Microbial Community Dynamics and the Effect of Geochemistry in Uranium Bioremediation Revealed by Functional Gene Array Analysis

L.Y. Wu[1,2,3], Z.J. Huang[2], T.J. Gentry[4,2], W-M Wu[5], J.D. Van Nostrand[1], C.W. Schadt[2], D. Watson[2], Z.L. He[1,2], F.W. Matthew[8], C. S. Criddle[5], J. Tiedje[6], T.C. Hazen[7,3], J. Zhou[1,2,3]

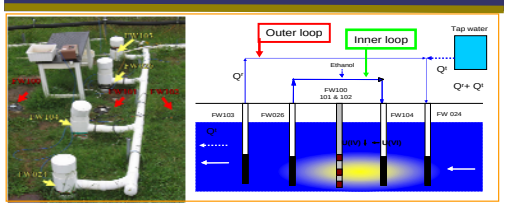
[1]University of Oklahoma, Norman, OK, [2]Oak Ridge National Laboratory, Oak Ridge, TN, [3]Virtual Institute for Microbial Stress and Survival, Berkeley, CA, [4]Texas A&M University, College Station, TX, [5]Stanford University, Stanford, CA, [6]Michigan State University, East Lansing, MI, [7]Lawrence Berkeley National Laboratory, Berkeley, CA, [8]Montana State University, Bozeman, MT

N-200 | http://vimss.lbl.gov/

ABSTRACT

A pilot-scale system was established to examine the feasibility of *in situ* bioremediation and immobilization of U(VI) at a highly contaminated aquifer at the U. S. DOE's Field Research Center, Oak Ridge, TN. An above-ground treatment system, including a denitrifying fluidized bed reactor, was used to pre-condition the groundwater to optimize subsurface U immobilization. Ethanol to stimulate microbial reduction of soluble U(VI) to insoluble U(IV). Three monitoring wells (FW101-2, 102-2, and 102-3) were analyzed using a functional gene array containing >24,000 probes covering 10,000 genes to examine the effect of geochemistry on the functional microbial community dynamics. Microarray results indicate that, during the U (VI) reduction period, both FeRB and SRB populations reached their highest levels on day 212 in FW102-3 and on day 255 in FW102-2 and FW101-2, followed by a gradual decrease over the next 500 days in all three wells and then a rebound on day 719. Mantel tests of functional genes versus the geochemical parameters showed a significant correlation in all three wells between pH and most of the functional gene groups (p-value, <0.01-0.1) detected. The U (IV) concentrations were significantly correlated with the microbial communities in wells FW101-2 and FW102-3 over the entire study period. Once the microbial population peaked, this correlation was also observed for FW102-2, and even stronger correlations were observed in both FW101-2 and 102-3. Chemical oxygen demand (COD) correlated with the microbial community structure only in well FW101-2. Neither nitrate nor sulfate showed a significant correlation in any of the wells until after the population peak, when significant correlations were observed in FW102-2 and 102-3. Canonical correlation analysis revealed similar correlations between the functional community and the geochemical variables. In addition to correlations with the geochemical parameters, principal components analysis showed that the microbial communities also varied both temporally and spatially.

BIOREMEDIATION SYSTEM



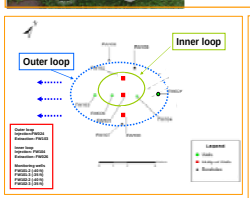


Figure 1. Test site for *in-situ* Uranium (IV) Bioremediation (Courtesy of Wei-Min Wu)**.** The test site is located at the DOE's Oak Ridge Field Research Center, Oak Ridge, TN. The subsurface is contaminated with uranium (up to 800 mg/kg) and nitrate (>200 mM) at pH 3.6. The bioremediation test system is composed of a nested well system (upper left and right) with resultant water flow from groundwater recirculation creating outer and inner loops. Treatment performance and microbial populations were monitored by sampling from wells (left).

