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AEROBIC BIODEGRADATION OF 1,1-DICHLOROETHYLENE IN SURFACE AND SUBSURFACE SOILS			
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Abstract
Laboratory studies were conducted to examine the aerobic biodegradation of 1,1-dichloroethylene in surface and subsurface soils having no previous exposure to the compound. Biodegradation accounted for disappearances equivalent to greater than 22% of initially applied 1 and 10 ppm (w/w) 1,1-dichloroethylene in a sandy loam soil after 185 days. In a subsurface sand soil, disappearance equivalent to approximately 6% of an initial 1 ppm (w/w) 1,1 dichlorethylene was attributed to biodegradation after 150 days. No evidence for biodegradation of 10 ppm 1,1-dichloroethylene was observed in the subsurface soil after the same 150 day period. The results of this study indicate that naturally occurring microorganisms in soil and ground water are capable of degrading 1,1-dichloroethylene without laboratory supplementation of exogenous organic nutrients or previous exposure history. The results further suggest that degradative potential may vary with soil type and 1,1-dichloroethylene concentration.

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Study Title

AEROBIC BIODEGRADATION OF 1,1-DICHLOROETHYLENE IN SURFACE AND SUBSURFACE SOILS

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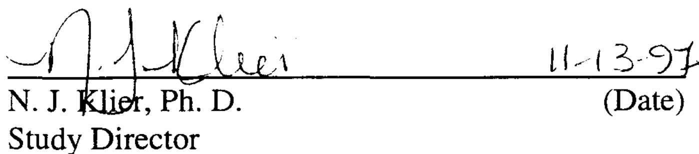
Title: AEROBIC BIODEGRADATION OF 1,1-DICHLOROETHYLENE IN SURFACE AND SUBSURFACE SOILS



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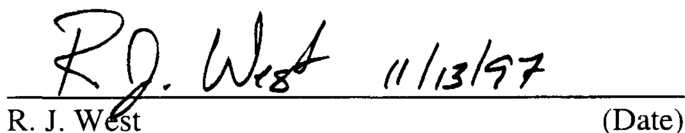

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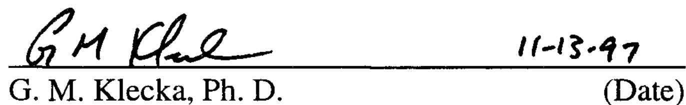

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SUMMARY

Laboratory studies were conducted to examine the aerobic biodegradation of 1,1-dichloroethylene in surface and subsurface soils having no previous exposure to the compound. Biodegradation accounted for disappearances equivalent to greater than 22% of initial concentrations of 1 and 10 ppm (w/w) 1,1-dichloroethylene in a sandy loam soil after 185 days. In a subsurface sand soil, disappearance equivalent to approximately 6% of an initial 1 ppm (w/w) 1,1-dichlorethylene was attributed to biodegradation after 150 days. No biodegradation of 10 ppm 1,1-dichloroethylene was observed in the subsurface soil after the same 150 day period. The results of this study indicate that naturally occurring microorganisms in soil and ground water are capable of degrading 1,1-dichloroethylene without laboratory supplementation of exogenous organic nutrients or previous exposure history. The data further suggest that degradative potential may vary with soil type and 1,1-dichloroethylene concentration.

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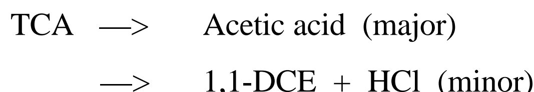
INTRODUCTION

Chlorinated aliphatic compounds (RCl's) have been used extensively in industry and agriculture over the past several decades and are widely detected in the environment. The one- and two-carbon halogenated compounds appear to be the most widespread (1). They include perchloroethylene, trichloroethylene, dichloroethylene, and vinyl chloride. Some of these compounds have been considered to be relatively persistent in the environment and are readily transported in ground water. In contrast, it is now known that a variety of RCl's, e.g. perchloroethylene (PCE), trichloroethylene (TCE), and trichloroethane (TCA) are degraded. Dichloroethylenes (1,1-, cis-1,2-, and trans-1,2-) found in the environment are most commonly the result of the degradation of these higher chlorinated compounds (2). The degradation processes which yield dichloroethylenes (DCE's) and other chlorinated compounds such as vinyl chloride (VC) include both biotic and abiotic mechanisms;

Biotic:

Abiotic:



A widely held belief is that, for highly chlorinated ethanes and ethenes, anaerobic biodegradation mechanisms are more favored than aerobic reactions (2). There have been numerous studies (3-6) illustrating the anaerobic transformation of the higher chlorinated compounds to lesser chlorinated products. As the degree of chlorine substitution decreases, however, the rates of anaerobic reactions also decrease.

