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Author
Chakraborty, Romy

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Microbial community changes during sustained Cr(VI) reduction at the 100H site in Hanford, WA

Romy Chakraborty[1], Eoin L Brodie[1], Boris Faybishenko[1], Yvette M Piceno[1], Lauren Tom[1], Swati Choudhuri[1], Harry R Beller[1], Jenny Liu,, Tamas Torok, Dominique C Joyner[1], Marcin P Joachimiak[1], Aifen Zhou[3], Joy D Van Nostrand[3], Joe Zhou[3], Phil E Long[2], Darrell R Newcomer[2], Gary L Andersen[1], Terry C. Hazen[1]

[1]Lawrence Berkeley National Laboratory, Berkeley, CA [2]Pacific Northwest National Laboratory, Richland, WA [3] University of Oklahoma, Norman

ENIGMA
Ecosystems and Networks Integrated with Genes and Molecular Assemblies

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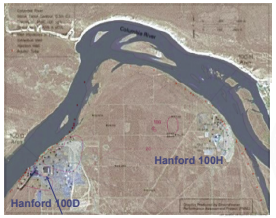
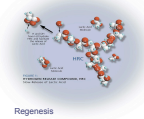
ABSTRACT

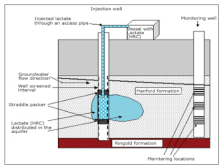
Hexavalent Chromium is a widespread contaminant found in soil, sediment, and groundwater. In order to stimulate microbially-mediated reduction of Cr(VI), a poly-lactate compound (HRC) was injected into the Chromium-contaminated aquifer at the Hanford (WA) 100H site in 2004. Cr(VI) concentrations rapidly declined to below the detection limit and remained so for more than three years after injection. Based on the results of the bacterial community composition using high-density DNA 16S rRNA gene microarrays, we observed the community to transition through denitrifying, iron-reducing and sulfate-reducing populations. As a result, we specifically focused isolation efforts on three bacterial species that were significant components of the community. Positive enrichments in defined anaerobic media resulted in the isolation of an iron-reducing *Geobacter metallireducens*-like isolate, a sulfate-reducing *Desulfovibrio vulgaris*-like strain and a nitrate-reducing *Pseudomonas stutzeri*-like isolate among several others. All of these isolates were capable of reducing Cr(VI) anoxically and have been submitted for genome sequencing to JGI. To further characterize the microbial, and geochemical mechanisms associated with *in situ* Cr(VI) reduction at the site, additional HRC was injected in 2008. The goal was to restimulate the indigenous microbial community and to regenerate the reducing conditions necessary for continued Cr(VI) bio-immobilization in the groundwater. Analysis of the microbial populations post-injection revealed that they recovered to a similar density as after the first injection in 2004. In this study, we present the results from our investigation into microbially-mediated Cr(VI) reduction at Hanford, and a comparison of the microbial community development following two HRC injections four years apart.

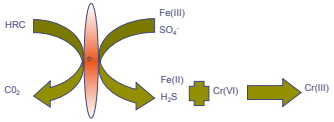


The DOE site at Hanford was established in 1943 to conduct research and development in nuclear energy technology.Chromium(VI) is a major contaminant at this site.



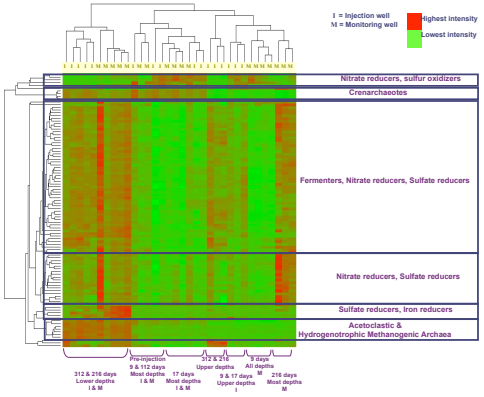
To stimulate bioremediation a poly-lactate compound HRC was injected into the chromium contaminated aquifers. It was expected that Cr(VI) would be immobilized by maintaining reduced conditions along with biological Cr(VI) reduction





Samples were monitored at regular intervals for microbial biomass and soluble Cr concentration. Water samples were also used to study geochemistry and isolate indigenous chromium reducers from the site.

