N-240: 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, stable isotope (^{13}C) 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}). For more information regarding site characteristics and HRC properties see poster N-230 (Hazen et al.). In addition to geochemical analyses, microscopic cell counts and stable isotope analyses, DNA and PLFAs were also extracted from filtered samples.

16S rRNA clone libraries were sequenced at JGI and assembled contigs checked for sequence quality (Phred Q20[1]) and chimeras using Bellerophon[2] before phylogenetic analysis by tree construction in ARB[3]. Rarefaction analysis was carried out using DOTUR[4].

High Density 16S Microarray analysis was performed using a custom made Affymetrix GeneChip[5]. The intensity (average difference) of OTUs with more than 90% positive probes were compared at 3 time points pre- and post HRC addition. Hierarchical clustering was performed to observe large scale relationships and principal components analysis of the most variable OTUs (top 10% standard deviation) was performed to identify dynamic organisms.

Results

Chromate analysis

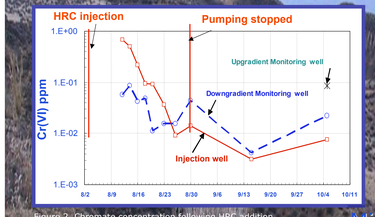


Figure 2. Chromate concentration following HRC addition

- Compared to up-gradient concentrations, chromate was successfully removed from groundwater
- Chromate remained below initial concentrations for over 2 months following a single injection of HRC®
- See poster N-230 for more geochemical/geophysical data

Microbial counts

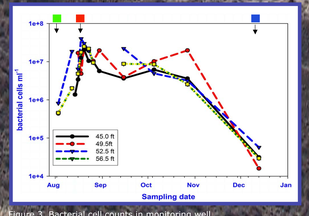


Figure 3. Bacterial cell counts in monitoring well

- Bacterial biomass was rapidly stimulated following HRC® injection
- Maximum biomass was observed 17 days following HRC® addition
- Bacterial biomass began to decline approx. 3 months after HRC injection

Clone library analysis

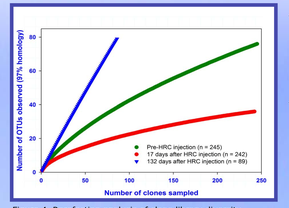


Figure 4. Rarefaction analysis of clone library diversity

- Rarefaction analysis of clone libraries demonstrated that while diversity decreased following HRC injection, 4 months later diversity was greater than the pre-injection groundwater
- None of the clone library rarefaction curves have reached an asymptote indicating insufficient sampling of these communities

- Pre-HRC groundwater samples dominated by *Comamonadaceae, Pseudomonadaceae, Moraxellaceae* and also numerous *Oxalobacteraceae, and Sphingomonadaceae.*
- Following HRC injection *Flavobacteriaceae* increase sharply with *Pseudomonadaceae* and *Comamonadaceae* remaining constant
- Diversity increases 4 months after HRC injection with decreased abundance of *Pseudomonadaceae, Comamonadaceae and Flavobacteriaceae,* while *Sphingomonadales, Rhodobacterales, Caulobacterales, Chloroflexi* and *Legionellales* all increase in relative abundance

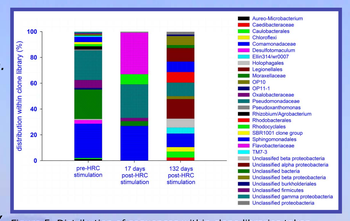


Figure 5. Distribution of sequences within clone libraries taken during chromate bioremediation

High Density DNA Microarray analysis

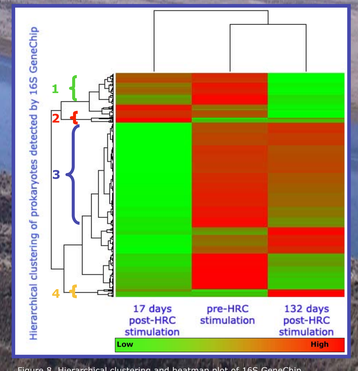


Figure 8. Hierarchical clustering and heatmap plot of 16S GeneChip analysis of microbial community sub-families detected during chromate bioremediation. PCA groups are indicated by brackets.

Figure 7. Workflow schematic for 16S GeneChip analysis

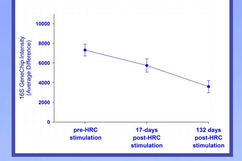
- Novel High Density Array contains 500,000 probes for 16S rRNA genes
- Each probe has corresponding mismatch probe (probe pairs)
- 9,000 sequence types (OTUs) are interrogated by a probe set containing at least 11 probe pairs
- 16S GeneChip has been validated with air, water and soil samples

- Clustering of High Density Array data shows pre-HRC community clustering with later 132 day post-HRC community indicating convergence
- A significant loss in diversity is suggested by large decreases in sub-family relative abundance following HRC injection
- Most dynamic sub-families indicated by PCA groups 1-4

Principal Components Analysis Microarrays

PCA Group 1

These bacteria decline following HRC addition and continue to decline over time

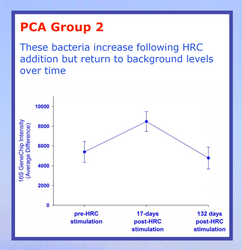


Phylum	Class	Order	Family
OP3	Unclassified	Unclassified	Unclassified
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae x 5
Proteobacteria	Betaproteobacteria	Burkholderiales	Burkholderiaceae
Proteobacteria	Gammaproteobacteria	Acidithiobacillales	Acidithiobacillaceae
Proteobacteria	Gammaproteobacteria	Thiotrichales	Thiotrichaceae
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Moraxellaceae
Proteobacteria	Gammaproteobacteria	Pseudomonadales	Pseudomonadaceae x 2

