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TCE Co-oxidation Rates and Quantification of Oxygenase Gene Abundances and Expression

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Background/Objectives. Assessment of chlorinated hydrocarbon degradation is often limited to evaluating biological reductive dechlorination. However, abiotic and aerobic, cometabolic mechanisms may make significant contributions to contaminant degradation particularly at sites undergoing monitored natural attenuation (MNA). The overall goal of ESTCP Project ER-201584 is to develop a complete MNA decision framework by validating analytical techniques and molecular biological tools to quantify the impacts of abiotic and cometabolic processes on TCE attenuation. The presentation will focus on links between observed TCE degradation rates and the abundance and expression oxygenase genes as determined by qPCR and RT-qPCR.

Approach/Activities. Groundwater samples were collected from four TCE impacted sites for quantification of TCE co-oxidation rates and the abundance and expression of a suite of oxygenase genes encoding enzymes capable of co-oxidation of TCE. More specifically, analysis included quantification of soluble methane monooxygenase (sMMO), phenol hydroxylase (PHE), toluene dioxygenase (TOD), ring-hydroxylating toluene-2-monooxygenase (RDEG), and toluene-4-monooxygenase (RMO) gene copies and transcripts. TCE co-oxidation rates were based on a ^{14}C-TCE tracer method (~335 mg/L TCE, ~1.1 x 10^6 dpm ^{14}C).

Results/Lessons Learned. To be useful for evaluation TCE co-oxidation rate constants, the target gene should be more abundant than all or most other biomarkers, the target gene must be expressed (mRNA transcripts), and the rate constant should increase with an increase in the abundance and expression of the target gene. At three of the sites (a total of 5 monitoring wells), TCE degradation rates were significantly greater than the accompanying sterile control microcosms. In each case, TCE degradation corresponded to elevated abundances and expression of specific oxygenase genes. In most wells with evidence of TCE degradation, phenol hydroxylase (PHE) gene copies were on the order of 10^3 to 10^4 gene copies/mL which are above average PHE abundances (60th to 95th percentiles). Moreover, PHE was frequently detected in RNA extracts from these samples confirming gene expression. Conversely, PHE abundances were generally below average (10^0 to 10^2 gene copies/mL) and expression was not typically quantified in samples which did not exhibit TCE degradation. At one location, TCE degradation rates were better linked to TOD and sMMO abundances and expression. TCE co-oxidation by aromatic oxygenases including toluene monooxygenases/phenol hydroxylases has been well established. Previously though, the common belief was that the natural substrate (toluene or phenol) was needed for inducing oxygenase gene expression and generation of reductants for growth and continued cometabolic activity. More recently however, reports of induction by TCE itself have become more common and significant TCE cometabolism has been demonstrated for toluene/phenol monooxygenase expressing pure cultures during growth on non-aromatic substrates. Combined with the results presented here, there is growing evidence not only that aerobic cometabolism can be a significant contributor to TCE attenuation but also that qPCR and RT-qPCR quantification of PHE, TOD, and sMMO gene copies and expression could provide useful information for predicting degradation rates.