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In contrast, biodegradation of the lower chlorinated compounds appears to occur more readily under aerobic conditions. Davis and Carpenter (7) demonstrated the aerobic degradation of vinyl chloride in soil-groundwater microcosm studies. The aerobic biodegradation of methylene chloride in soil (8) has also been shown. Although trichloroethylene biodegradation has been extensively studied under anaerobic conditions, TCE has also been observed to degrade aerobically by a co-oxidation process in the presence of methyltrophic bacteria (9-11). Co-oxidation of both cis-1,2-dichloroethylene and trans-1,2-dichloroethylene by methylotrophs has also been reported (12-14). Aerobic biodegradation of trichloroethylene, vinyl chloride, and cis-1,2-dichloroethylene has also been documented using propane-oxidizing bacteria (15).

Very little information exists in the literature about the aerobic degradation of dichloroethylenes in the absence of co-oxidative conditions. A single study is known which investigated aerobic biodegradation of 1,1-dichloroethylene (1,1-DCE). Tabak et al. (16) measured biodegradation of the compound at initial concentrations of 5 and 10 mg/L in static reaction flasks containing a dilute domestic wastewater inoculum. They concluded that 1,1-DCE was subject to significant biodegradation after gradual (7 day) adaptation periods. However, this study failed to account for abiotic losses of the compound, such as adsorption to biosolids and evaporation. Further, biodegradation of 1,1-DCE in soil cannot be inferred from the apparent biodegradation observed in an aqueous wastewater. Because of the potential for 1,1-DCE to be introduced to soil environments, the fate and lifetime of the compound in such systems is of interest.

MATERIALS AND METHODS

Soil Collection

Surface and subsurface soils were collected from two separate locales; 1) a sandy loam surface soil from an uncultivated area near a landfill in Midland, MI, and 2) an aquifer sand from Norman, OK. The surface soil has been previously classified in the Kingsville series (17). The upper six inches of soil and vegetation were cleared away via shovel and

discarded. Soil was then collected from a depth of six to twelve inches via shovel and transferred into a plastic-lined cardboard container. The plastic liner was sealed and the container was stored in the lab at 5°C until used. The soils was sieved through U.S.A. Standard Testing Sieve #10 (2 mm) prior to use in experiments.

The subsurface sand was collected from a site upgradient of the Norman, OK landfill with the aid of a backhoe. A pit was excavated to the water table (approximately 6 to 8 feet deep). Soil and ground water samples were brought to the surface in the backhoe bucket. Soil samples were then transferred into sterile glass quart jars and sealed with screw-top lids under zero headspace. The soil samples were then packed in blue ice and shipped by overnight delivery to the investigating lab in Midland, MI, where they were stored at 5°C until used.

Soil moisture was determined for each of the two soils by gravimetric analysis (18). In addition, soil samples were submitted to Midwest Labs (Omaha, NE) for texture and gross organic and inorganic content analysis according to conventional methods (19). Standard plate counts (20) were used to approximate the total heterotrophic bacterial populations of both the subsurface and surface soils.

Test Chemical

1,1-Dichloroethylene (vinylidene chloride, 99%, Lot #'s 04614KY and 05017DZ) was obtained from the Aldrich Chemical Company, Milwaukee, WI. All other chemicals used in reagent and test system preparation were of at least reagent grade and obtained from commercial sources. Water used in reagent and test system preparation was purified using a MilliQ water treatment system (Millipore Corp.).