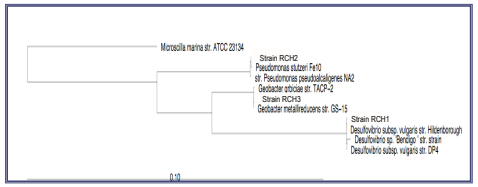
PHYLOGENETIC MICROARRAY-2004



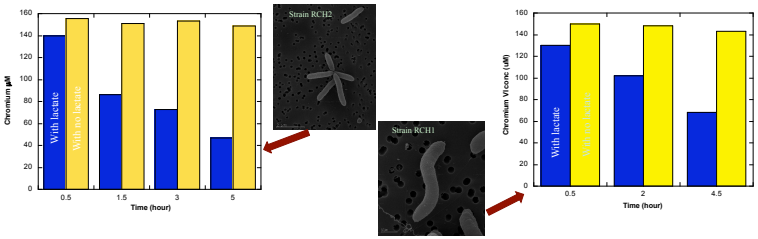
The biomass of significant organisms such as *Desulfovibrio spp.* and *Geobacter spp.* went up post-stimulation and continued to remain high.

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

ISOLATION, CHARACTERIZATION



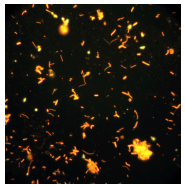
Based on the phylochip data, anaerobic enrichments were initiated to study the dominant species in pure culture. Three organisms were isolated, an Iron-reducer, a sulfate reducer and a nitrate reducer named strain RCH3, strain RCH1 and strain RCH2 respectively. Phylogenetic analyses based on partial 16S rDNA sequencing revealed strain RCH3 was 99% similar to *Geobacter metallireducens*, strain RCH1 was 99.9% similar to *Desulfovibrio vulgaris Hildenborough* and strain RCH2 was 99% similar to *Pseudomonas stutzeri*. Both strain RCH3 and strain RCH2 were able to utilize glycerol as carbon and energy source. Glycerol is a component of HRC. However strain RCH3 could not utilize lactate contrary to strains RCH1 and RCH2. Lactate is also a major component of HRC. All strains were capable of Fe(III) reduction and Cr(VI) reduction. Detailed experiments were set up to study direct biological enzymatic Cr(VI) reduction by the isolates. Strain RCH1 has been whole genome sequenced by JGI , while strains RCH2 and RCH3 are in the process of being completed.



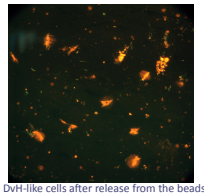
Cell suspension experiments were carried out with strains RCH1 and RCH2 to determine their ability of Chromium(VI) reduction. With both strains, Chromium(VI) concentrations decreased with time when 10mM Lactate was supplied as the sole electron donor. When lactate was left out, little or no reduction of Chromium took place, demonstrating biological reduction of Cr(VI).

IMMUNO-MAGNETIC SEPARATION

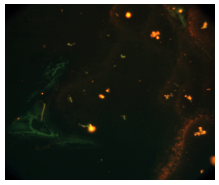
Immunomagnetic separation (IMS) has been shown highly efficient for recovering microorganisms from heterogeneous samples. We are developing a field-deployable version of IMS that enables detection of target microorganisms (in this case Desulfovibrio spp) from environmental water samples which will be then processed for transcriptomics and metabolomic studies. Anti-*Desulfovibrio vulgaris* antibodies were raised in rabbit, collected, and purified. Antibodies were then labeled with the unique biotin ligand. After antigen-antibody reaction, *D. vulgaris* cells were targeted and captured. After immunocapture using beads and subsequent release from beads, the sample was enriched only in curved, vibrio like cells of DvH indicating a successful application of the IMS technique.



