Title
Assessment of Bioreduction of Cr(VI) Using PLFA Analysis

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Q 177/442 Assessment of Bioreduction of Cr(VI) Using PLFA analysis

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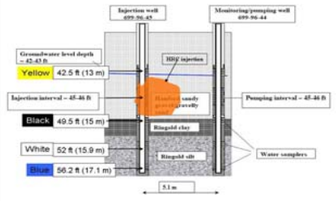
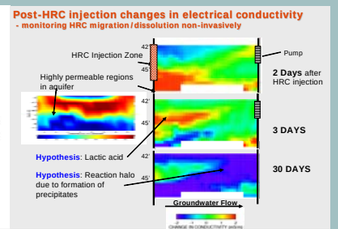
Abstract

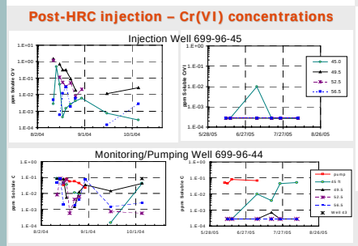
Cr(VI) is a widespread groundwater contaminant. To stimulate bioreduction of Cr(VI) in the groundwater at the Hanford 100H field site, 18 kg of HRC® was injected into the aquifer. HRC® consists of polylactate esterified to a glycerol backbone that slowly releases lactic acid providing a source of carbon. 10 g of 13C-labeled polylactate was added to the HRC® to give it a d13C value of ~40‰ (versus an unlabeled value of -15‰). Phospholipid fatty acid (PLFA) extracts of biomass in the samples were analyzed to assess the shift in microbial community structure in the extracted groundwater collected from 4 depth intervals in the injection well and in an extraction well located 5 m down-gradient from the injection well. Standard geochemical parameters (dissolved O_2, Eh, anion chemistry, etc.) and the d13C values of dissolved inorganic carbon (DIC), dissolved organic carbon, cell counts, and 16s rDNA analyses are also being monitored. The d13C values of the primary PLFA peaks (common to many organisms) showed a 13C-enrichment, but reached values (>200‰) much higher the bulk d13C of the labeled HRC®, reflecting the faster dissolution rate for the 13C-labeled polylactate relative to the HRC®. After the d13C of the PLFA peaked, it quickly returned to background values (-15‰) indicating rapid turnover of biomass in the system. Several PLFA peaks specific to organisms known to metabolize glycerol (e.g., Flavobacteria) increased significantly but did not show the same increase in d13C. Biomarkers PLFA associated with Desulfobacter, 10Me16:0, were identified and demonstrated 13C-enrichment. These data are being used to quantify the changes in biological activity resulting from HRC® injection.

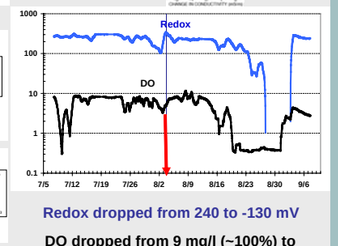
Field Site and Monitoring Data

Poly-lactate HRC with 13C labeled lactate included was injection into well 45. Well 44 was the pumping, or extraction well. The HRC was injected on 8/03/2004 and breakthrough was detected in the pumping well 15 days later, 8/18/2004. Pumping stopped after 2 weeks. Sampling of all depths of injection and extraction well was done periodically weekly.