Biodegradation Experiments

Biodegradation of 1,1-DCE was examined in microcosms consisting of 8 g (dry wt) of the Kingsville surface soil or 10 g (dry wt.) Norman aquifer sediment suspended in 10 mL of

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an aqueous 0.01 M $(NH_4)_2HPO_4$ solution (sterile, pH 7.0). Microcosms were constructed in 23 x 75 mm glass headspace autosampler vials (Hewlett-Packard) which allowed for direct analysis of the volatile test compound without further manipulation of the soil/water mixtures. 1,1-DCE was added as a 2,000 mg/L aqueous stock solution to each microcosm to achieve initial DCE concentrations of 1 or 10 ppm on a total weight basis. Prior to addition of the test chemical, the reaction mixtures were purged with high purity oxygen gas to ensure maintenance of aerobic conditions over the expected duration of the experiments. Teflon-lined butyl rubber septa and aluminum crimp seals were used to seal the microcosms immediately after addition of the test chemical. Microcosms were incubated at 25°C in darkness, with slight agitation provided by horizontal placement on a gyratory shaker.

Biologically inhibited controls were included in the study to monitor for non-biological losses of the test compound. Controls were prepared for each soil type and 1,1-DCE concentration using various sterilization treatments. Three sets of surface soil (Kingsville) controls were prepared using formaldehyde (2% total wt.), $HgCl_2$ (0.023% total wt.), and a combination of autoclaved soil (1 hour treatments at 121 ^{o}C, 15 psi on three consecutive days) and $HgCl_2$ (0.023% total wt.). Two sets of subsurface (Norman) soil controls were prepared using gamma-irradiated soil (5 MRad) and autoclaved soil (1 hour treatments at 121 ^{o}C, 15 psi on three consecutive days). Aside from the various sterilization treatments, the control microcosms contained the same soil/water weights and headspace volumes as the analogous viable microcosms.

Analytical Methods

Duplicate killed and viable microcosms from each experimental set were analyzed at selected time intervals using a static headspace/gas chromatographic procedure. Microcosm slurries were homogenized prior to analysis by manual shaking. Headspace samples were generated using a Hewlett-Packard Model 19395A automatic headspace sampler which directly heated the microcosms at 70°C over a 1 to 6 hour equilibration

period. Headspace samples (1 mL automated injections) were then analyzed using a Hewlett-Packard Model 5890 gas chromatograph with flame ionization detection. Separation of 1,1-DCE was achieved on a 30 meter x 0.53 mm fused silica column containing a GSQ porous polymer media (J&W Scientific, Folsom, CA). The concentrations of 1,1-DCE in the microcosms were determined by comparison to external standards containing 1 and 10 mg/L 1,1-DCE in the 0.10 M ammonium phosphate buffer solution. Concentrations of 1,1-DCE at each sampling point were expressed as a percentage of (Day 0) measured values.

RESULTS

Soil Characterization

Physical and chemical characteristics of the soils used in this study are summarized in Table 1. The Kingsville soil was characterized as a sandy loam soil with a cation exchange capacity of 9.6 mEq/100g, while the subsurface Norman aquifer material was classified as a sand with a cation exchange capacity of 7.6 mEq/100g. Organic matter contents of the Kingsville and Norman soils were 4.8 and 0.26 % (wt.) respectively. Despite differences in texture and organic matter content, the major inorganic nutritional parameters of the two soils were quite similar. Nitrate concentrations in the soils ranged from 18 to 21 ppm while phosphorous concentrations ranged from 6 to 11 ppm. Potassium ranged from 32 to 71 ppm. Standard plate counts of the total heterotrophic microbial population in the Kingsville sandy loam soil produced approximately 3×10^7 colony forming units per gram (CFU/g) dry wt., while the Norman soil produced approximately 1×10^5 CFU/g.

Abiotic Losses of 1,1-DCE

Biodegradation of 1,1-DCE in the soil microcosms was indicated by increased disappearance of the compound relative to biologically inhibited controls. Since primary biodegradation (1,1-DCE disappearance) was the only possible measure of biodegradation, it was important to accurately measure attenuation of the compound in

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the absence of biological activity. Therefore, biologically inhibited control microcosms were prepared in an identical fashion to that of the viables, using several different sterilization treatments. The inclusion of these different treatments allowed some assessment of the impact the treatments themselves might have on abiotic removal processes (e.g.adsorption to soil).