METHODS

- **Sampling and DNA extraction.** Groundwater samples (2 L) were taken from the sampling wells FW101-2, FW102-2 and FW102-3 during the period from day 163 through 719. Samples were filtered and DNA was extracted from the filters using a freeze-grind method (Zhou et al., 1996).
- **50mer Oligonucleotide Functional gene array.** The second version of a functional gene array (FGAII, Table 1) was used to monitor microbial community dynamics during bioremediation in the groundwater recirculation system.
- **DNA amplification, labeling, and hybridization.** 100 ng of DNA from each sample was amplified using phi29 DNA polymerase, labeled with cy5 and hybridized to FGA II slides.
- **Microarray scanning and data processing.** Hybridized microarray slides were scanned using a ScanArray® 5000 and the image displays were analyzed by quantifying the pixel density (intensity) of each spot using ImaGene™ version 5.0. Empty and poor spots were removed before the signal intensities were normalized by the mean signal across the slide; then outliers (at p<0.01) and minorities (only 1 of the three replicates was present) were also removed.
- **Data Analysis.** Functional gene diversity was calculated using Simpson's reciprocal index (1/D) and Shannon-Weaver index (H). Cluster analysis was performed using the pairwise average-linkage hierarchical clustering algorithm in the CLUSTER software. Several multivariate statistical methods, Mantel test, DCA and CCA analysis, were employed to analyze the microarray data.

RESULTS: Overall Picture of the Communities

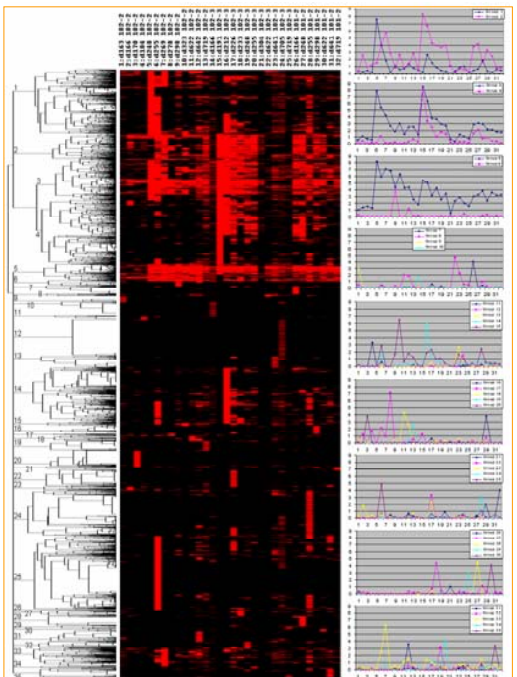


Figure 1. Clusters of functional gene groups detected. The clusters show the different functional groups within the communities during uranium bioremediation. The genes detected can be clustered into 35 distinct groups, which can be further sorted into 2 main sections. The first section, composed of genes which were shared by all wells and all time points, includes the first five groups; the second section includes the remaining 30 groups, the genes in which were to some extent unique to individual samples. The line graphs show average signal intensity of each gene group for each sample. The bar graph below shows the gene composition differences in the two sections.

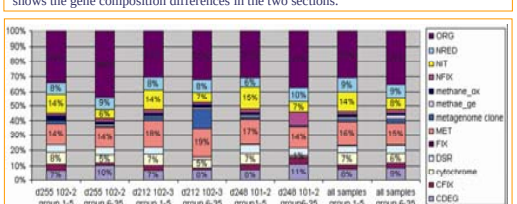


Figure 2. Bar graph of functional genes in the two sections from different wells. The bar graph shows the gene composition differences in the two gene sections (Figure 1). The multiple bars of both sections for the microbial communities from the three wells at their population peaks show that there were more cytochrome genes and nitrification genes, less organic contaminates degradation genes and carbon degradation genes in the shared sections than those in the unique sections. The differences of *dsr* genes and metal resistance genes were not consistent in all three wells.

