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Results

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

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ABSTRACT

A plug-cored system was established for bioremediation of subsurface U(VI) reduction by injection of ethanol at the U.S. DOE’s Fernald Research Center (FRC) in Oak Ridge, TN. After a U(VI) reduction was achieved, the stability of the bioreduced area was examined by means of ethanol injections (retesting) and reoxidation of the area by introducing dissolved oxygen (DO) for two months and then ethanol injections were resumed. Tapping 2.0 is a comprehensive 15 μm 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 bioaugmented microbial community structure and function. The bioreduced U(VI) area was stable during the resting period. After DO was introduced into the subsurface, monitoring wells (FW101-2) located closest to the injection well saw a greater increase in DO (2 mg L−1) than the well located further away (FW102-3: 0.45 mg L−1). Based on canonical correspondence analysis and Mantel test results, ethanol showed the greatest correlation to community structure, although subtle differences correlated with changes in the functional community. Detrended correspondence analysis showed a shift towards a different community structure after ethanol injections resumed compared to the period 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, testing time, or ethanol addition, while dissimilarity sulfite reduction (dsr) genes grouped only by testing time or ethanol addition. However, the relative abundance of dsr genes increased with the relative abundance of cytochrome c genes, while changes in the functional/community structure were identical in both wells, despite differences in temporal controls.

BACKGROUND

5-3 Waste Ponds. The four defined 5-3 waste collection ponds were constructed in 1955 (left). Effluent wells, consisting primarily of metal actinides, nitrate, nitrite, and radionuclides (U, Th), were discharged into the ponds until 1983. Biological activity was reduced by treatment and acidified to pH 3–4.5. The ponds were further neutralized and decontaminated 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 characterized by high concentrations of uranium and other contaminants.

METHODS

Groundwater recirculation system. The Stanford-Oak Ridge project, located adjacent to the 5-3 ponds, was started to establish contamination stability and bioremediation of contaminated groundwater. The system consists of two injection and two extraction wells and several monitoring wells in a test area. An above-ground treatment system was used to produce a mixture of ethanol and oxygen at a known ratio and deliver this mixture to injection wells. Oxygen was released by a bioremediation plant when oxygen was required in the groundwater. The microbial community within the vicinity of the area was monitored using GeoChip 2.0.

mpl file cannot be rendered

RESULTS

Results of array data from the two monitoring wells. Samples taken during the control (no ethanol addition) and reoxidation periods (injection of sulfuric acid) 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.

Cluster analysis (left) and relative abundances (right) of individual genes from wells FW102-3 and FW101-2. For well FW102-3, most of the genes clustered on ethanol injection, so error of 8/992 (ethanol). Samples from well FW102-2 showed a similar level of increase in both wells on day 901 and 360. Differences in the relative abundance of cytochrome c genes was not affected by any of the experimental periods, but did increase with and without ethanol addition. Differences in 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 decrease in the relative abundance of metal resistance 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, sulfur, U, and temperature were the most significant environmental variables, with U, COD, and temperature being the most important.

SUMMARY

Uranium 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 and FW102-3. DO did not affect the relative abundance of genes, but did affect the relative abundance of metal resistance genes.

REFERENCES

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 GenePix software (Axon Instruments Inc.).

Akkaya and others. 2000. Measurements of dissolved oxygen (DO) and Eh in a subsurface environment using a commercially available Eh probe.

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