Changes in a Bioreduced, Uranium-Contaminated Subsurface during Periods of Resting, Reoxidation, and Recovery

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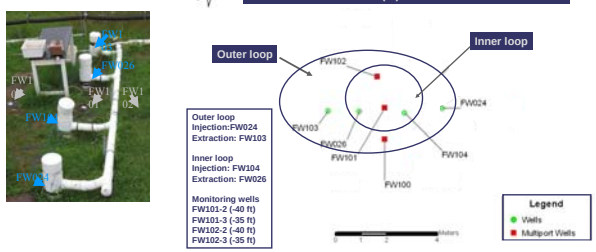
ABSTRACT

A pilot-scale system was established for biostimulation of subsurface U(VI) reduction by injection of ethanol at the U.S. DOE's Field Research Center (FRC) in Oak Ridge, TN. After U(VI) reduction was achieved, the stability of the bioreduced area was examined by suspension of ethanol injections (resting state) and reoxidation of the area by introducing dissolved oxygen (DO) for two months and then ethanol injections were resumed. Geochip 2.0, a comprehensive 50mer microarray containing probes for genes involved in the geochemical cycling of N, S, and C, metal resistance and contaminant degradation, was used to monitor the dynamics of the groundwater microbial community structure and function. The immobilized U was stable during the resting state. After DO was introduced to the reduced area, the monitoring well (FW101-2) located closest to the injection well, had a greater increase in DO (2 mg L^{-1}) than the well located further away (FW102-3; <0.4-0.5 mg L^{-1}). Based on canonical correspondence analysis and Mantel test results, ethanol showed the greatest correlation to community structure, although sulfide did correlate with changes in the functional community. Detrended correspondence analysis showed a shift towards a different community structure after ethanol injections resumed compared to the periods of starvation and exposure to DO. Changes in the functional community structure were similar in the two wells; however, the community in FW101-2 was more affected by DO than in FW102-3. Hierarchical clustering showed that cytochrome c genes grouped based on DO exposure, resting state, or ethanol addition, while dissimilatory sulfite reductase (*dsr*) genes grouped only by resting state or ethanol addition. However, the relative abundance of *dsr* genes did decrease when DO levels increased while the relative abundance of cytochrome genes seemed unaffected by changes in DO. Overall, results indicated that ethanol was the main factor affecting community structure, although some changes could be attributed to DO.

BACKGROUND

S-3 Waste Ponds. The four unlined S-3 waste collection ponds were constructed in 1951 (left). Effluent waste, consisting primarily of nitric acid, nitrate, metals, and radionuclides (U, Tc), were discharged into the ponds until 1983. The ponds were neutralized and denitrified in 1984 and then capped in 1988. The site is currently covered with asphalt and serves as a parking lot (right). Waste from the ponds seeped into the groundwater and has contaminated the surrounding area, resulting in a site with low pH (3.4-3.6), high U (50 mg L^{-1}), and high nitrate (8-12 g L^{-1}). (Oak Ridge Field Research Center, 2007)



Groundwater recirculation system. The Stanford-ORNL project, located adjacent to the S-3 ponds, was started to examine the feasibility of *in situ* bioremediation of contaminated groundwater. The system consists of two injection and two extraction wells and several monitoring wells in a nested design. An above ground treatment system was used to reduce nitrate and other contaminants in the groundwater and treated/clean water was reinjected to further reduce the contaminants within this system. Ethanol was injected intermittently to serve as an electron donor and promote reduction of residual nitrate and immobilize U. Concentrations of U were reduced to below EPA drinking water standards (30 µg L^{-1}). This study examined changes in the functional community when ethanol injections were temporarily stopped and when dissolved oxygen levels were allowed to increase.

METHODS

Groundwater samples (2 L) were taken from wells FW101-2 and FW102-3 during periods of resting, reoxidation and recovery. Samples were filtered and DNA was extracted from the filters using a freeze-grid method (Zhou et al., 1996). An aliquot of DNA (50 ng) was amplified using a modified rolling circle amplification (Wu et al., 2006) and labeled with cyanine 5.

