Microarray-based analysis of microbial community composition and dynamics in uranium bioremediation

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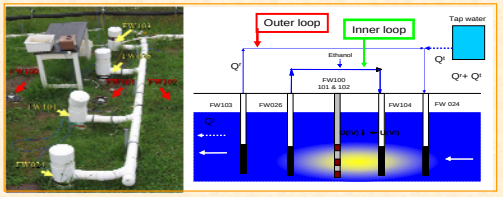
ABSTRACT

A field-scale system is being used for evaluating in situ biological reduction and immobilization of U(VI) at a highly contaminated aquifer in the DOE ERSP Field Research Center, Oak Ridge, TN. Bioremediation of this uranium (U)-contaminated site is complicated by the presence of other contaminants such as high concentrations of nitrate, chlorinated solvents, and heavy metals, and extremely acidic conditions. Above-ground treatment of groundwater, including nitrate removal via a denitrifying fluidized bed reactor (FBR) pre-conditions the groundwater for subsurface uranium immobilization. Treated water was then injected into the subsurface with ethanol to stimulate microbial reduction of U(VI) to insoluble U(IV). The microbial community dynamics from one of the 4 frequently sampled monitoring wells (FW 102-3) was intensively analyzed with a functional gene array containing >24,000 probes and covering 10,000 genes in >150 gene categories. The microarray data indicated that during the uranium reduction period, both FeRB and SRB populations reached their highest levels at Day 212, followed by a gradual decrease over 500 days. The uranium concentrations in the groundwater were significantly correlated with the total abundance of c-type cytochrome genes (r=0.73, p<0.05) from Geobacter-type FeRB and Desulfovibrio-type SRB, and with the total abundance of *dsrAB* (dissimilatory sulfite reductase) genes (r=0.88, p<0.05). The Mantel test of microarray data and chemical data also indicated that there was significant correlation between the differences of uranium concentrations and those of total c-cytochrome gene abundance (r=0.75, p <0.001) or *dsrAB* gene abundance (r=0.72, p<0.01). The changes of more than dozen of individual c-type cytochrome genes from *Geobacter sulfurreducens* and *Desulfovibrio desulfuricans* showed significant correlations to the changes of uranium concentrations among different time points. Also the changes of more than 10 *dsrAB*-containing populations, including both cultured (e.g. *Desulfovibrio desulfuricans*, *Desulfovibrio termitidis*, *Desulfotomaculum kuznetsovii*, and *Thermosedulfovibrio yellowstonii*) and non-cultured SRB were significantly related to the changes in uranium concentrations, indicating their importance in uranium reduction. Interestingly, as expected, the changes of several *dsrAB*–containing sulfate-reducing populations previously recovered from this site (e.g., FW003269B, FW300181B) showed significant correlations to the differences of uranium concentrations.

BIOREMEDIATION SYSTEM



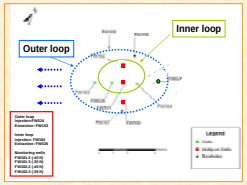


Figure 1. Test site of Uranium (IV) Bioremediation. The test site is located at DOE Oak Ridge Field Research Center, Oak Ridge, TN. The subsurface of the site is contaminated with uranium up to 800 mg/kg and nitrate (>200 mM) at pH 3.6. The bioremediation test is performed with a nested well system (upper left, and right). The treatment system is composed of outerloop and innerloop. Treatment performance and microbial populations were monitored by sampling from wells (left).

METHODS

- **Sampling and DNA extraction.** Groundwater samples (2L) 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 functional gene array (FGAII, table 1.) was used to monitor the dynamic of the microbial communities in the bioremediation system during the process.
- **DNA amplification, labeling, and hybridization.** 100ng of DNA of 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 1of 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.

