

Background

The Department of Energy site at Hanford, WA, has been historically impacted by U and O from the nuclear weapons industry. In an attempt to stimulate microbial remediation of these metals, in-situ lactate enrichment experiments are ongoing. In order to bridge the gap from the laboratory to the field, we inoculated triplicate anaerobic, continuous-flow glass reactors with groundwater collected from well Hanford 100-H in order to obtain a stable, enriched community while selecting for metal-reducing bacteria. Each reactor was fed from a single carboy containing defined media with 30 mM lactate at a rate of 0.223 ml/min under continuous nitrogen flow at 3 ml/min. Cell counts, organic acids, gDNA (for qPCR and pyrosequencing) and gases were sampled during the experiment. Cell counts remained low (less than 1x10⁷ cells/ml) during the first two weeks of the experiment, but by day 20, they reached a density greater than 1x10⁸ cells/ml. Metabolic analysis showed a decrease in the lactate concentrations over time. Pyruvate concentrations ranged from 20-40 uM the first week of the experiment then was undetectable after day 10. Likewise, formate appeared in the reactors during the first week with concentrations of 1.48-1.55 mM at day 7 then the concentrations decreased to 0.69-0.95 on day 10 and were undetectable on day 15. Acetate was present in low amounts on day 3 (0.15-0.33 mM) and steadily increased to 3.35-5.22 mM over time. Similarly, carbon dioxide was present in low concentrations early on and increased to 0.26-0.35 mM as the experiment progressed. No silos were able to detect low amounts of methane (0.20-0.80) during the first week of the experiment, but by day 10 the methane was undetectable. From these results and pyrosequencing analysis, we conclude that a shift in the microbial community dynamics occurred over time to eventually form a stable and enriched microbial community. Comprehensive investigations such as these allow for the examination of not only which nutrient source will accelerate site remediation, but also provide insight to evaluate remediation strategies through which enriched community members are important for bioremediation.

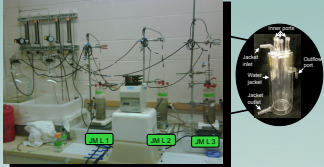


Fig. 1. The continuous flow culture instrumentation operates multiple reactors. The setup provides continuous culture under strict anaerobic conditions and is compatible with regular sampling of the consortium without contamination and exposure to oxygen.

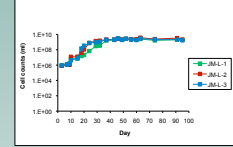


Fig. 2. Cell count data for microbial consortium in triplicate anaerobic continuous flow reactors inoculated with groundwater from well Hanford H-100.

Metabolites

Table 1: Metabolite Levels Below Detection in each Bioreactor

Metabolite	Detection Limit
Hydrogen	< 25 nM
Pyruvate	< 1.0 uM
Formate	< 1.0 uM
Succinate	< 0.5 uM
Fumarate	< 0.5 uM

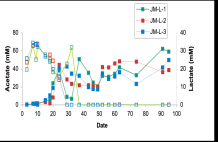


Fig. 3. Acetate and lactate concentrations from the triplicate anaerobic continuous flow reactors inoculated with groundwater from Hanford well H-100.

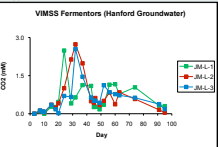


Fig. 4. Acetate and lactate concentrations from the triplicate anaerobic continuous flow reactors inoculated with groundwater from Hanford well H-100.

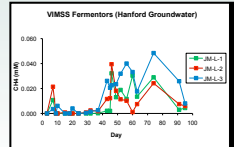


Fig. 5. Acetate and lactate concentrations from the triplicate anaerobic continuous flow reactors inoculated with groundwater from Hanford well H-100.

Bacterial Community Composition

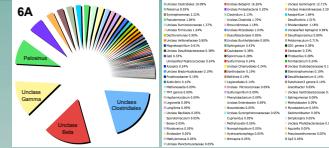
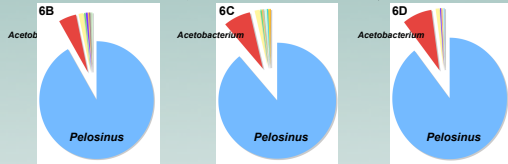


Fig. 6A. Bacterial community composition from pyrosequencing analysis of initial groundwater sample taken from Hanford well H-100.

Fig. 6B-C. Bacterial community composition from pyrosequencing analysis of groundwater sample from Hanford well H-100 after 95 days in triplicate lactate-enriched continuous flow reactors.



Archaeal Community Composition

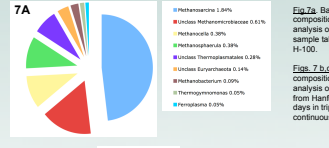


Fig. 7A. Archaeal community composition from pyrosequencing analysis of initial groundwater sample taken from Hanford well H-100.

Fig. 7B-D. Archaeal community composition from pyrosequencing analysis of groundwater sample from Hanford well H-100 after 95 days in triplicate lactate-enriched continuous flow reactors.

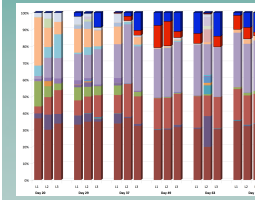


Fig. 8. Total microbial community composition from pyrosequencing analysis of selected dates from a triplicate continuous flow reactor experiment of lactate-enriched Hanford well H-100 groundwater sample.

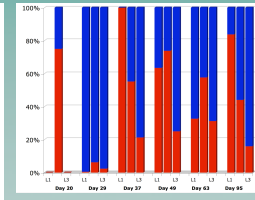


Fig. 9. Archaeal community composition from pyrosequencing analysis of selected dates from a triplicate continuous flow reactor experiment of lactate-enriched Hanford well H-100 groundwater sample.

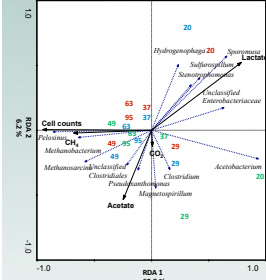


Fig. 10. Triplot of redundancy analysis (RDA) of the relative abundances of microbial genera determined by pyrosequencing analysis on selected dates from triplicate continuous flow reactor experiment of lactate-enriched Hanford well H-100 groundwater sample. Dashed arrows (blue) indicate genera associated with the variation in microbial community composition. Solid (black) arrows indicate metabolite data significantly associated with the variation.

Summary

- Inoculated triplicate lactate-enriched anaerobic continuous-flow reactors with groundwater from Hanford well H-100
- Quantified cell counts and metabolites throughout experiment
- Analyzed microbial community composition through pyrosequencing analysis
- Achieved a stable and metabolically defined microbial community (including two methanogen species)
- Currently working with isolates from reactors (SRB, IRB and methanogens) to determine metal reduction capacity

ACKNOWLEDGEMENTS

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