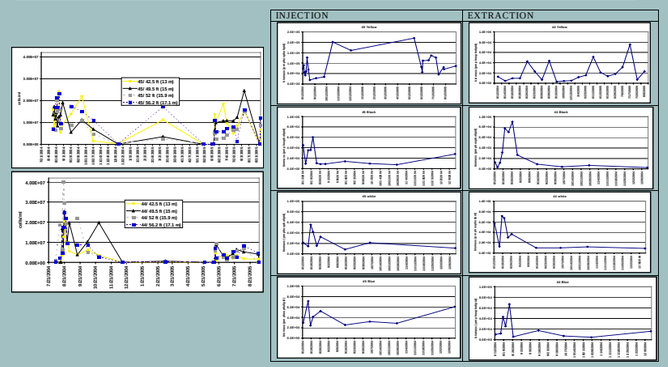




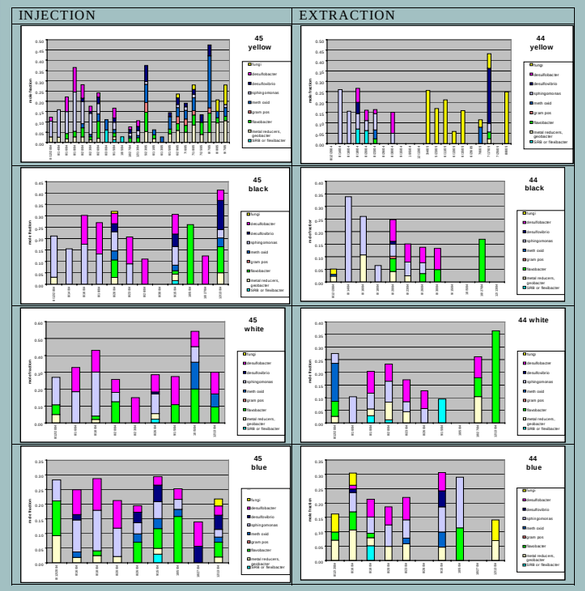
Biomass Estimates

Biomass was measured by total picomole (pm) lipid recovered from the samples. The concentration of lipid ranged from 1 x 10^4 to 2.5 x 10^5 pm per sample. Also shown below are the cell counts for the same samples which have similar trends to the PLFA data, with the exception of the large biomass increase in the 45 yellow samples midway through the sampling cycle.

Samples at 8/3/2004 represent background concentrations of microbes. The injection of the HRC stimulated the microbial community for a period of several days while pumping was active, but the values returned to near background levels with exception to the topmost sampling depth. Restimulation of microbial growth was observed during the 2nd pumping test started in early June 2005.

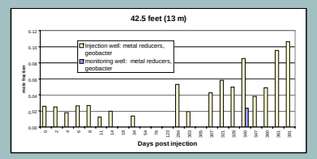


Microbial community analysis: Signature Lipid Biomarkers

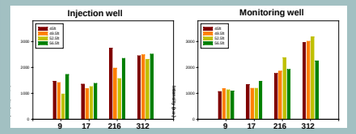


These plots identify the signature biomarkers for the samples, the common lipids have been omitted. The scale is the mole fraction of each individual lipid in the total sample. The biomass was low in all samples which made quantification of the samples difficult. Samples dates with no data were below the detection limit. Biomarkers for metal and sulfur reducing bacteria were found at all sample depths but not all sample times. The injection of HRC appears to have stimulated the growth of these microorganisms immediately after injection and during pumping. The sampling ports for the shallowest depth in both the injection and extraction well show data until 9/2005 while the other depths have data only until the end of 2004, with the remaining data pending analysis. The extraction well, 44, predominately had fungal biomarkers after March 2005.

PLFA (15:1w8c – metal reducers)

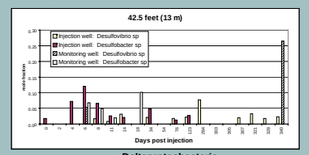


Deltaproteobacteria (*Geobacteraceae*)

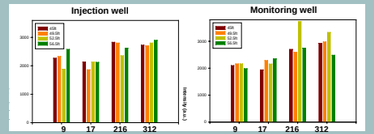


Biomarkers for Geobacter were consistently found in the PLFA analysis of samples from the 42.5 ft depth from the injection well. They were only found at 340 days in the monitoring well. Lack of detection of these organisms most likely due to insufficient sample volume. Phylochip analysis of the samples found these types of organisms present and increasing in both wells post HRC injection. Continuous presence of iron reducer may help maintain chromium reducing conditions by producing Fe(II)

PLFA (i17:1w7c – desulfovibrio; 10me 16:0 – desulfobacter)

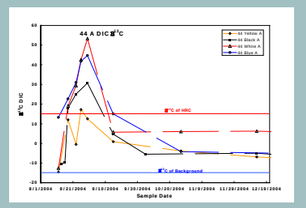


Deltaproteobacteria (*Desulfovibrionaceae*)

