N-118: Phylogenetic and Functional Gene Microarray Analysis Demonstrates Direct and Indirect Mechanisms for Sustained Chromium Bio-Immobilization

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BACKGROUND

During a field-scale chromium treatability study at the Hanford 100H site in Washington, a single dose of a slow release electron donor (HRC) was applied to a contaminated aquifer to stimulate microbial reductive precipitation of hexavalent chromium Cr(VI). Here we present analysis of microarray-based prokaryotic population dynamics and correlations with geochemical observations following this application over one year.

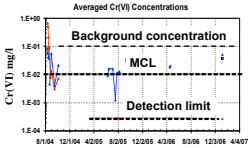
METHODS

A high-density 16S rRNA phylogenetic microarray (PhyloChip; 1,2) and a functional gene array (GeoChip; 3) were used to analyze groundwater samples from multiple depths in injection and monitoring wells taken at intervals pre- and post-HRC injection. Following filtration, genomic DNA was extracted and PCR amplicons or MDA-amplified community-DNA were analyzed by microarray hybridization. A range of geochemical and geophysical parameters were also monitored.

Schematic of PhyloChip process

RESULTS

Chromium immobilization



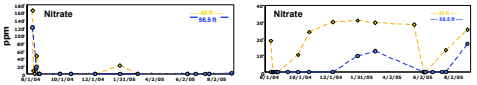
- Following HRC injection reducing conditions had rapidly established with a corresponding decline in DO, Eh and nitrate.
- Cr(VI) concentrations declined steadily over 6 weeks and remained below up-gradient concentrations.

RESULTS

Injection well

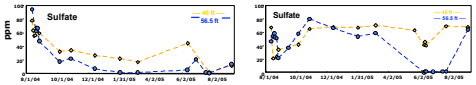
Monitoring well

Nitrate reduction



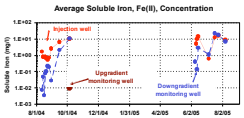
- Nitrate concentrations declined in the injection well at upper and low depths
- Upper depths of monitoring well showed fluctuation nitrate concentrations with upper depths showing consistently higher concentrations

Sulfate reduction



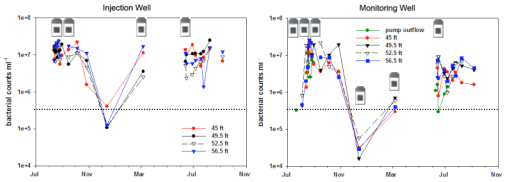
- Sulfate concentrations also declined in the injection well with most reduction occurring at lower depths
- Like nitrate concentrations, sulfate concentration was variable in the monitoring well with upper depths typically showing higher concentrations

Iron reduction



- Soluble Fe(II) concentrations increased over time in both injection and monitoring wells
- Elevated Fe(II) concentrations one year later suggests continued Fe(III) reduction

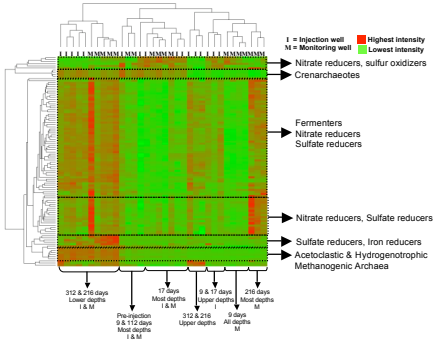
Direct microscopic cell counts



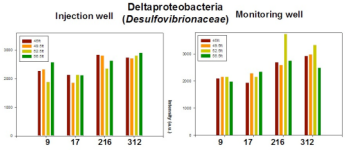
- Bacterial biomass enriched rapidly by 2 orders of magnitude – remained elevated over one year later
- Possible recharge events were noted in December when bacterial counts returned briefly to background numbers
- GeneChip symbol denotes time points where array analysis was performed

RESULTS

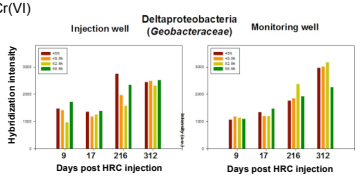
PhyloChip prokaryotic community analysis



- PhyloChip data demonstrates depth stratified microbial communities
- Temporal shifts in composition correspond to changing geochemistry.



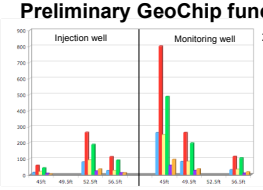
- *Desulfovibrio* and other sulfate reducing bacteria remain enriched at least one year following HRC injection
- Hydrogen sulfide produced by sulfate reduction can abiotically reduce Cr(VI)



- *Geobacteraceae* iron reducing bacteria remain enriched at least one year following HRC injection
- Fe(II) produced by iron reduction can abiotically reduce Cr(VI)

RESULTS

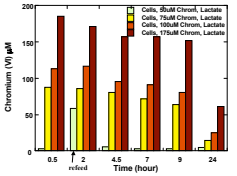
Preliminary GeoChip functional analysis



21 months after HRC injection

- GeoChip detects putative enrichment in chromium tolerance/reduction genes associated with *Pseudomonas**
- Nitrate reduction, sulfate reduction, methanogenesis genes also detected in monitoring well.

* GeoChip analysis is preliminary and requires further validation



- Tolerance and ability to reduce Cr(VI) was tested with in a *Pseudomonas* isolate.
- Cr(VI) reduction occurred at all concentrations tested and no chromium toxicity effects were observed even at concentrations of 175 mM.

See poster Q200 for full details this and other Cr(VI) tolerant isolates

CONCLUSIONS

The combination of phylogenetic and functional gene arrays represents a complementary high-throughput approach to elucidating mechanisms responsible for contaminant immobilization in the subsurface.
A sustained enrichment of iron and sulfate reducing bacteria was observed over 2 years, suggesting indirect chromium immobilization through interaction with reactive iron or sulfide by-products. Nitrate reducers such as *Pseudomonas* also remained elevated over the two years and FGA array data demonstrated a sustained enrichment of *Pseudomonas* chromate reductase genes suggesting direct reduction of chromate may also be significant in chromium immobilization. Based on this data, organisms representing each of these functional groups have been isolated and characterized (see poster Q200).

ACKNOWLEDGMENTS

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