Evaluation of Natural Attenuation of 1,4-Dioxane in Groundwater Using a ^{14}C Assay

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Background/Objectives. Monitored Natural Attenuation (MNA) is a preferred remedy for sites contaminated with 1,4-dioxane due to its low cost and limited environmental impacts compared to active remediation. Having a robust estimate of the rate at which biodegradation occurs is an essential component of assessing MNA. The objective of this study was to develop and validate a laboratory assay using ^{14}C-labeled 1,4-dioxane to measure rate constants for biodegradation based on accumulation of ^{14}C products.

Approach/Activities. The assay consists of 160 mL serum bottles containing 100 mL of groundwater. The serum bottles were filled in the field and sealed with Teflon-faced septa, leaving air in the headspace. They were then placed on ice and shipped overnight to the laboratory, where they were warmed gradually overnight. Custom-synthesized ^{14}C-1,4-dioxane received from the vendor was purified by passage through a high performance liquid chromatography (HPLC) column and the fraction when 1,4-dioxane eluted was collected. The HPLC separated 1,4-dioxane from the solvent in which it was synthesized (butanol) and lowered the level of ^{14}C impurities to below 1% of the total ^{14}C activity. Approximately 0.27 µCi of purified 1,4-dioxane was added to the serum bottles. Once per week, a 5 mL sample was removed, the pH was lowered with HCl, and the liquid was sparged to strip off $^{14}CO_2$, which was then trapped in an alkaline solution and counted for ^{14}C activity. The acidified sample was neutralized and then passed through a selective solid phase sorbent cartridge to remove unreacted 1,4-dioxane; soluble degradation products were collected and the ^{14}C products were quantified. The assay was validated with cultures that are known to metabolize and cometabolize 1,4-dioxane (*Pseudonocardia dioxivorans* CB1190 and propane grown ENV487, respectively). Controls with acetylene added and incubation under anaerobic conditions were used to demonstrate that biodegradation of 1,4-dioxane was mediated by monooxygenases. Treatments with groundwater and cultures were accompanied by filter sterilized controls, to assess the background level of ^{14}C product formation via autoradiolysis.

Results/Lessons Learned. Groundwater samples were collected from 10 sites in a variety of locations across the US. Of the 54 groundwater samples evaluated, statistically significant rate constants were determined with the ^{14}C assay for 24. The median rate constant was 0.0.0138 yr^{-1} (half-life = 50 years); the maximum rate constant was 0.367 yr^{-1} (half-life = 1.9 years). The lowest rate constant measured was 0.0021 yr^{-1} (half-life >300 years), indicating the high level of sensitivity of the assay. The results confirmed that biodegradation of 1,4-dioxane is occurring at 9 of the 10 sites sampled, although there was considerable variability in the level of activity. In microcosms that contained sediment, the rate constants were not always higher in comparison to those with only groundwater. Nevertheless, the presence of sediment is advisable when that is an option, since it will reduce the chances of negative or low rate constants due to a lack of the required microbes and/or inadequate nutrients. Rate constants increased when nutrients were added to groundwater along with CB1190 and ENV487. Overall, the results demonstrate that the ^{14}C assay reported in this study is an effective tool that can contribute to assessment of the applicability of MNA as a remedy for 1,4-dioxane.