ENIGMA: Microbial Community Dynamics in Groundwater and Surrogate Sediments During HRC® Biostimulation of Cr(VI)-Reduction

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Project Goals: The elucidation of bacterial community dynamics for both groundwater and sediment-associated communities over time and space during biostimulation for chromate reduction in relation to geochemical variables.

The Hanford 100-H site is a chromium-contaminated site that has been designated by the Department of Energy Environmental Management as a field study site for *in situ* chromium reduction. In August 2004, the first injection of hydrogen release compound (HRC®) resulted in an increase of microorganisms and a reduction of soluble chromium(VI) to insoluble chromium(III). Little is understood about the microbial community composition and dynamics during stimulation. The aim of this study is to compare microbial communities of groundwater and soil samples across time and space during a second injection of HRC®. A second injection occurred November 2008 and geochemical data collected throughout the study showed an overall decrease in nitrate, sulfate, and chromium(VI). Spatial and temporal water and soil samples (n=34) were collected pre-and post-injection from four wells at the field site. Soil columns constructed from stainless steel mesh were lined with nylon mesh and filled with Hanford soils from the 100-H site. The soil columns were used to represent not only the microbes flowing through the soil via groundwater, but the microbes that require a matrix in order to grow. DNA was extracted from each of the samples and SSU rDNA gene fragments was sequenced via multiplex pyrosequencing. Sequences were refined by length, primer errors, and Ns, and sequences with a high percentage of low Phred quality score values were removed. Python scripts were developed to filter the pyrotag data with respect to quality scores, and the filtering technique was validated with environmental samples. Soil samples differed from the corresponding groundwater (even at the phyla level) and were more diverse ($p=0.001$). While many of the populations were observed in both groundwater and surrogate sediments, the respective matrices appeared to enrich for particular OTUs. Predominant populations for the sediments were *Psuedomonas*, *Acidovorax*, *Clostridium*, *Aquaspirillum*, *Methylibium*, *Anaeromyxobacter* while predominant populations for groundwater were *Psuedomonas*, *Pleomorphomonas*, *Ramlibacter*, *Arthrobacter*, and *Herbaspirillum*. Genera observed only in the sediment included *Marinomonas* while genera observed only in the groundwater included *Desulfonauticus*, *Desulfomicrobium*, and *Syntrophobacter*. Results do not indicate a large shift in dominant organisms in soil from pre- to post- injection, and this may be due to the organisms remaining dominant from the first stimulation. However, a prevalence of core genera and rare genera were observed across 34 samples while urban and rural genera were less abundant. There was a shift from *Acidovorax* to *Aquaspirillum* from upstream (non-stimulated) to downstream

soil both pre- and post-injection. Surrogate soil samples indicated similar changes in the soil community in the injection (Well 45) and downstream (Well 41) wells across time, while water samples seem to indicate more of a pre- and post-injection grouping instead of gradual changes across time. Furthermore, while post-injection soil samples indicate a continuing dominance of *Aquaspirillum*, corresponding water samples indicate *Pseudomonas* as a dominant genus. For each well, HRC® injection resulted in increased diversity, but the greatest changes during stimulation occurred in the populations of mid-dominance either between wells or across time. These organisms could be important to consider as possible indicator species in future work.

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