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Aerobic Biodegradation of Vinyl Chloride in Groundwater Samples

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Studies were conducted to examine the biodegradation of ^{14}C-labeled vinyl chloride in samples taken from a shallow aquifer. Under aerobic conditions, vinyl chloride was readily degraded, with greater than 99% of the labeled material being degraded after 108 days and approximately 65% being mineralized to $^{14}CO_2$.

Widespread use of chlorinated aliphatic hydrocarbons has stimulated considerable interest in the processes which determine the environmental fate of these compounds. Because of their relatively high aqueous solubilities and persistence in soil, chlorinated aliphatic hydrocarbons have been detected in groundwater (15). In particular, vinyl chloride has received increased attention as a groundwater contaminant, since it is both toxic and carcinogenic to humans (13). The presence of vinyl chloride in groundwater has been reported and was proposed to originate from degradation of higher chlorinated aliphatic hydrocarbons, such as trichloroethylene and tetrachloroethylene (7–9). Additional laboratory studies have firmly established that vinyl chloride can result from reductive dechlorination of trichloroethylene, tetrachloroethylene, and dichloroethylene (4, 14).

Biotransformation of vinyl chloride under methanogenic conditions has been reported, although degradation was shown to be relatively slow and incomplete (1, 4). Less information is available on the aerobic biodegradation of vinyl chloride. Hartman et al. (6) isolated a *Mycobacterium* strain which used vinyl chloride as the sole carbon and energy source for growth. Roberts et al. (11) have observed degradation of vinyl chloride in an aquifer enriched with methane and oxygen. However, no degradation occurred without methane enrichment.

The purpose of this study was to examine the aerobic degradation of vinyl chloride by naturally occurring microorganisms in groundwater. Laboratory studies were conducted with soil-water microcosms prepared from authentic aquifer material.

Aquifer material was obtained from a site located on the northern bank of the South Canadian River from an area bordering the municipal landfill in Norman, Okla. (2). The water table at the site has been reported to be quite shallow and ranges from approximately 0.6 to 1.5 m below the surface (12). The sample site did not receive leachate from the adjacent landfill and was chosen to represent an aerobic portion of the aquifer. Subsurface soil samples were obtained from approximately 0.5 to 1.0 m below the surface and transferred to sterile glass jars. Groundwater was collected by digging a hole approximately 1 to 2 m deep and allowing the hole to fill. Groundwater was then bailed into sterile one-gallon (3.784-liter) glass bottles. The samples were chilled and sent to the laboratory in Midland, Mich.

Analysis of the subsurface soil for organic and inorganic contents and soil texture was performed by A & L Midwest Laboratories, Inc. (Omaha, Nebr.), by standard methods (3). The total number of bacterial cells and the number of viable microorganisms associated with the subsurface soil were determined by the method of Ghiorse and Blackwill (5).

Biodegradation of ^{14}C-labeled vinyl chloride was examined in microcosms prepared with the subsurface soil and groundwater. Samples were prepared in 30-ml serum bottles by combining 20 g (wet weight) of solids with 20 ml of groundwater which had been sterilized by filtration through a 0.45-μm-pore-size filter. To ensure aerobic conditions, the microcosms were sparged for 5 min with 100% O_2 before addition of the labeled material. The bottles were then supplemented with an aqueous solution of ^{14}C-labeled vinyl chloride (specific activity, 0.53 mCi/mmol; Dupont, NEN Research Products, Boston, Mass.) to yield a final concentration of either 0.1 or 1.0 ppm (wt/wt or grams of vinyl chloride per gram of soil and water) and sealed with a Teflon-faced butyl rubber septum and an aluminum crimp seal. Reaction mixtures also contained resazurin (0.0002%) as a redox indicator. Autoclaved controls were included to monitor abiotic degradation, as well as loss of test material from the microcosms. All samples were incubated at 20°C in the dark and agitated on a tissue culture rotator which continually rolled the bottles at 1 rpm.

Analysis for ^{14}C-labeled vinyl chloride in the aqueous fraction was performed by high-performance liquid chromatography. Before analysis, the samples were chilled on ice for approximately 30 min. Chromatography was performed with a ZORBAX octyldecylsilane column (4.6 mm by 25 cm; Dupont) with acetonitrile-water (50:50) as the mobile phase delivered at 1.0 ml/min by a Waters 510 solvent delivery system. Radioactive compounds were detected by an on-line radioactivity monitor unit (Berthold 506A).

Total radioactivity in the aqueous fraction was determined by liquid scintillation counting. Triplicate samples of the aqueous fraction (200 μl) were counted in 10 ml of liquid scintillation counting cocktail (Aquasol; Dupont, NEN).

Mineralization of ^{14}C-labeled vinyl chloride to $^{14}CO_2$ in the reaction mixtures was determined during the study. $^{14}CO_2$ was collected by passing N_2 gas (250 to 350 ml/min) through the slurry mixtures, which had been acidified with 200 μl of concentrated phosphoric acid. The purged gas was collected in a series of two traps, each containing 10 ml of a 1 N potassium hydroxide solution. One-milliliter portions from the combined traps were analyzed by liquid scintillation counting. $^{14}CO_2$ production was confirmed by adding barium nitrate to the trap solution, mixing it for 30 min, and determining the radioactivity in the solution after removal of the precipitate (10).

