Changes in Microbial Community Structure During Biostimulation for Uranium Reduction at Different Levels of Resolution

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Former radionuclide waste ponds at the ERSP-Field Research Center in Oak Ridge, TN pose several challenges for uranium bioremediation. The site is marked by acidic conditions, high concentrations of nitrate, chlorinated solvents, and heavy metals. A series of re-circulating wells serve to create a subsurface bioreactor to stimulate microbial growth for in situ U(VI) immobilization. Well FW-104 is the injection well for the electron donor (i.e., ethanol); well FW-026 is the extraction well for the recirculation loop; well FW-101 and FW-102 are the inner zones of biostimulation; and FW-024 and FW-103 are upstream and downstream wells, respectively, which are the outer protective zones. Microbial community composition and structure of groundwater from the wells were analyzed via clonal libraries of partial SSU rRNA gene sequences, a phylogenetic chip array (Bacteria and Archaea), and a functional gene chip array over time. LIBSHUFF analysis for the clonal libraries of the re-circulating wells showed that over each phase of manipulation for uranium immobilzation, the bacterial communities of the inner zones of biostimulation were more similar to each other than those of the outer protective zones. The outer protective zones were more similar to the injection well. LIBSHUFF analyses for the clonal libraries from FW-104 (injection), FW-101 and FW-102 (biostimulation) showed that bacterial communities of the three wells were initially similar but developed changes through time. FW-101 and FW-102 bacterial communities developed changes in parallel, while those of FW-104 showed gradual changes. Diversity indices showed that bacterial diversity tended to increase during the initial phase of uranium bioreduction and decreased toward the end of uranium bioreduction (i.e., low U(VI) levels). As uranium levels declined, increasing *Desulfovibrio*- and *Geobacter*-like sequences were detected from the clonal libraries; moreover, *Desulfovibrio*-like sequences predominated over time. The results were further confirmed via RT-PCR, and RT-PCR results correlated with OTU and PhyloChip distributions for *Desulfovibrio*. PhyloChip analyses also demonstrated the presence and dynamics of both acetoclastic and hydrogenotrophic methanogens. The results indicated that the microbial community composition and structure changed upon stimulating for uranium bioreduction conditions, and that sequences representative of the sulfate-reducers *Desulfovibrio* spp. and metal-reducers *Geobacter* spp. were detected in wells that displayed a decline in U(VI). In addition, when electron donor was added to the subsurface, community diversity increased with a subsequent decline in U(VI) levels. However, when levels of potential electron acceptors decreased, community diversity also decreased. 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 27,000 probes covering 10,000 genes and >100 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 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 *dsr*AB (dissimilatory sulfite reductase) genes ($r=0.88$, $p<0.05$). 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 *dsr*AB gene abundance ($r=0.72$, $p<0.01$). The changes of more than a dozen 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 *dsr*AB-containing populations, including both cultured (e.g. *Desulfovibrio* spp., *Desulfotomaculum*, and *Thermosedulfovibrio*) and non-cultured SRB were significantly related to the changes in uranium concentrations. These results suggested the importance of these functions for *in situ* uranium reduction. Interestingly, the changes of several *dsr*AB sequences previously recovered from this site (e.g., FW003269B, FW300181B) showed significant correlations to the changes in uranium levels.