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

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Field-scale bioremediation projects use reactive transport models for design and interpretation, and these models require stoichiometric parameters and empirically verified kinetic expressions for microbial growth and U(VI) reduction. Uncertainty regarding the ecophysiology of U(VI)-reducing bacteria and the rates of their associated reactions hinder modeling. Accordingly, we characterized kinetics and composition of a U(VI)-reducing community derived from sediment from Area 3 of the Oak Ridge Field Research Center, the location of an ongoing field experiment. Here, we summarize findings for the metabolism of this enrichment, kinetic parameters for its growth and U(VI) reduction, and the results of a 16S rDNA clone library.

Sediment was inoculated into a defined sulfate-reducing medium with ethanol as an electron donor, under electron donor-limiting conditions. Enrichment cultures were maintained anaerobically in serum bottles at 30°C. Ethanol was converted to acetate and hydrogen concurrently with the reduction of sulfate. Hydrogen was subsequently consumed, but acetate persisted. The growth yield was 0.13 g VSS/g COD and the maximum specific rate of ethanol utilization was 2.8 g COD/g VSS·d, values within the range expected for sulfate-reducing bacteria. The half-saturation coefficient for ethanol was too small to be determined from substrate depletion curves. Little activity was observed in control bottles that lacked sulfate, indicating endogenous decay was not significant in the time frame of the experiment. The fermentation of ethanol to acetate and hydrogen was observed in control bottles that lacked sulfate, as were acetogenesis and methanogenesis. Late log-phase cells were harvested, washed, and resuspended in buffer, and their rate of U(VI) reduction was measured under nongrowth conditions. The second-order rate coefficient for U(VI) reduction was 181 L/g VSS·d, which is at the low end of values reported for pure cultures of U(VI)-reducing bacteria. Most clones from the 16S rDNA library (75%) were related to *Bacillus* spp., and a significant number were related to *Bacteroides* and *Desulfovibrio* spp. (14% and 10%, respectively). *Bacteroides* species are known to produce acetate during fermentation, and *Desulfovibrio* species oxidize one mole of ethanol to one mole of acetate during sulfate reduction, the same stoichiometry observed for the enrichment. Kinetic parameters and community composition will be compared with parameters and community composition results for the field. The observed detection of methane is of concern for field applications. Methanogens compete for electron donors with U(VI)-reducing populations, and methane gas can block flow paths, preventing the delivery of chemicals to contaminated regions.