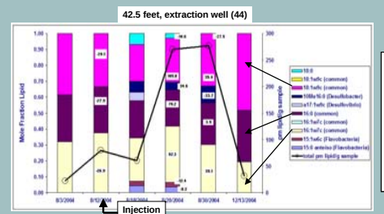


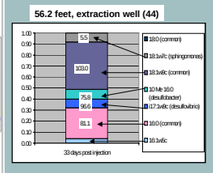
Biomarkers for sulfate reducers were found in the PLFA analysis of samples from the 42.5 ft depth from the injection well. As found for Geobacter, the detection was inconsistent due to low sample biomass. Phylochip analysis of the samples found desulfovibrio-type organisms present and increasing in both wells post HRC injection. The PLFA analysis identified desulfobacter lipids which were not found in the phylochip analysis. Presence of sulfate reducers will aid in Cr reduction by maintaining lower redox conditions.

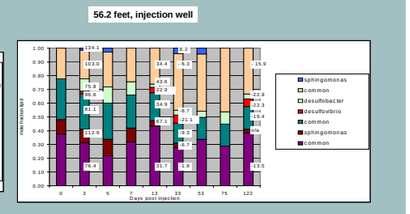
^{13}C Phospholipid Analysis

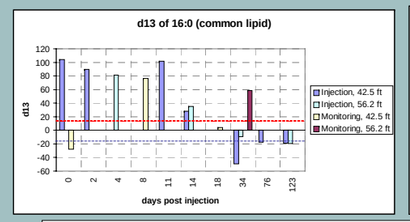


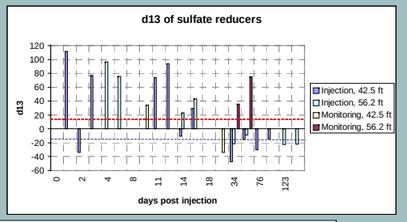
Injection of ^{13}C labeled lactate combined with PLFA analysis can potentially provide information on which organisms are actively consuming the labeled material. The figure below shows the PLFA from the 42.5 ft depth of the monitoring well. The maximum shift occurs about the same time as the measured DIC shift. General bacterial biomarkers indicate rapid enrichment in ^{13}C. The ^{13}C ratio is greater than expected (overall spiked HRC ratio was 15 per mil). ^{13}C polylactate used as spike was not esterified to glycerol backbone and was released and consumed more rapidly. Biomarkers for *Flavobacteriaceae* increased following injection but showed minimal enrichment with ^{13}C. *Flavobacteria* do NOT typically utilize lactate, but may use glycerol (backbone, unlabeled). Similar analysis of the extraction well at the lowest sampled depth showed similar patterns at 33 days that the upper depth showed at 7 days post injection, suggesting that the labeled lactate had migrated downward.











Biomarkers for Sulfate reducers were found in most of the samples. Isotope analysis of these lipids as well as 16:0, a lipid common to most bacteria, show enrichment of ^{13}C quickly at the uppermost sampling point. At later times, the shift observed in the in the lower wells was more positive than the upper wells because of the downward drift of the unbound labeled lactate. The red dashed line at d13 of 15 represents the shift of the injected lactate.

Summary

Monitoring data indicates that the HRC injected into the subsurface at Hanford 100 H stimulated the growth of anaerobic bacteria and caused reducing conditions conducive to metal reduction in the subsurface. Several species of metal reducing bacteria, including Geobacter and Desulfovibrio, were shown to be present in the microbial community. PLFA analysis indicates that the sulfate reducers were active and quickly assimilated the injected HRC. After two weeks when pumping stopped, the available labeled HRC was consumed at the upmost wells and the isotope signal was lost.

Subsequent pumping tests have demonstrated re-stimulation of growth as quantified by total cell counts and 13dC analysis. PLFA analysis and isotope shits in the recovered lipids is still pending on these samples.

Acknowledgments

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http://www-esd.lbl.gov/ERT/hanford100h/index.html