RESULTS: Population Dynamics in Time and Space

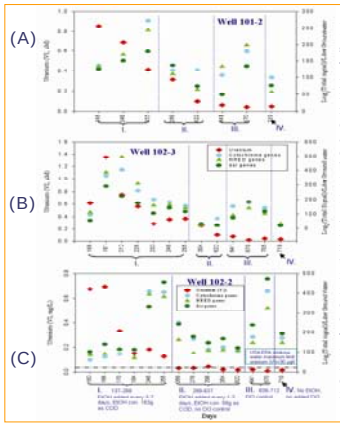


Figure 3. Scatter plots showing population changes.

The scatter plots show the population changes of metal, nitrate/nitrite, and sulfate reducing bacteria (represented by changes in cytochrome, NRED, and dsr genes, respectively) during uranium (VI) bioremediation in sampling wells FW102-2 (A), FW102-3 (B), and FW101-2 (C).

All populations of these bacteria increased in the early stage, reaching a peak around day 255, then dropping due to a decrease in the ethanol injection rate, DO infiltration (DO was not controlled), and depletion of other nutrients.

The populations re-bounded from day 642 to 712, when DO infiltration was controlled, and dropped again when the ethanol injection stopped.

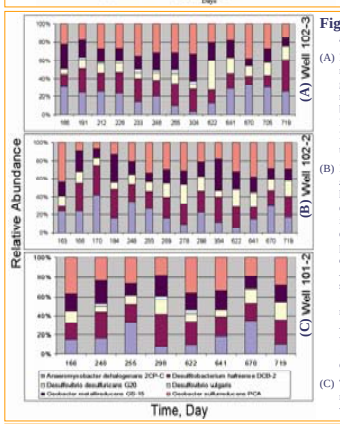


Figure 4. Population changes among metal reducing bacteria.

(A) Populations represented by the relative abundances of cytochrome C genes detected among different metal reducing bacteria changed during bioremediation of uranium (VI) in FW102-3. The population changes in FW102-3 appeared to occur earlier than those in the other two wells.

(B) In FW102-2, *Geobacter sulfurreducens* PCA showed the highest relative abundance initially, dropped while the other strains increased, and then increased again on day 255 and stabilized. The proportion of *Anaeromyxobacter dehalogenans* 2CP-C, and *Desulfitobacterium hafniense* DCB-2 showed an initial increase from day 166 to 184 and then dropped. The relative population changes of *Desulfovibrio desulfuricans* G20, *D. vulgaris*, and *G. metallireducens* GS-15 were relatively stable over the time course examined.

(C) The relative population changes of metal reducing bacteria in FW101-2 were similar with those in FW102-2.

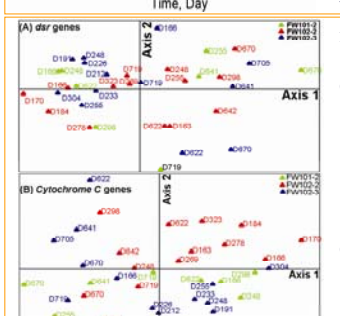


Figure 5. DNA microarray data for dsr and cytochrome C genes.

(A) The DCA ordination plot of dsr genes shows that the sulfate reducing bacteria changed over time during the bioremediation process in all three wells in a similar manner with a few exceptions. The plot also shows that the sulfate reducing communities in the three wells were different to some extent. Samples from the same well were expected to group together.

(B) Similarly, the DCA ordination plot of cytochrome C genes shows the same distribution across space and time.