As shown in Figs. 1-4, there were very little differences in the attenuation of 1,1-DCE among the various sterilization treatments. In the Kingsville soil, the combination of autoclaving and $HgCl_2$ treatment consistently showed the least disappearance (i.e. highest concentrations over time) of 1,1-DCE at both tested concentrations (Figs. 1,2). Conversely, $HgCl_2$ treatment alone consistently showed the largest degree of 1,1-DCE disappearance among the sterilization treatments. These results indicate that autoclaving the soil had some impact on abiotic attenuation of 1,1-DCE in the Kingsville soil. Sterilization treatment in an autoclave has been previously shown to affect the sorptive characteristics of soils (21). The extremes of temperature and pressure encountered are believed to impact the physical and chemical nature of the natural organic matter associated with soil. The adsorption of hydrophobic, chlorinated aliphatic compounds to soil is due in part to interaction with this soil organic matter. Therefore, the observed differences in 1,1-DCE attenuation between the autoclaved/$HgCl_2$ and the $HgCl_2$ treatments might be explained by alteration of the soil organic matter, as it is apparent that sorption of the compound to soil was at least partly responsible for the observed disappearance in the killed soil microcosms.

Differences in 1,1-DCE disappearance between the autoclaved and gamma-irradiated Norman soil controls were less pronounced than observed between Kingsville soil treatments. Neither the autoclaving nor the gamma-irradiation treatment consistently showed increased or decreased attenuation of 1,1-DCE in the Norman soil (Figs. 3,4). Although the Norman microcosms contained 25% (dry wt.) more soil than the Kingsville microcosms, the compound was removed to a lesser extent in killed Norman soil than in killed Kingsville soil. For example, after 70 days, disappearance of an initial 1 ppm 1,1-

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DCE in all Norman controls averaged 39%. Likewise, disappearance of an initial 10 ppm 1,1-DCE averaged 28% after 70 days. In the Kingsville soil, disappearance of 1 ppm of the compound in all controls averaged 54% after 74 days. At the 10 ppm initial concentration, disappearance in the Kingsville controls averaged 38%. This observation further supports the role of adsorption in disappearance of 1,1-DCE in the killed control microcosms. A greater extent of 1,1-DCE adsorption to the Kingsville soil is to be expected, since it contained 4.8% (wt.) organic matter as opposed to 0.26% organic matter in the Norman soil (Table 1).

Biodegradation

The concentrations of 1,1-DCE in the various killed control microcosms were averaged at each sampling time point. Biodegradation of the compound was then determined from the difference of this average control concentration and the 1,1-DCE concentration measured in corresponding viable microcosms. Biodegradation of 1,1-DCE was most pronounced in the Kingsville soil, as an additional 22 and 23% of the initial 1 and 10 ppm 1,1-DCE, (respectively) was removed in viable microcosms relative to the average removal in controls after 185 days (Figs. 1,2). At an initial concentration of 1 ppm, disappearance of 1,1-DCE in the viable microcosms began to diverge from the controls after 6 days. However, between 37 and 74 days were required before removal of 10 ppm 1,1-DCE in viable reactions surpassed that in the controls. This indicates that a lag period was required prior to onset of biodegradation, and that the lag period was extended with increased concentration of 1,1-DCE.

Differences between the Norman viable and control soil reactions were less apparent, indicating that little or no biodegradation of 1,1-DCE occurred in this soil after 150 days (Figs. 3,4). After 131 days, disappearance of the initial 1 ppm 1,1-DCE in viable microcosms began to diverge from that in the killed controls (Fig. 3). This indicates that a small amount ($\leq$ 6% of initial concentration) of the material may have biodegraded after this time period. However, to evidence for biodegradation of an initial 10 ppm 1,1-DCE was observed in the Norman soil (Fig. 4).

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DISCUSSION

Chlorinated aliphatic compounds have been widely detected in the environment. Higher chlorinated compounds are known to biodegrade under anaerobic conditions to produce lower chlorinated homologs as intermediates. Many of these lower chlorinated compounds can be co-oxidized by aerobic microorganisms such as methane-oxidizers (12,13,22-26), toluene degraders (27-29) and phenol degraders (10,27,30) that produce monooxygenase or dioxygenase enzymes. Co-oxidation refers to the concomitant oxidation of nongrowth substrates when they are present in a medium in which one or more different compounds are furnished for growth (31). In order to degrade the chlorinated compound the primary substrate must be present. Inhibition may occur, however, when there is competition for the enzyme between the growth substrate and the chlorinated compound.

