Alternative Electron Donor Utilization in the Reductive Dechlorination Processes by Organisms in the Class *Dehalococcoidia*

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Background/Objectives. A superfund site located in south Louisiana (USA) contains a mixture of chlorinated alkanes and alkenes. In support of the remedial technology decision-making at the site, a combination of culture-based and DNA-based studies have been performed, which resulted in isolation and characterization of a novel reductively dechlorinating genus, *Dehalogenimonas*. Three species within the genus *Dehalogenimonas* have been formally characterized and taxonomically described, *D. lykanthroporepellens* (BL-DC-9^T), *D. alkenigignens* (IP3-3^T), and *D. formicexedens* (NSZ-14^T). Previous research identified hydrogen (H_2) as the sole electron donor known to support reductive dehalogenation within the class *Dehalococcoidia,* including *Dehalogenimonas* spp. and *Dehalococcoides mccartyri* strains. To understand currently employed biostimulation approaches and potentially identify alternative approaches, alternatives substrates were tested to assess their viability as electron donors to support reductive dehalogenation.

Approach/Activities. Isolated cultures of *Dehalogenimonas* spp. were spiked with known concentrations of chlorinated solvents of interest at the site, such as 1,2-dichloroethane (1,2-DCA) and 1,2-dichloropropane (1,2-DCP), and were amended with H_2 in the gas headspace (10%:10%:balance, H_2:CO_2:N_2) as a positive control or one of the following alternative substrates: citrate, formate, methyl ethyl ketone, propionate, pyruvate, starch, succinate, and yeast extract. Potential electron donor substrates were added at a concentration of 5 mM in an initial screen for positive and negative results assessed via gas chromatography (GC) analysis to measure parent compound degradation and daughter product formation. More detailed follow-up experiments were carried out to verify alternative electron donor utilization using quantitative real-time PCR (qPCR) to assess growth and ion chromatography (IC) to assess changes in electron donor concentration. Genome sequences generated from DNA extracts from isolate cultures were annotated and analyzed to assess for genetic markers associated utilization of alternative electron donors in organohalide respiration.

Results/Lessons Learned. Analysis of *Dehalogenimonas* type strain cultures (isolates) amended with 1,2-DCP and formate demonstrated reductive dehalogenation in the absence of H_2. 1,2-DCP concentrations (parent compound) were observed to reduce over time with a near stoichiometric increase of propene (daughter product). Formate concentration concurrently decreased during the dehalogenation of 1,2-DCP. Genus-specific primers were used in qPCR to demonstrate both that growth was not supported solely on formate in the absence of a chlorinated alkane as an electron acceptor and that growth was support during dehalogenation of 1,2-DCP with formate in the absence of hydrogen. Comparable growth yields were observed for *Dehalogenimonas* type strains using either formate or H_2 as an electron donor. Utilization of formate as an electron donor was further supported via genomic analyses and identification of putative selenocysteine-containing formate dehydrogenases, as well as selenocysteine-specific elongation factors and selenocysteine insertion sequence (SECIS) elements. Elucidation of formate-supporting reductive dechlorination provides further insight into the biostimulation processes occurring at the superfund site and may justify alternative biostimulation amendment strategies for chlorinated solvent contaminated sites.