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

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**ABSTRACT**

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. 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.

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).

Immunomagnetic separation (IMS) has been shown highly efficient for recovering microorganisms from heterogenous 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 then be processed for transcriptomics and metabolomics 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, DvH vulgaris cells were targeted and captured. After immunocapture using beads and subsequent release from beads, the sample was enriched only in cured, vibrio like cells of DvH indicating a successful application of the IMS technique.

**CONCLUSIONS**

- Phyllospheric data suggests that the increased chromium immobilization coincided with the increase of the Desulfovibrio, Geobacter, Pseudomonas and Dichelosoma 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 and in media 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 injection in 2004.

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