communities.

SUMMARY

- **Uranium can be reduced and immobilized *in situ* by delivery of electron donor (ethanol) and the immobilized U remained stable under anaerobic conditions.**
- **Both DO and nitrate reoxidized and remobilized uranium in bioreduced zone.**
- **After re-oxidation, the reduced zone can be restored by delivery of ethanol donor.**
- **Microorganisms related to U(VI) reduction, Fe(III) reduction, sulfate reduction and re-oxidation were detected.**
- **Reoxidation by DO impacted the microbial community in the subsurface.**
- **Invasion of nitrate contaminated water caused the re-oxidation of bioreduced U(IV) and Fe(II) solids. Removal of nitrate from subsurface is essential for bioreduction and immobilization of uranium at the IFC site, Oak Ridge, TN.**

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