Some chlorinated compounds, however, have also been demonstrated to act as sole carbon sources for some microorganisms under aerobic conditions. Several microorganisms have been isolated that can use a number of chlorinated compounds such as 1-monohalo-n-alkanes and some α,ω-dichloroalkanes as growth substrates (32, 33). Hartman, et al. (34) has described an aerobic vinyl chloride degrading organism. There have also been examples of microorganisms that utilize other halogenated compounds as primary substrates. Pignatello (35) has reported the microbial degradation of dibromoethane in aquatic environments. However, there is very little information on the potential for aerobic biodegradation with dichloroethylenes as a sole carbon source.

The purpose of this study was to examine the aerobic degradation of 1,1-dichloroethylene by naturally occurring microorganisms in soils without the addition of exogenous organic nutrients. The experimental results indicate that 1,1-DCE was degraded to varying degrees by the natural microbial populations in the two soils studied. In the Kingsville surface soil, the extent of biodegradation observed for 1 ppm 1,1-DCE was very similar to that observed for 10 ppm 1,1-DCE. This is in contrast to biodegradation of cis- and trans-1,2-DCE observed in a similar sandy loam surface soil. Klier et al. (36) showed that the rates

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and extents of biodegradation of these compounds was decreased by increasing concentration from 1 to 10 ppm.

Differences in aerobic degradation rates between similar isomeric forms of chlorinated compounds have been noted in previous studies. In general, the rate of transformation is observed to be faster when the compounds are less chlorinated and the halogens are more distributed on the molecule (13). Therefore, biodegradation of 1,1-DCE would be expected to occur more slowly than trans- and cis-1,2-DCE. This was found to be true for biodegradation of these compounds in soil, as very little or no biodegradation of 1,1-DCE was observed in the Norman soil, while Klier et al. (36) showed approximately 15 % degradation of cis-1,2-DCE in the same soil after 180 days. The trans-1,2-DCE isomer was also biodegraded in the Norman soil, but with rates and extents lesser than that of the cis- isomer. Hopkins, et al. (30) noted that methanotrophs degraded t-DCE to a greater degree than c-DCE. The opposite, however, was true for phenol utilizers. They concluded that small changes in molecular structure can result in large differences in transformation rates depending upon the degradation mechanism. Jannsen, et al. (37) also found that t-DCE was degraded more efficiently than c-DCE by a culture of methanotrophic bacteria. Malachowsky, et al. (15), however, present degradation results using propane-oxidizing bacteria in which c-DCE is more effectively degraded. In fact, these bacterial strains had no degradative effect on t-DCE. It appears evident that, depending upon the microorganisms involved, even slight differences in structure can significantly affect transformation potential.

Rates of DCE degradation were also observed to vary with soil type. The extent of biodegradation for 1,1-DCE in the Norman aquifer material was observed to be lesser than in the Kingsville sandy loam soil. The same observation was made for biodegradation of cis- and trans-1,2-DCE in the Norman soil and a similar sandy loam surface soil (36). The lower rates of biotransformation in the subsurface sand may be attributed to substantially lower microbial populations compared to the surface sandy loam soil. The microbial population in the Kingsville surface soil was determined to be approximately 100-fold

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greater than in the Oklahoma aquifer material. Higher concentrations of microorganisms in surface soils compared to subsurface soils have been well documented. For example, Federle, et al. (38) identified a marked vertical discontinuity (at a depth of 2 to 3 m) in the distribution of active microorganisms at a site in Wisconsin. They observed that microbial biomass and activity decreased by 10- to 100-fold below 3 meters, then exhibited little variation with depth. The slower rates observed in the Oklahoma material in these studies may therefore be due to significantly lower numbers of microorganisms capable of degrading the dichloroethylene.

Several studies have indicated that another potential reason for variability between soil systems may be due to the prior exposure history of the soil. It has been observed that some compounds elicit an enhanced degradative response from indigenous microorganisms following short periods of exposure. Pentachlorophenol-degrading bacteria have been isolated from water (39,40), soil (40,41) and sewage (40) after exposure to the compound. Spain and Van Veld (42) have also shown that pre-exposure of microbial communities to chemicals can result in increased rates of degradation. Davis and Madsen (8) demonstrated that soil preadapted (exposed) to methylene chloride exhibited increased rates of biodegradation for that compound. They attributed this increase in degradation rate to increased numbers of microorganisms capable of mineralizing methylene chloride. Nishino (43) conducted a comparison study examining the biodegradation of chlorobenzene (CB) by indigenous bacteria at four different CB-contaminated sites. Degradation of CB in previously contaminated soil was compared to unexposed soil at the same site. CB-degrading microorganisms were readily isolated from the contaminated soils and results indicated that indigenous degradative populations developed where there was chronic CB contamination of soil and groundwater. By analogy, it would be expected that surface and subsurface soils with a history of exposure to 1,1-DCE would exhibit more rapid rates of biodegradation than reported here.