Labeled samples were hybridized to the GeoChip 2.0 (He et al., 2007). The GeoChip consists of >24,000 probes for genes involved in the geochemical cycling of carbon, nitrogen, and sulfur, as well as genes for metal reduction and resistance and organic contaminant degradation. Hybridizations were carried out in triplicate at 42 °C.

Arrays were imaged and analyzed using ImaGene software (v.6.1.0, Biodiscovery Inc.)

Spots with signal-to-noise ratios (SNR) of ≥1.5 were considered positive. If at least 1/3 (minimum of 2 probes) of the probes for a particular gene were positive, the gene was considered positive.

Statistical analysis was performed using PCOrd (MjM Software, Gleneden Beach, OR) or Canoco (Version 4.5, Biometris – Plant Research International, The Netherlands)

RESULTS

Summary of GeoChip 2.0 probes and sequence information

Gene category	No. gene subcategories	No. probes
Carbon degradation	6	2954
Carbon fixation	6	844
Methane reduction and oxidation	2	773
Metal resistance and reduction	17	3950
Nitrogen cycling	12	5663
Organic remediation	95	8741
Sulfur cycling	2	1616
Total	292	24541

Major geochemical concentrations at each timepoint examined

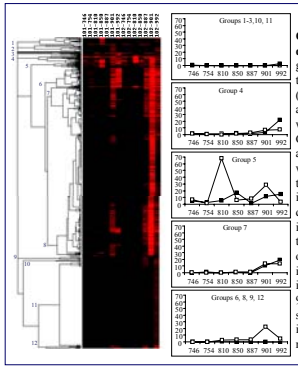
FW101-2

Operational Day	746	754	810	850	887	901	992
Sulfate	111.5	101.4	120.1	56.0	68.8	95.2	41.4
Sulfide	0.3	1.0	0.9	0.02	0.2	1.5	23.5
Uranium	0.1	0.1	0.04	0.2	0.2	0.3	0.04
COD[a]	3	44	3	3	39	126	130
Fe(II)	1.26	1.2	1.33	3.95	3.22	0.82	2.09
pH	6.00	5.98	5.83	6.00	5.97	6.25	6.69
Temperature (°C)	19.7	19.6	17.30	14.3	14.3	13.7	16.1

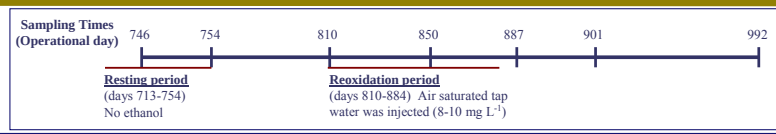
FW102-3

Operational Day	746	754	810	850	887	901	992
Sulfate	112.5	96.3	136.2	64.4	36.5	67.4	61.5
Sulfide	0.3	2.3	0.4	0.2	3.5	6.0	6.1
Uranium	0.1	0.1	0.1	0.2	0.2	0.2	0.2
COD	7	52	3	3	57	67	15
Fe(II)	2.52	2.5	0.57	4.0	3.45	2.5	3.76
pH	6.00	5.83	5.73	5.63	5.43	5.99	5.85
Temperature (°C)	19.7	19.6	17.30	14.3	14.3	13.7	16.1

[a] COD, chemical oxygen demand. COD was used to monitor ethanol concentration.

