Characterization of a Sulfate- and U(VI)-Reducing Enrichment from Area 3 of the Oak Ridge Field Research Center

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Objectives

› Develop a sulfate-reducing enrichment from the location of the Oak Ridge FRC Area 3 field experiment

› Assess the capacity of the enrichment community for U(VI) reduction

› Characterize the metabolic activity of the enrichment community

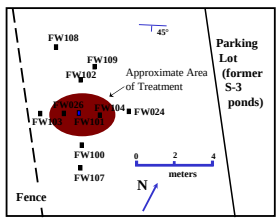
› Kinetically model microbial growth and U(VI) reduction by the enrichment

› Investigate the enrichment's community structure

Background

Figure 1. The former S-3 ponds at Oak Ridge. Millions of liters per year of radioactively contaminated waste were disposed in this series of unlined ponds over several decades. The ponds were drained and capped in 1983, but radioactive and acidic contamination has leached into the subsurface.

Figure 2. Location of the near-source zone biostimulation experiment and associated wells. The field site is near the former S-3 ponds in Area 3 of the Oak Ridge Field Research Center (FRC). The treatment strategy involves the subsurface amendment of ethanol to stimulate anaerobic conditions and microbial U(VI) reduction for bioremediation.



Enrichment Development

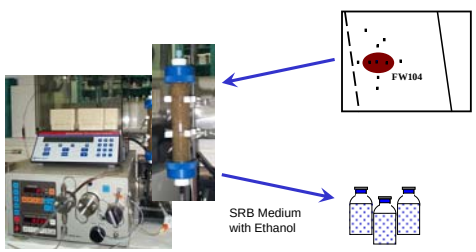


Figure 3. Development of the enrichment. A core from the inner injection well of the field site (FW-104) was used in a column experiment to demonstrate the stimulation of microbial U(VI) reduction by the amendment of ethanol. Sediment from the column experiment was used to inoculate enrichments in sulfate-reducing media. The enrichment was maintained anaerobically under a headspace of 80:20 N_2:CO_2 in a medium of ~6 mM sulfate and ~10 mM ethanol.

Characterization of Metabolism

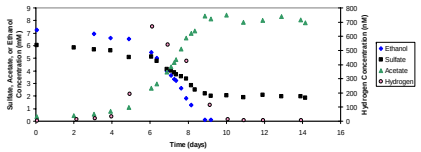


Figure 4. Metabolism of enrichment in an example culture. Serum bottles of media were inoculated with the enrichment culture (7%) on Day 0. Bottles contained ethanol, sulfate and viable bacteria. Ethanol was incompletely oxidized to acetate concurrently with the reduction of sulfate, and hydrogen accumulated intermediately.

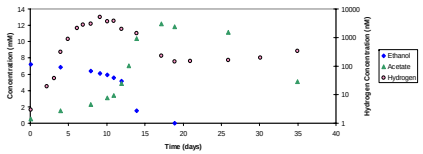


Figure 5. Metabolism of enrichment without sulfate in an example culture. Serum bottles of media were inoculated with the enrichment culture (7%) on Day 0. Bottles contained ethanol and viable bacteria but no sulfate. Hydrogen accumulated to a higher concentration than in bottles with sulfate, and was not completely utilized. Ethanol was incompletely oxidized to acetate and some acetate was subsequently consumed.

Kinetics of Microbial Growth

Yield was calculated for two viable enrichments assuming ethanol served as the electron donor and was incompletely oxidized to acetate. The results are shown in Table 1.

Table 1. Yield to protein ratios and yield calculation.