Table 1 List of major functional markers on the FGA II

Gene category	Example of key enzyme (gene)	Total probe#
Nitrogen cycling		**5310**
Nitrogen fixation	Nitrogenase (*nifH*)	1225
Denitrification	Nitrate reductase (*narG*, *napA*, *nasA*), nitrite reductase (*nirS*, *nirK*), nitric oxide reductase (*norB*), nitrous oxide reductase (*nosZ*)	2306
Nitrification	Ammonium monooxygenase (*amoA*), hydroxylamine oxidoreductase (*hao*)	347
Nitrogen mineralization	Urease (*ureC*), glutamate dehydrogenase (*gdh*)	1432
Carbon cycling		**4599**
Carbon fixation	Rubisco (*cbbL*, *rbcL*), AcI (*acIB*), CODH, FTHFS	1018
Cellulose degradation	Cellulase, endoglucanase	1285
Lignin degradation	Laccase, mannanase	513
Chitin degradation	Endochitinase (*chiA*), exochitinase	744
Methane production	Methyl coenzyme M reductase (*mcrA*)	437
Methane oxidation	Methane monooxygenase (*pmoA*)	336
Others	Lignin peroxidase (*lip*), pectinase, cellobiase	266
Sulfate reduction	Sulfite reductase (*dsrAB*), APS (*apsA*)	**1615**
Phosphorus utilization	Exopolyphosphatase (*ppx*), phytase	**145**
Metal reduction and resistance		**4546**
Arsenic resistance	Arsenate reductase (*arsC*, *arsB*, *arsC*)	877
Cadmium resistance	Cadmium transporter (*cadA*, *cadB*, *cadC*)	282
Chromium resistance	Chromium/chromate transporter (*chrA*)	319
Mercury resistance/reduction	Mercuric ion reductase/transporter (*mer*, *merA*, *merB*)	548
Nickel resistance	Nickel transporter (*nccA*), permease (*nreB*)	140
Zinc resistance	Zinc resistance protein (*znt*A)	128
Other metal resistance/reduction	cobalt resistance proteins, selenium reductase, etc.	2252
Contaminant degradation		**8028**
Benzene, toluene, ethylbenzene, and xylene (BTEX) & related aromatics	Benzene 1,2-dioxygenase (*ben*), ethylbenzene dehydrogenase (*ebd*), benzylsuccinate synthase (*bss*), xylene monooxygenase (*xyl*), benzoyl-CoA reductase (*bad*), and catechol 1,2-dioxygenase (*cat*, *tfd*).	4176
Chlorinated aromatics	Chlorophenol reductive dehalogenase (*cpr*)	90
Nitroaromatics	Nitrobenzene nitroreductase (*nbz*), 4-nitrobenzaldehyde dehydrogenase (*ntn*), p-nitrobenzoate reductase (*pnb*)	152
Polycyclic aromatic hydrocarbons (PAHs)	Naphthalene dioxygenase (*nah*), PAH ring-hydroxylating dioxygenase (*pdo*)	741
Polychlorinated biphenyls (PCBs)	Biphenyl dioxygenase (*bph*)	388
Chlorinated solvents (e.g. PCE)	PCE/TCE reductive dehalogenase (*rdh*, *pceA*, *tecA*)	232
Other organic compounds/by-products	Alkane hydroxylase (*alk*), homogentisate 1,2-dioxygenase (*hmg*), vanillate O-demethylase oxygenase (*van*), etc.	2249
Total		***24243***

RESULTS: The population changed during the proces

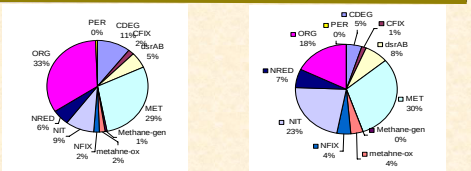


Figure 2. **Community shifts in well 102-2.** Community shift was observed from day 163 (left) to day 184 during (right) early bioremediation phase in sampling well FW102-2. For example, the proportion of organic degradation (ORG) genes decreased while the proportion of metal resistant (MET) including cytochrome c genes, nitrate/nitrite reducing (NRED) genes increased.

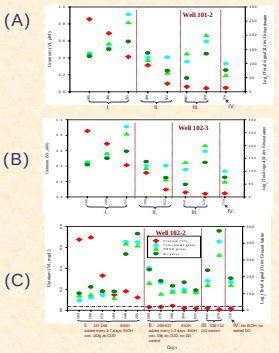


Figure 3. **Scatter plots showing the population changes.** These scatter plots show the population changes of metal, nitrate/nitrite, and sulfate reducing bacteria during the uranium (VI) bioremediation process in three sampling wells, FW102-2 (A), FW102-3 (B), and FW101-2 (C). All populations of these bacteria were increasing at the early stage and reached a peak around day 255, then dropped due to decrease of ethanol injection rate, DO infiltration (DO was not controlled), and the depletion of other nutrients. The populations re-bounced from day 642 to 712, when DO infiltration was controlled, and dropped again when the ethanol injection stopped.