The physical, chemical, and biological characteristics of the subsurface soil and groundwater are summarized in

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TABLE 1. Subsurface soil and groundwater characteristics

Parameter	Subsurface soil	Groundwater
Texture	Sand	
Composition (%)		
Sand	93	
Silt	1	
Clay	6	
pH	8.6	7.6
% Organic carbon	0.24	
Dissolved organic carbon concn (ppm)		48
Sulfate concn (ppm)		27
Nitrate nitrogen concn (ppm)	6	<0.2
No. of bacteria/g (dry wt) by:		
Direct count (acridine orange)	9.77×10^7	
Total heterotrophic count (plate count)	3.01×10^4	

Table 1. The solids, which contained relatively little organic carbon, were classified as a sand on the basis of low levels of silt and clay. The total number of microorganisms associated with the solids, as determined by acridine orange direct counting, was similar to that observed by Beeman and Sulflita (2) in solids from anaerobic areas within the same aquifer. The level of viable microorganisms associated with the solids determined by a standard plate count (5) was several orders of magnitude lower than that determined by direct counting.

Biotransformation of ^{14}C-labeled vinyl chloride at two different concentrations was examined under aerobic conditions in the groundwater microcosms. ^{14}C-labeled vinyl chloride (1.0 ppm) was readily degraded in the groundwater samples (Fig. 1). No adaptation or observable lag occurred before the transformation of vinyl chloride, and approximately 25% of the test material was degraded during week 1

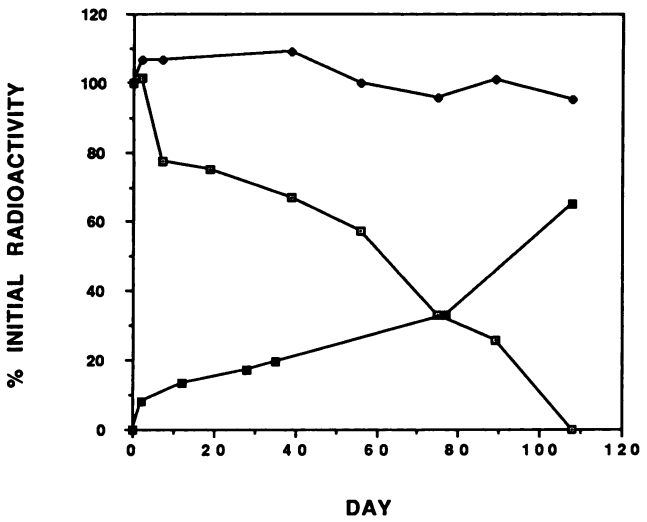

FIG. 1. Biodegradation of ^{14}C-labeled vinyl chloride at 1 ppm in aquifer microcosms. Symbols: ◆, sterile samples; ⊡, viable samples; ■, $^{14}CO_2$ produced by the viable samples.

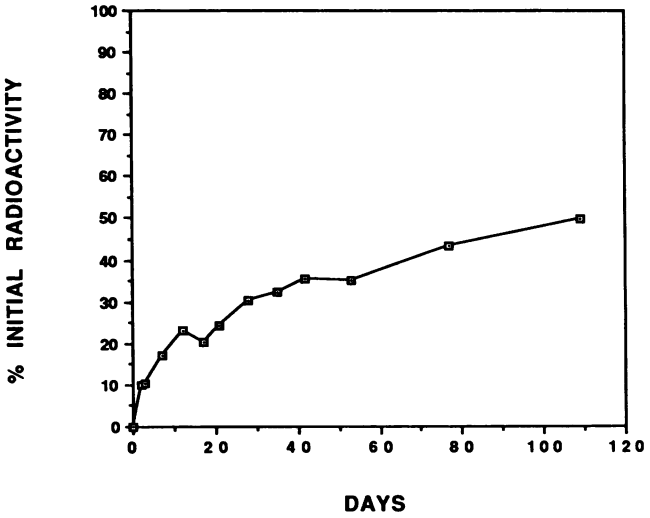

FIG. 2. Biodegradation of ^{14}C-labeled vinyl chloride at 0.1 ppm in aquifer microcosms. The amounts of $^{14}CO_2$ produced by the viable samples are shown. Less than 1% $^{14}CO_2$ was detected in the sterile controls.

of incubation. After 108 days, greater than 99% of the test material was degraded in the biologically active samples. Vinyl chloride degradation was biologically mediated, since greater than 95% of the labeled material was recovered from the aqueous fraction in the sterile controls. Mineralization accounted for much of the loss, since approximately 65% of the labeled material was recovered as $^{14}CO_2$ after 108 days of incubation.

To determine whether biodegradation would occur at lower concentrations of vinyl chloride, additional microcosms were prepared as previously described and spiked with the ^{14}C-labeled material at 0.1 ppm. Degradation of the test material at this concentration was monitored by $^{14}CO_2$ production only. The microorganisms present in the aquifer material were capable of mineralizing vinyl chloride at concentrations of 100 ppb and below (Fig. 2). After 109 days, approximately 50% of the labeled material was recovered as $^{14}CO_2$. Mineralization was not observed in the sterile controls.

The results of this study demonstrate that vinyl chloride can be rapidly degraded under aerobic conditions. The absence of an observable lag or adaptation period was unexpected, since the aquifer at this site had no known previous exposure to vinyl chloride or other chlorinated solvents. Thus, the ability of microorganisms associated with soil and groundwater to degrade vinyl chloride may be widespread. These results are consistent with those of other investigators (6) who have reported the occurrence of vinyl chloride-degrading microorganisms associated with soil.

This investigation is the first report of aerobic biodegradation of vinyl chloride in environmental samples. Although there have been several reports on vinyl chloride biodegradation (1, 11), previous studies have relied on addition of exogenous nutrients, such as methane, to demonstrate degradation.

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