In conclusion, biodegradation may represent a key process for the removal of 1,1-DCE from aerobic soil environments. There are several factors that appear to affect the

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transformation of the compound in soils. These include soil type and biomass concentration, as well as previous exposure to the compound (i.e. acclimation). It is evident, however, that the aerobic biodegradation of dichloroethylenes to non-toxic products is possible in natural soil and groundwater systems.

ARCHIVING

Permanent records of all data generated during the course of this study, the protocol, protocol revisions/changes, and the final report are archived at Health and Environmental Research Laboratories, The Dow Chemical Company.

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REFERENCES

1. Westrick, J. J., J. W. Mello and R. F. Thomas. 1984. The Ground-Water Supply Survey. J. Am. Wat. Works Assoc. 76: 52-59.

2. Vogel, T. M., C. S. Criddle, and P. L. McCarty. 1987. Transformation of halogenated aliphatic compounds. Environ. Sci. Technol. 21: 722-736.

3. Freedman, D. L. and J. M. Gossett. 1989. Biological reductive dechlorination of tetrachloroethylene and trichloroethylene to ethylene under methanogenic conditions. Appl. Environ. Microbiol. 55: 2144-2151.

4. Vogel, T. M. and P. L. McCarty. 1985. Biotransformation of tetrachloroethylene to trichloroethylene, dichloroethylene, vinyl chloride and carbon dioxide under methanogenic conditions. Appl. Environ. Microbiol. 49: 1080-1083.

5. Vogel, T. M. and P. L. McCarty. 1987. Abiotic and biotic transformations of 1,1,1-trichloroethane under methanogenic conditions. Environ. Sci. Technol. 21: 1208-1213.

6. Tiedje, J. M., S. A. Boyd, and B. Z. Fathepure. 1987. Anaerobic degradation of chlorinated aromatic hydrocarbons. Develop. Ind. Microbiol. 27: 117-127.

7. Davis, J. W. and C. L. Carpenter. 1990. Aerobic biodegradation of vinyl chloride in groundwater samples. Appl. Environ. Microbiol. 56: 3878-3880.

8. Davis, J. W. and S. S. Madsen. 1991. The biodegradation of methylene chloride in soils. Environ. Toxicol. Chem. 10: 463-474.

DOW CONFIDENTIAL - Do not share without permission

- DOW CONFIDENTIAL -

9. Wilson, J. T. and B. H. Wilson. 1985. Biotransformation of trichloroethylene in soil. Appl. Environ. Microbiol. 49: 242-243.

10. Nelson, M. J. K., S. O. Montgomery, E. J. O'Neill, and P. H. Pritchard. 1986. Aerobic metabolism of trichloroethylene by a bacterial isolate. Appl. Environ. Microbiol. 52: 383-384.

11. Little, C. D., A. V. Palumbo, S. E. Herbes, M. E. Linstrom, R. L. Tyndall, and P. J. Gilmer. 1988. Trichloroethylene biodegradation by a methane-oxidizing bacterium. Appl. Environ. Microbiol. 54: 951-956.

12. Moore, A. T., A. Vira, and S. Fogel. 1989. Biodegradation of *trans*-1,2-dichloroethylene by methane-utilizing bacteria in an aquifer simulator. Environ. Sci. Technol. 23: 403-406.

13. Semprini, L., P. V. Roberts, G. D. Hopkins, and P. L. McCarty. 1990. A field evaluation of in-situ biodegradation of chlorinated ethenes. Part 2, The results of biostimulation and biotransformation experiments. Ground Water. 28: 714-727.

14. Semprini, L. and P. L. McCarty. 1991. Comparison between model simulations and field results for in-situ biorestoration of chlorinated aliphatics. Part 1. Biostimulation of methanotrophic bacteria. Ground Water. 29: 365-374.

15. Malachowsky, K. J., T. J. Phelps, A. B. Teboli, D. E. Minnikin and D. C. White. 1994. Aerobic mineralization of trichloroethylene, vinyl chloride, and aromatic compounds by *Rhodococcus* species. Appl. Envir. Microbiol. 60: 542-548.

