Growth of Anaerobic Cultures under Diluted Conditions to Monitor Changes in Activity and Microbial Community Structure

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ABSTRACT

Chlorinated aliphatic hydrocarbons (CAHs) such as trichloroethene (TCE) were commonly used organic solvents, and their improper storage and disposal resulted in widespread groundwater contamination. Anaerobic bioremediation is a promising solution to this problem and is investigated at Oregon State University. Sequential dehalogenation of TCE into *cis*-dichloroethene (cDCE) and vinyl chloride (VC), and finally ethene is receiving special attention because ethene is a non-toxic end product of this process. *Dehalococcoides Mccartyi* is recognized for its ability to obtain energy for growth from this process, which is called halorespiration, and it is the only known organism with this ability. The Evanite (EV) culture that contains *D. Mccartyi* is grown in 2-Liter (EV-2L) and 5-Liter (EV-5L) reactors (chemostats) in the OSU Bioremediation laboratory, and is the focus of the following study. The objectives of this study were to 1) compare how two different cultures transform TCE in batch reactors containing diluted culture samples from the chemostat, 2) observe overall effect of dilution on microbial activity, 3) observe evidence of microbial growth, and 4) compare how different electron donors affect kinetics and growth. The end goal of this study is to construct a small-scale version of the reactors already under operation.

Experiments were performed with culture samples obtained under anaerobic conditions from the chemostats, and the samples were then diluted in ratios of 1:5, 1:10, and 1:100 with standard growth media. Over the course of months, gas samples were taken regularly from each bottle to monitor the mass of CAHs, ethene, and H_2. These data were utilized to determine when hydrogen (H_2) should be added and provide evidence that growth was occurring. The data was also used to estimate transformation rates using a rate model programmed in Excel.

Objective 1) was satisfied with CAH, ethene, and H_2 mass monitoring. The biggest risk when diluting these cultures was the possibility of "shocking" the bacteria with a new environment, which is made apparent in slow or zero dehalogenation. This did not occur, as both cultures survived even the highest dilution. Dilution did lower dehalogenation rates—theoretically, relative to the dilution ratio as a result of lower biomass. Objective 3) was also satisfied with mass monitoring. It was anticipated that the step from VC to ethene would be the slowest, but would provide the greatest opportunity to demonstrate growth. More TCE was added over the course of the experiments with the objective of observing changes in transformation rates. In many cases, ethene production rates increased after the second addition of TCE, indicating microbial growth.

Objective 4) was satisfied by constant H_2 mass monitoring, as the presence of H_2 was required as an electron donor for growth. H_2 was added and was viable until pressure built up and disrupted atmospheric conditions. Formate as a direct H_2 source and propionate as an H_2 source via fermentation were used in place of H_2 gas. While both sources worked, the extremely slow release of H_2 by propionate limited dehalogenation compared to formate.

Objective 2) is ongoing because microbial population is being monitored at Stanford University using new DNA-based molecular methods on samples taken from the large-scale dilutions. They will examine changes in population size and community structure over the course of the experiments.