Enrichment	Protein/VSS	Yield (g VSS/g BOD)
Viable 1	0.32	0.112
Viable 2	0.26	0.153
Average	0.29	0.133

Ethanol utilization curves for 6 viable enrichments were fit using a least-squares analysis to the Monod model:

$$-\frac{dC}{dt} = \frac{q_{max} X_a C}{K + C}$$

C = ethanol concentration (mM)
t = time (days)
q_{max} = maximum specific rate of ethanol utilization (mmol ethanol/mg VSS/day)
X_a = concentration of active biomass (mg VSS/L)
K = half-saturation coefficient for ethanol (mM)
Y = yield (mg VSS/mmol ethanol)

$$q_{max} = 0.088 \pm 0.017 \frac{\text{mmol}}{\text{mg VSS - d}} = 2.82 \frac{\text{g BOD}}{\text{g VSS - d}} \quad (n = 6)$$

$$\mu_{max} = 0.37\ d^{-1}$$

$$t_{1/2} = 2.7 \text{ days}$$

Uranium Reduction

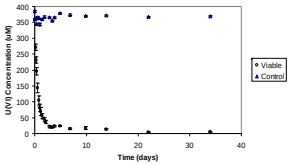


Figure 6. U(VI) reduction by the enrichment culture under nongrowth conditions. Cells were washed three times in bicarbonate buffer and resuspended in buffer. Ethanol and U(VI) were added, and U(VI) was measured over time. Data points represent the averages of triplicates, and error bars represent one standard deviation.

Kinetics of Uranium Reduction

The reduction of U(VI) was modeled with a pseudo first-order equation. The second-order rate coefficient was converted into a pseudo first-order coefficient using the measured concentration of biomass in the experiment.

$$-\frac{dU}{dt} = kUX \qquad -\frac{dU}{dt} = k'U$$

U = U(VI) concentration (uM)
t = time (days)
k = second-order rate coefficient (L/mg VSS/hr)
k' = pseudo first-order rate coefficient (1/hr)
X = concentration of biomass (mg VSS/L)

k = second - order rate coefficient
$= 181 \pm 10 \frac{L}{\text{g VSS - d}}$

k' = pseudo first - order rate coefficient
$= 0.205 \pm 0.012\ hr^{-1}$

Assuming a cell concentration of ~10^6 cells/mL, a reasonable estimation for the field site, the half-life for U(VI) is approximately 4 days.

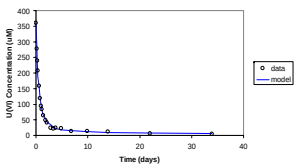


Figure 7. Model and data for U(VI) reduction by an example viable enrichment.

Acknowledgements

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› We would like to thank David Watson of the Oak Ridge Field Research Center for providing samples.

Community Analysis

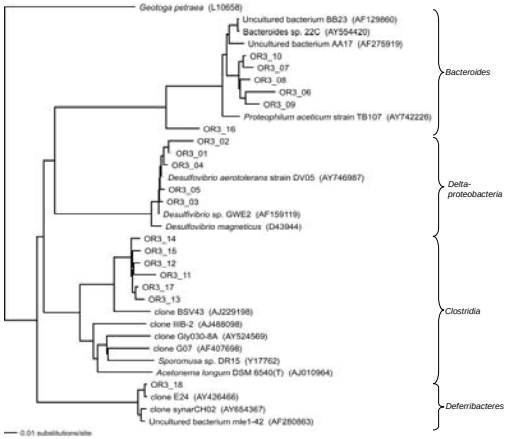

Figure 8. Distance neighbor-joining tree of 16S rRNA gene sequences of clones from the enrichment culture. Representative clones are labeled in the tree with the prefix OR3. The tree was constructed with 776 aligned base pairs. GenBank accession numbers of the three nearest neighbors of clone sequences, as determined by a BLAST search, are shown in parentheses. *Geotoga petraea* was used as an outgroup.

Table 2. Phylogenetic affiliation of clones from the 16S rRNA library.

Bacterial class	No. of 16S rRNA clones	% of total
Deltaproteobacteria	15	10
Bacteroides	22	14
Deferribacteres	1	1
Clostridia	115	75

Conclusions

› The enrichment incompletely degraded ethanol to acetate concurrently with the reduction of sulfate.

› The pseudo first-order rate coefficient for U(VI) reduction by the enrichment was 0.205 hr^{-1}.

› Kinetic parameters describing microbial growth were obtained and will be used to guide modeling efforts for the field experiment. The doubling time for growth was 2.7 days.

› Most clones from a 16S rRNA library of the enrichment were related to gram-positive fermenting organisms. Clones related to *Desulfovibrio* species were also detected. Species of the genera *Desulfovibrio* are known to reduce U(VI).