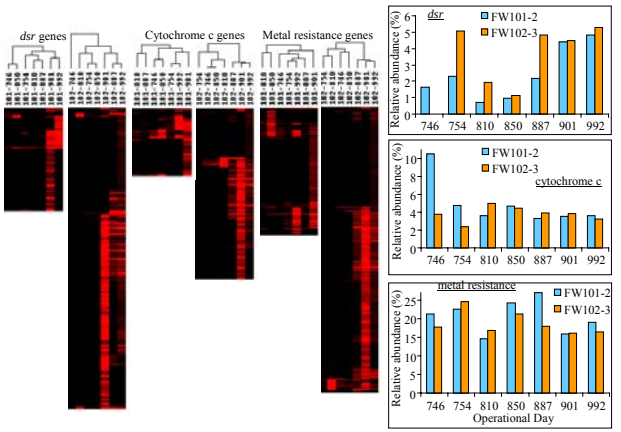


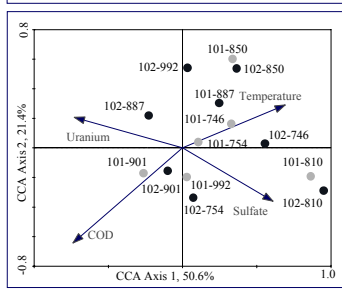
Cluster analysis of all genes detected. A total of twelve gene groups were detected. Several of these groups showed similar patterns (graphs). Genes in groups 1, 2, 3, 10, and 11 showed little change in either well over the course of the study. Group 4 showed an increase in average signal intensity on day 992, with a greater increase in FW101-2 than FW102-3. Group 5 showed an increase in average signal intensity on day 810 and 901 for FW102-3 while increases in FW101-2 were less than that observed in FW102-3 and occurred at a later timepoint. Genes in group 7 showed a similar level of increase in both wells on days 901 and 992. Finally, groups 6, 8, 9, and 12 showed an increase in average signal intensity on day 901 for FW102-3, but not in FW101-2.

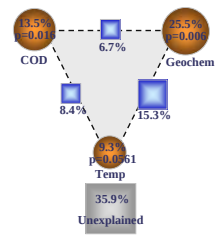


Cluster analysis (left) and relative abundances (right) of individual gene groups from wells FW101-2 and FW102-3. For well FW102-3, most of the genes clustered based on ethanol injection (d 746-850, no ethanol; d 887-992, ethanol). Samples from well FW102-2 clustered less clearly, suggesting that other factors, such as DO or other environmental factors, affected microbial communities in addition to ethanol.

The relative abundance of *dsr* genes decreased during the reoxidation period, suggesting DO had an affect on this gene group. However, cluster analysis did not show a similar response as no separate DO cluster appeared. The relative abundance of cytochrome c genes was not affected by any of the experimental periods, yet distinct with and without ethanol clusters were evident for FW102-3. The relative abundance of metal resistance genes decreased after ethanol addition was resumed, but did not appear to be affected by DO. However, FW101-2 showed a separate cluster during the reoxidation period while FW102-3 showed with and without ethanol clusters.

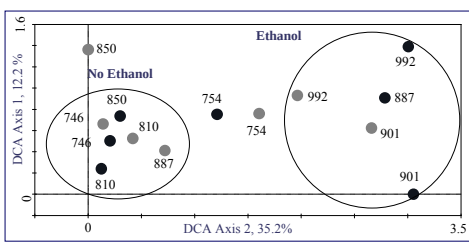






Canonical correspondance analysis (CCA) and variation partitioning analysis (VPA). CCA was done using all functional genes detected and environmental variables. Environmental variables were chosen based on significance calculated from individual CCA results and from variance inflation factors calculated during the CCA. Based on CCA results, sulfate, U, COD, and temperature were the most significant environmental variables, with U, COD, and temperature being the most important.

VPA results calculated from partial CCA results indicated that about 75% of the variation observed could be explained by the environmental variables examined. A majority of the variation could be explained by geochemistry (U and sulfate) and the combination of geochemistry and temperature (~41%).



Detrended correspondence analysis (DCA) results of array data from the two monitoring wells. Samples clustered into two main groups (circled) – one of wells during resting (no ethanol addition) and reoxidation periods during which no ethanol was injected (right) and the other after oxygen control was reestablished and ethanol injections were restarted (left). This suggests that the communities were significantly altered by resumption of ethanol injections.

SUMMARY

- ✓ Ethanol increased diversity and richness in both wells and appeared to be the main factor in overall community structure.
- ✓ The impact of DO on the communities could also be observed, although additional factors appeared to be influencing the communities in FW101-2, as well.
- ✓ DO did affect the relative abundance of *dsr* genes, but did not appear to affect the relative abundance of cytochrome c and metal resistance genes.
- ✓ Temperature, sulfate, uranium, and COD were the most significant environmental variables examined in this study. The geochemical variables U and sulfate explained ~25% of the variation in functional genes observed and an additional 20% in combination with COD or temperature.

ACKNOWLEDGEMENT

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