

## Use of a $^{14}\text{C}$ Assay to Determine Rates of TCE Co-oxidation in Groundwater

**James C. Mills** (jcmills@g.clemson.edu) and David L. Freedman  
(Clemson University, Clemson, SC, USA)

John T. Wilson (Scissortail Environmental, Ada, OK, USA)

Todd H. Wiedemeier (Wiedemeier and Associates, Denver, CO, USA)

**Background/Objectives.** Bacteria that degrade natural organic matter in groundwater contain oxygenases that can co-oxidize trichloroethene (TCE). This degradation pathway is promising for large dilute plumes, but its evaluation is limited because the density of the bacteria with oxygenase enzymes has not been correlated to field scale rates of degradation. ESTCP funded a project (ER-201584) that includes evaluation and quantification of the aerobic TCE co-oxidation process at five sites. This abstract describes the portion of the study that focused on determination of first-order rate coefficients for co-oxidation of TCE.

**Approach/Activities.** Groundwater samples were collected in 160 mL serum bottles from five locations across the US; three or four wells were sampled at each site, with triplicate bottles per well. The serum bottles were shipped on ice to Clemson University, warmed to room temperature overnight, and then evaluated for the rate of co-oxidation using  $^{14}\text{C}$ -labeled TCE. Custom-synthesized  $^{14}\text{C}$ -TCE was received in acetonitrile. A stock solution was prepared by dissolving the  $^{14}\text{C}$ -TCE/acetonitrile mixture in TCE-saturated water. TCE in the stock solution was purified by passage through a packed column on a gas chromatograph. As purified TCE eluted from the column, it was injected into a serum bottle, resulting in addition of  $\sim 1 \mu\text{Ci}$  of  $^{14}\text{C}$ -TCE and an increase in the concentration of TCE by  $\sim 300 \mu\text{g/L}$ . Aqueous samples (3 mL) were removed over a 40 day incubation period to measure accumulation of  $^{14}\text{C}$  degradation products. This was accomplished by raising the pH of the sample to  $>10$  in order to retain  $^{14}\text{CO}_2$  and  $^{14}\text{C}$ -labeled non-volatile products; unreacted  $^{14}\text{C}$ -TCE was removed by vigorously sparging the sample with  $\text{N}_2$ .  $^{14}\text{CO}_2$  formation was confirmed by precipitation with barium. A model was used to determine first-order degradation rate coefficients based on product accumulation and accounting for volumetric changes in the serum bottles due to sampling and subsequent changes to the distribution of TCE between the aqueous phase and headspace. A propanotrophic culture that cometabolizes TCE was used to validate the assay.

**Results/Lessons Learned.** Of the 19 wells evaluated at five sites, six wells at three of the sites had  $^{14}\text{C}$  product accumulation rates that exceeded the accumulation rate in distilled deionized water controls or filter-sterilized groundwater controls. First-order rate coefficients ranged from 1.59 to  $0.033 \text{ yr}^{-1}$ , which is equivalent to half-lives of 0.44 to 21 yr. A few of the wells in which co-oxidation occurred had volatile organic contaminants in addition to TCE; their presence may have induced the oxygenases needed for TCE co-oxidation. Quantification of rate coefficients below  $\sim 0.07 \text{ yr}^{-1}$  was hindered by autodegradation of  $^{14}\text{C}$ -TCE in the water controls. Use of filter-sterilized groundwater appears to reduce product accumulation in the controls, especially when the groundwater contains sufficient alkalinity to quench the hydroxyl radicals formed during autoradiolysis of  $^{14}\text{C}$ -TCE.  $^{14}\text{CO}_2$  represented  $\sim 57\text{-}97\%$  of the  $^{14}\text{C}$  products that accumulated in the groundwater samples, indicating that oxidation of TCE was nearly complete in the six wells that exhibited activity.

Additional efforts under ER-201584 involved quantification of five oxygenase genes by qPCR analyses for DNA and mRNA. Overall, the  $^{14}\text{C}$  product accumulation results correlate well with the molecular data.