Association between qPCR Analyses for Oxygenase Enzymes and Rate Constants for Cooxidation of TCE in Groundwater

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Background/Objectives. Our goal is to use qPCR analyses to evaluate the potential contribution of aerobic biological cooxidation to natural attenuation of TCE in groundwater. Bacteria that harbor and express oxygenase enzymes can fortuitously metabolize TCE under aerobic conditions. It should be possible to use a qPCR assay for genes that code for oxygenase enzymes to predict the rate of TCE cooxidation. The utility of the qPCR assays to predict biological cooxidation of TCE was evaluated by comparing the gene abundance and rate constants for TCE cooxidation in samples of groundwater to rate constants that might be expected based on the kinetics of TCE cooxidation in pure culture studies.

Approach/Activities. Water samples were collected from 19 wells at five locations, and the rate of aerobic cooxidation of TCE in the water samples was measured using a newly developed assay. In the assay, the bacteria in the groundwater were presented with ^{14}C labelled TCE. The degradation of the TCE was measured as the accumulation of radiolabel that was not volatile and could not be purged from a water sample. Assays were conducted for the following oxygenase enzymes using the following qPCR primers: phenol hydroxylase (PHE), ring hydroxylating toluene monooxygenase (RDEG), toluene dioxygenase (TOD), xylene monooxygenase (TOL) and soluble methane monooxygenase (sMMO). We summed the abundance of the gene copies, and then calculated a normalized rate constant for each groundwater sample by dividing the experimental rate constant by the abundance of gene copies in the water sample.

Published values for the Michaelis-Menten kinetic constants for TCE cooxidation by pure strains of bacteria that expressed toluene-2-monooxygenase, toluene-4-monooxygenase, toluene-2,3-dioxygenase and soluble methane monooxygenase were used to estimate rate constants that might be expected for TCE cooxidation in groundwater. A first-order rate constant (normalized to the crude protein in the culture) was calculated by dividing V_{max} by K_m. Then the normalized first-order rate constant was multiplied by an estimate of the quantity of protein per gene copy in the groundwater bacteria to calculate an expected rate constant normalized to the abundance of gene copies. Four the four enzymes, the normalized first-order rate constants only varied by a factor of two.

Results/Lessons Learned. There were eight wells where it was possible to extract a rate constant with the ^{14}C assay. The first-order rate constants for biological cooxidation varied from 2.65 per year to 0.0065 per year, corresponding to half-lives of 0.27 years to 107 years. The sum of gene copies amplified by the PHE, RDEG, TOD, TOL, and sMMO primers varied from 8E+04 to 1E+02 per mL. For seven of the wells, the experimentally determined rate constants were in reasonable agreement with the range of the expected rate constants. At one site, the experimental rate was an order of magnitude lower than the range of expected rate constants based on pure cultures. The qPCR assay can identify sites where natural cooxidation of TCE may be occurring, but the actual rate of cooxidation should be confirmed by the ^{14}C assay before natural cooxidation is used as part of a monitored natural attenuation remedy.