Title
Chromium Bio-Immobilization at the Hanford 100H site: Comprehensive Molecular Analysis of Microbial Population Dynamics

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Background
The focus of these studies is to further understand the coupled hydraulic, geochemical, and microbial conditions necessary to maximize Cr(VI) bioreduction and minimize Cr(III) reoxidation in groundwater. Here we present data regarding a comprehensive analysis of microbial populations during a field-scale treatability study. We have combined a suite of molecular tools including 16S rDNA microarrays, clone libraries, quantitative PCR, stable isotope and PLFA analyses.

Methods
At the Hanford 100H field site, two new wells were drilled and equipped: injection Well 699-96-45 and a monitoring and pumping Well 699-96-44. Samples were taken at intervals pre- and post-injection of a 13C labeled slow release polylactate compound (HRC) used to stimulate indigenous microbial populations of low initial densities (<10^4 cells ml^-1). In addition to geochemical analyses, microscopic cell counts and stable isotope analyses, DNA and PLFAs were also extracted from filtered samples.

Results
Cell counts, DNA and PLFA data showed stimulation of microbial populations over several orders of magnitude with densities reaching a maximum of 2x10^7 cells ml^-1 13-17 days after the injection. This corresponded with the onset of reducing conditions, and removal of Cr(VI) from solution. Analysis of 13C enrichment in PLFAs was used to identify broad groups of organisms involved in metabolism of the polylactate substrate. 16S rDNA microarray analysis and clone libraries showed enrichment of specific bacterial and archael populations including Pseudomonas, Dechloromonas, Geobacter, Desulfovibrio with archaeal populations being dominated by Crenarchaeotes.

Quantitative real time PCR was used to confirm array indicated population changes.

Conclusions
We have developed and applied a suite of advanced molecular tools for comprehensive monitoring of microbial population dynamics during a field-scale remediation study. Connecting this data with geochemical and geophysical analyses will lead to a better understanding of processes controlling metal stability in the subsurface.