RESULTS: Cluster Analysis

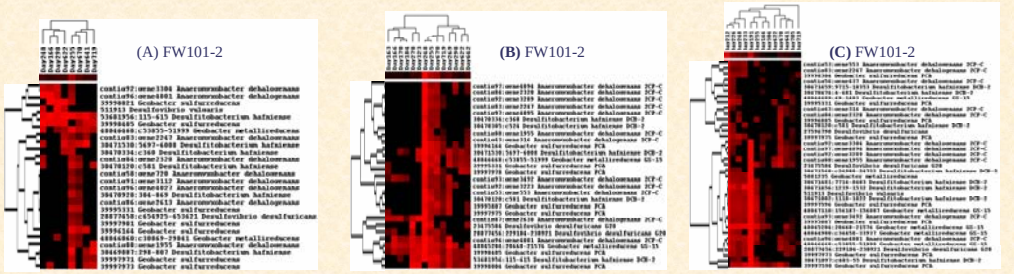


Figure 4. Clusters of Cytochrome genes. These clusters show population changes of the metal reducing bacteria with cytochrome C genes in sample wells FW101-2 (A), FW102-2 (B), and FW102-3 (C). The cytochrome genes shown in these clusters were significantly correlated to the reduction of uranium (VI) process in the wells, including those from *Anaeromyxobacter dehalogenans*, *Geobacter sulfurreducens*, *Geobacter metallireducens*, *Desulfovibrio vulgaris*, *Desulfitobacterium hafniense*. Based on the cytochrome genes detected and correlated to uranium (VI) concentration in the system, the samples were grouped basically by time; however the samples from middle stages were more similar, and the samples from early and late stages shared some commons.

RESULTS: CCA and DCA analysis.

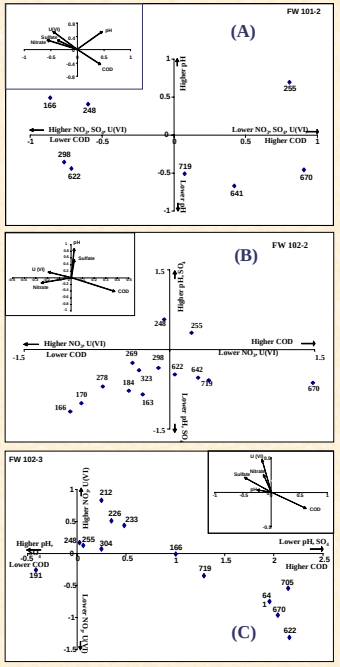


Figure 5. Canonical Correspondence Analysis (CCA) of array hybridization data and geochemical data (A, B, C).

(A) FW101-2.. Days 166, 248, 298, and 622 were all associated with increasing nitrate and sulfate, Days 166 and 248 were also associated with increasing U(VI), Days 255 was associated with pH and COD, and Days 641, 670, and 719 were strongly associated with COD.

(B) FW102-2. The early time periods (with the exception of 248 and 255) were distributed along a gradient of nitrate and U(VI), Days 248 and 255 were more correlated to sulfate and pH (primarily pH); the later time periods were distributed along a gradient of COD; and there is a temporal gradient that is likely being strongly influenced by the decreasing nitrate and U(VI) and increasing COD.

(C) FW 102-3. The gradient of communities is moving away from the geochemistry and toward the COD; Day 191 is highly correlated to pH and nitrate; Day 166, a bit of an outlier, is highly correlated to axis 1 and is being pulled by very high nitrate, but low U(VI) and pH; and all the other communities are along a changing gradient beginning with high geochemistry and low COD, to the later time points where geochemistry is much lower and COD much higher.

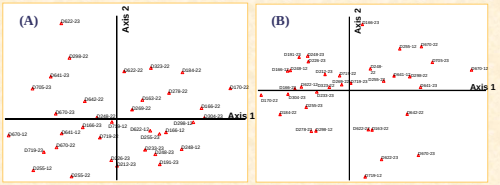


Figure 6. Detrended Correspondence Analysis (DCA) for cytochrome C genes (A) and sulfate reducing genes (B) across the sampling wells. In the figure the number after the dash, 23 stand for FW102-3, 22 for FW102-2, and 12 for FW101-2. The DCA analysis of the microarray data was able to reduce the data to two components that explained a large amount of the variation in the hybridization signals. Though no distinct clustering was found, the samples are distinctly divided by time on Axis 1 and site on Axis 2.

CONCLUSIONS

- The microbial communities in this system changed from their original composition. The total populations in all three sampling wells were increasing during the early stage (day 163-255) and reached the peak around day 255, then dropped due to decrease of ethanol injection rate, and the presence of DO (DO was not controlled), and the depletion of other nutrients. The populations rebounced from day 642 to 712, when DO infiltration was controlled, and dropped again when the ethanol injection stopped.
- Basically the populations changed proportionally, however, shift of organic degradation, metal resistant, and nitrate/nitrite reducing populations was observed from day 163 to day 184.
- Results of CCA analyses indicate the strong correlations between the chemistry parameters and the microbial community changes.
- DCA analyses show that while the populations changed with the time, in some extent, the communities in different wells remained different.
- Form the results of Mantel tests, *Anaeromyxobacter dehalogenans*, *Geobacter sulfurreducens*, *Geobacter metallireducens*, *Desulfovibrio vulgaris*, *Desulfitobacterium hafniense* were main

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