Microbiome Composition Resulting from Different Substrates Influences Trichloroethene Dechlorination Performance

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Background/Objectives. Reductive dehalorespiration is a microbial energy-conserving metabolic process mediated by organohalide-respiring bacteria (OHRB) to eliminate halogens from organohalides with hydrogen acting as the electron donor and the organohalide as the electron acceptor under anaerobic conditions. This distinct microbial redox process is a cost-effective and environmentally friendly method to remediate chlorinated solvents such as trichloroethene (TCE) in groundwater.

With a genome of small size (approximately 1.2 to 1.4 Mb), *Dehalococcoides* is auxotrophic, and lacks the ability to synthesize several growth factors, such as cobamide, biotin, and thiamine. It has been reported that microbial consortia in the bioreactors were able to perform reductive dechlorination of TCE without exogenous vitamins or amino acids.

In this study, we evaluated the effects of substrate type on the function and structure of the resulting fermentation microbiome, without cobalamin supplementation, by conducting complete reductive dechlorination of TCE with three electron donors: acetate, a simple substrate; soybean oil, a complex substrate with slow H_2 release; and molasses, a complex substrate with rapid H_2 release.

Approach/Activities. Three anaerobic reactor systems (working volume, 5 L) were established in parallel in an ambient (28-30 °C) fume hood with acetate, emulsified soybean oil, and molasses (designated as the AS, EOS, and MS reactors, respectively) as the electron donors in a modified TCE-containing medium without cobalamin or amino acid supplements. Simultaneous substrate feeding with a 2 L substrate bottle and supernatant extraction into a 2 L effluent bottle were performed using a peristaltic pump in batch mode.

The reactors were seeded with 2 L of the TCE-degrading consortium that was obtained from the groundwater of the TCE-contaminated site. The site had received the emulsified-soybean-oil-based biostimulation without supplement of cobalamin or amino acids. Prior to this study, the inoculated sludge had been acclimated to emulsified soybean oil (40 mg-C/day) and TCE (0.5 mmole) under neutral conditions for 10 days. To ensure uniform start-up conditions, the acclimated sludge was thoroughly mixed before distribution to each reactor. The constant doses of substrates (40 mg-C/day) and various TCE loads (0.025-0.2 mmol/[L day]) were supplied to the reactors in the context of different hydraulic retention time (HRT) of 15.6 to 23.2 days. The electrons supplied to the reactors (6.67 to 18.7 mmol/day) were higher than those required for TCE dechlorination to ethene.

Results/Lessons Learned. The molasses-fed reactor exhibited superior performance and dechlorination of TCE loadings to ethene, and the oil-fed reactor exhibited a high growth rate of the key OHRB, *Dehalococcoides*. This finding suggests an effect of the substrate on reductive dechlorination and the growth of *Dehalococcoides*. The results of 16S rRNA gene amplicon sequencing demonstrated that the molasses reactor had the greatest phylogenetic diversity and microbial richness, followed by the oil and acetate reactors. Principal coordinate analysis revealed various trajectories of the microbiome dynamics within the three reactors, resulting in distinct community structures separated in the ordination space. The predicted functions of the

three reactor microbiomes were distinguished on the basis of vitamin and amino acid metabolisms as well as the fermentation pathways. In addition to the diversified hydrogen-producing pathways, the molasses-induced microbiome exhibited high potential to synthesize the cobalamin, which may account for its high *Dehalococcoides* activity and thus effective dechlorination performance. The substrate dependence of microbiomes may provide insight into strategies of exogenous amino acid supplementation to benefit *Dehalococcoides* growth. This study adds novel insight into the interplay of hydrogen-releasing substrates and OHRB. The results may contribute to the development of tailored and cost-effective management for the reductive dechlorination of chlorinated solvents in bioremediation.