- Mostly gamma and beta-proteobacteria
- Sulfur oxidizers decline
- Some iron oxidizers decline *Comamonadaceae* (*Leptothrix*)
- Some nitrate reducers decline

PCA Group 2

These bacteria increase following HRC addition but return to background levels over time

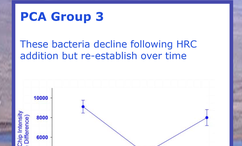


Phylum	Class	Order	Family
Bacteroidetes	Bacteroidetes	Bacteroidales	Unclassified
Bacteroidetes	Flavobacteria	Flavobacteriales	Unclassified
Bacteroidetes	Flavobacteria	Flavobacteriales	Flavobacteriaceae x 2
Cyanobacteria	Cyanobacteria	Chloroplasts	Chloroflaceae
Proteobacteria	Betaproteobacteria	Rhodocyclales	Rhodocyclaceae
Proteobacteria	Betaproteobacteria	Burkholderiales	Comamonadaceae x 2

- Bacteroidetes increase in response to HRC
- *Flavobacteriaceae* do not typically oxidize lactate or acetate, but may utilize glycerol – an unlabeled component of the HRC compound
- Different genera within *Comamonadaceae* (*Acidovorax*)

PCA Group 3

These bacteria decline following HRC addition but re-establish over time

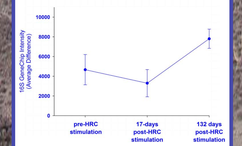


Phylum	Class	Order	Family
Chloroflexi	Unclassified	Unclassified	Unclassified
Chloroflexi	Anaerolineae	Chloroflexi-1a	Unclassified
Chloroflexi	Anaerolineae	Unclassified	Unclassified
OP3	Unclassified	Unclassified	Unclassified
Proteobacteria	Alphaproteobacteria	Azospirillales	Magnetospirillaceae
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylocystaceae
Proteobacteria	Alphaproteobacteria	Rhizobiales	Methylobacteriaceae
Proteobacteria	Alphaproteobacteria	Unclassified	Unclassified
Proteobacteria	Alphaproteobacteria	Sphingomonadales	Sphingomonadaceae x 2
Proteobacteria	Unclassified	Unclassified	Unclassified
Actinobacteria	Actinobacteria	Actinomycetales	Microbacteriaceae
Firmicutes	Clostridia	Clostridiales	Peptostreptococcaceae
Firmicutes	Bacilli	Lactobacillales	Lactobacillaceae

- Many oligotrophic bacteria decline with HRC injection

PCA Group 4

These bacteria show a slight decline following HRC but increase above background levels with time



Phylum	Class	Order	Family
Bacteroidetes	Sphingobacteria	Sphingobacteriales	Flexibacteraceae
Chlamydiae	Chlamydiae	Chlamydiales	Chlamydiaceae
Proteobacteria	Gammaproteobacteria	Legionellales	Legionellaceae
Proteobacteria	Unclassified	Unclassified	Unclassified

- Increase in *Legionellaceae, Chlamydiaceae* and *Flexibacteriaceae*

PLFA analysis

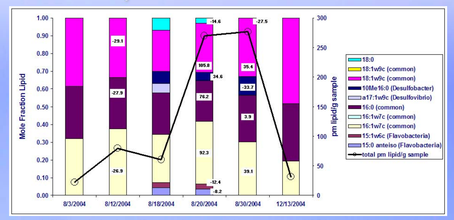


Figure 4. PLFA analysis of specific groundwater samples pre and post HRC injection. Numbers in boxes indicate $\delta^{13}C$ ratio (per mil) detected in specific PLFA peaks.

- Carbon appears to take approx. 17 days to reach monitoring well 5 m from injection well and be incorporated into biomass.
- General bacterial biomarkers indicate rapid enrichment in ^{13}C to a ratio greater than the spiked HRC ratio (15 per mil).
- Biomarkers for *Flavobacteriaceae* increased following injection but showed minimal enrichment .

Discussion

Chromate reduction was successful using slow release polylactate HRC and addition of a universally 13C labeled polylactate spike allowed tracking of carbon progress and consumption. Following HRC injection most baseline microbes (many oligotrophs) declined. The ^{13}C label in HRC was rapidly metabolized and incorporated into most bacterial biomarkers resulting in a ^{13}C ratio that was substantially greater than that of the spiked HRC spike reflecting the properties of the polylactate spike which was not incorporated into the glycerol backbone of the HRC compound making it more readily soluble. An example of the complementarity of the approaches used in this study can be seen with *Flavobacteraceae*. This organism was identified by clone and array analysis of increasing following HRC injection however only array analysis detected this group of bacteria pre-HRC addition. Furthermore, PLFA data indicate that compared to most bacteria in the sample, *Flavobacteriaceae* did not assimilate significant quantities of ^{13}C despite their increase in number. *Flavobacteriaceae*) typically do not utilize lactate or acetate but are capable of utilizing glycerol which forms the backbone of the HRC compound and which was not labeled.

Conclusions

The combined approach of clone libraries (relative abundance) and High Density Arrays (high resolution prokaryotic dynamics) enabled accurate monitoring of population dynamics during this field trial. Array analysis has been shown to correctly detect most bacteria, including whole phyla that are missed by clone analysis. Coupled with ^{13}C PLFA, these methods were used successfully to follow carbon assimilation and induced community shifts during remediation.

References

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Acknowledgements

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