ENIGMA Environmental: Succession of Hanford Groundwater Microbial Communities During Lactate Amendment and Electron-acceptor Limitation

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Project Goals

Bioremediation strategies involving *in-situ* microbial stimulation for Cr(VI) - reduction/immobilization are ongoing, but determining their relative success is complex. By using controlled laboratory conditions, the influence of individual variables on the successful community structure, dynamics and the metal-reducing potential can be studied. The goal of the current work was to determine the impact of lactate stimulation during sulfate limitation on the succession of a native microbial community. Triplicate anaerobic, continuous-flow reactors were inoculated with Cr(VI) contaminated groundwater from the Hanford, Washington 100-H area and incubated for 95 days to obtain stable, enriched communities. The microbial community structure shifted with a significant loss of diversity. Final communities were dominated by *Pelosinus* spp. and to a lesser degree, *Acetobacterium* spp. with small levels of other organisms including methanogens. The resultant diversity decreased from 63 genera within 12 phyla to 11 bacterial genera (from 3 phyla) and 2 archaeal genera (from 1 phylum). Isolation efforts attained four new strains of *Pelosinus* spp. Three of the 4 *Pelosinus* strains were capable of Cr(VI)-reduction and one also reduced U(VI). Under the tested conditions of limited sulfate, it appears that the sulfate-reducers, including *Desulfovibrio* spp., were outcompeted even though they are capable of fermentative growth. These results suggest that lesser-known organisms, such as *Pelosinus* spp., may play a more important role in metal-reduction than currently suspected.

Currently, a similar bioreactor experiment is underway and builds from the above findings. Hanford groundwater was enriched in duplicate bioreactors with lactate and; 1) no Cr(VI) to emulate the earlier work and establish a baseline, 2) 0.1 mg/L Cr(VI) to reflect the low plume concentrations nearing the Columbia river and 3) 3.0 mg/L Cr(VI) to reflect the source metal concentration. Multiple levels of analysis are now underway after a 105 day experiment. These include temporal measurements of; 16S rRNA gene pyrosequencing, gas and organic acid quantification by GC and HPLC, Geochip, Phylochip, metagenomics, metatranscriptomics, metaproteomics, soluble Fe(III), HFO, Cr(VI) and U(VI) reduction assays, metal uptake characteristics for 36 metals, small metabolite quantification, and fluorescent antibody cell counts for species of *Desulfovibrio*, *Geobacter*, *Methanococcus*, and *Pelosinus*. These results will help to determine if and how the added complexity of Cr(IV) will influence the microbial

community structure and metabolism as well as revealing the any acclimation of the community to Cr and quantifying the relative reduction potential.

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