RESULTS: Effects of Environmental Elements

Table 1. Mantel Test for Correlations between Functional Microbial Communities and Environmental Variables in FW102-2

	nitrate		sulfate		U(IV)		COD		pH	
	r value	p value	r value	p value	r value	p value	r value	p value	r value	p value
Day163-719										
CDEG	-0.101	0.589	-0.070	0.640	-0.117	0.626	0.054	0.297	0.322	0.062
DSR	-0.090	0.546	-0.098	0.762	-0.098	0.532	0.025	0.358	0.366	0.047
cytochrome	-0.092	0.532	-0.118	0.778	-0.112	0.620	-0.032	0.520	0.444	0.025
MET	-0.079	0.509	-0.095	0.715	-0.082	0.526	0.012	0.371	0.346	0.069
methane	-0.270	0.921	-0.238	0.963	-0.218	0.856	0.030	0.380	0.236	0.120
methaneOx	0.136	0.211	0.105	0.190	0.076	0.277	-0.081	0.709	0.348	0.027
NRED	-0.138	0.664	-0.135	0.790	-0.102	0.528	-0.030	0.511	0.462	0.014
ORG	-0.122	0.618	-0.135	0.812	-0.144	0.646	0.025	0.347	0.364	0.052
Day255-719										
CDEG	-0.101	0.711	0.530	0.021	0.600	0.044	0.065	0.235	0.365	0.135
DSR	0.269	0.158	0.502	0.018	0.614	0.045	0.045	0.299	0.451	0.061
cytochrome	0.358	0.076	0.537	0.017	0.715	0.005	-0.075	0.644	0.599	0.005
MET	0.351	0.090	0.514	0.013	0.666	0.017	0.019	0.341	0.461	0.031
methane	0.258	0.164	0.464	0.042	0.479	0.043	0.008	0.392	0.347	0.083
methaneOx	0.444	0.013	0.638	0.001	0.670	0.002	0.187	0.058	0.392	0.055
NRED	0.386	0.084	0.624	0.002	0.842	0.002	-0.009	0.388	0.596	0.011
ORG	0.231	0.208	0.451	0.041	0.645	0.026	0.015	0.306	0.496	0.034

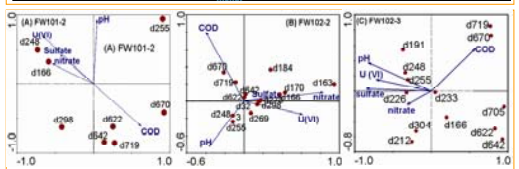


Figure 6. Biplots of CCA results. The biplots show the correlations of microbial communities and the environmental variables. The microbial communities closely correlated with COD in FW101-2 (**A**), closely correlated with pH, COD, and nitrate in FW102-2 (**B**) and FW102-3 (**C**).

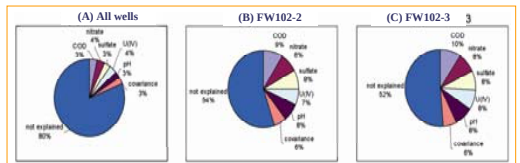


Figure 7. Pie graphs of variation partitioning. The variation which could be explained by environmental variables was partitioned using partial CCA. The proportions explained by each environmental variables are shown in the pie for the entire community in all wells and for FW102-2 and FW102-3 alone.

CONCLUSIONS

- The microbial communities did respond to the bioremediation treatment. Populations in all three wells increased until around day 255, then dropped due to lower ethanol concentration, DO increase, and depletion of other nutrients. A rebounce occurred when DO was controlled but dropped again when the ethanol injection stopped.
- *Anaeromyxobacter dehalogenans*, *Geobacter sulfurreducens*, *G. metallireducens*, *Desulfovibrio vulgaris*, and *Desulfitobacterium hafniense* were the main constituents of the metal reducing bacteria detected.
- Based on DCA results, the communities remained somewhat spatially distinct although the populations changed temporally.
- Results of CCA indicate strong correlations between the environmental parameters (primarily COD, pH, and nitrate) and the microbial community composition. Mantel tests indicated that sulfate and pH were the most important environmental variables, although none of the environmental variables explained greater than 10% of the variation observed.

ACKNOWLEDGEMENT

ESPP2 (MDCASE) is part of the Virtual Institute for Microbial Stress and Survival (VIMSS) supported by the U. S. Department of Energy, Office of Science, Office of Biological and Environmental Research, Genomics:GTL Program through contract DE-AC02-05CH11231 between Lawrence Berkeley National Laboratory and the U. S. Department of Energy.