Hanford 100H water sample concentrated by centrifuging down to 1ml
Approx cell count: 1.8X 10^7/ml



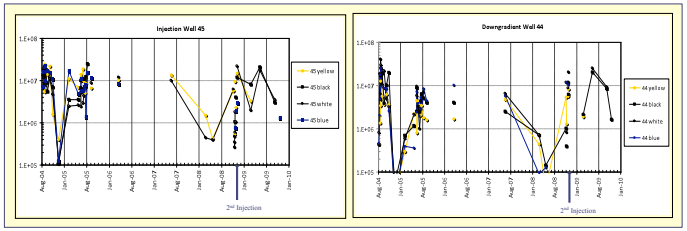
DvH-like cells after release from the beads.
Approx cell count: 10^5cells/ml



Non DvH-like cells post IMS
Approx cell count:1.8X 10^6/ml

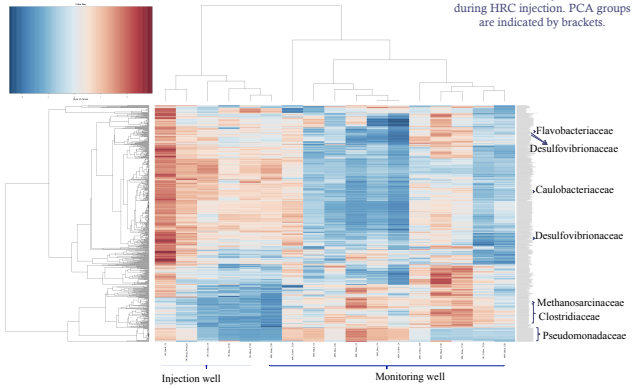
Efficiency of cell isolation and recovery (*i.e.,* release of the microbial cells from the beads following separation) was followed by microscopic imaging and acridine orange direct counts (AODC). AODC analysis of the samples post-separation of DvH cells show absence of DvH cells, and the presence of other cell morphologies.
Preliminary Geochip analyses of DvH cells post IMS from field samples showed down regulation of several energy metabolism genes which is consistent with geochemical and phylochip data from the site.

PHYLOGENETIC MICROARRAY-2008



Cell numbers were monitored by AODC. The biomass increased in the lower part of the Hanford formation 5-6 days after the HRC injection to the same level as that in 2004.

Hierarchical clustering and heatmap plot of 16S GeneChip analysis of microbial community OTUs detected during HRC injection. PCA groups are indicated by brackets.



CONCLUSIONS

- Phylochip data suggests that the increased chromium immobilization coincided with the increase of the *Desulfovibrio, Geobacter, Pseudomonas* and *Dechloromonas* strains following HRC injection in 2004.
- Enrichments set up with water samples led to the isolation of a *Geobacter* species, a *Pseudomonas* species and a *Desulfovibrio* species from the site.
- All the isolates grew best at 0.5% salinity in media and at circumneutral pH, and were all able to reduce metals like Iron(III) and Chromium(VI), demonstrating Cr(VI) immobilization at the Hanford 100H site could be mediated by direct microbial metabolism apart from indirect chemical reduction of Cr(VI) by end products of microbial activity.
- Targeting specific microbes (sulfate reducing bacteria) from the water samples was successful using the IMS protocol. Geochip analyses for specific functional genes from targeted DvH-like cells was successfully analyzed.
- The microbial community in the monitoring well differs from that of the injection well post the second HRC injection. The microbial community post the second injection is not enriched with sulfate reducing bacteria, methanogens or Iron reducing bacteria contrary to observations during the first inhection in 2004.

ACKNOWLEDGEMENT

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