16. Tabak, H. H., S. A. Quave, C. I. Mashni, and E. F. Barth. 1981. Biodegradability studies with organic priority pollutant compounds. Journal WPCF 53: 1503-1518.

DOW CONFIDENTIAL - Do not share without permission

- DOW CONFIDENTIAL -

17. Hutchinson, D. E. 1979. Soil survey of Midland County, Michigan. U. S. Dept. of Agriculture, Natural Resources Conservation Service, Washington, D.C.

18. The Dow Chemical Company. 1997. Determination of soil moisture content. Environmental Chemistry Research Laboratory, Standard Operating Procedure ENV-CHM-017.03. August 11, 1997.

19. Black, C. A., D. D. Evans, J. L. White, L. E. Ensminger and F. E. Clark (Eds.). 1965. Methods of Soil Analysis. American Society for Agronomy, Madison, WI.

20. Gerhardt, P., R. Murray, R. Costilow, E. Nester, W. Wood, N. Krieg, and G. B. Phillips (Eds.). 1991. Manual of Methods for General Bacteriology. American Society for Microbiology, Washington, D.C.

21. Wolf, D. C., T. H. Dao, H. D. Scott, and T. L. Lavy. 1989. Influence of sterilization methods on selected soil microbiological, physical, and chemical properties. J. Environ. Qual. 18: 39-44.

22. Fogel, M. M., A. R. Taddeo and S. Fogel. 1986. Biodegradation of chlorinated ethenes by a methane-utilizing mixed culture. Appl. Envir. Microbiol. 51: 720-724.

23. Henson, J. M., M. V. Yates, and J. W. Cochran. 1987. Metabolism of chlorinated aliphatic hydrocarbons by a mixed bacteria culture growing on methane. Abstr. Q-97, Annual Meeting Amer. Soc. Microbiol., Atlanta, GA. p. 298.

DOW CONFIDENTIAL - Do not share without permission

- DOW CONFIDENTIAL -

24. Henson, J. M., M. V. Yates, J. W. Cochran, and D. L. Shakelford. 1988. Microbial removal of halogenated methanes, ethanes, and ethylenes in an anaerobic soil exposed to methane. FEMS Microbiology Ecology. 53: 193-201.

25. Strand, S. E. and L. Shippert. 1986. Oxidation of chloroform in aerobic soil exposed to natural gas. Appl. Envir. Microbiol. 52: 203-205.

26. Oldenhuis, R., R. L. J. M. Vink, D. B. Janssen and B. Witholt. 1989. Degradation of chlorinated aliphatic hydrocarbons by *Methylosinus trichosporium* Ob3b expressing soluble methane monooxygenase. Appl.. Envir. Microbiology. 55: 2819-2826.

27. Nelson, M. K. J., S. O. Montgomery, W. R. Mahaffey, and P. H. Pritchard., 1987. Biodegradation of trichloroethene and involvement of an aromatic biodegradative pathway. Appl. Envir. Microbiology. 53: 949-954.

28. Nelson, M. J. K., S. O. Montgomery and P. H. Pritchard. 1988. Trichloroethylene metabolism by microorganisms that degrade aromatic compounds. Appl. Envir. Microbiol. 54: 604-606.

29. Wackett, L. P. and D. T. Gibson. 1988. Degradation of trichloroethylene by toluene dioxygenase in whole-cell studies with *Pseudomonas putida F1*. Appl. Envir. Microbiol. 54: 1703-1708.

30. Hopkins, G. D., L. Semprini, and P. L. McCarty. 1993. Microcosm and in situ field studies of enhanced biotransformation of trichloroethylene by phenol-utilizing microorganisms. Appl. Envir. Microbiol. 59: 2277-2285.

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31. Leadbetter, E. R. and J.W. Foster. 1959. Oxidation products formed from gaseous alkanes by the bacterium *Pseudomonas methanica*. Arch. Biochem. Biophys. 82: 491.

32. Stucki, G., U. Krebser, and T. Leisinger. 1983. Bacterial growth on 1,2-dichloroethane. Experientia. 39: 1271-1273.

33. Janssen, D. B., A. Scheper, L. Dijkhuizen and B. Witholt. 1985. Degradation of halogenated aliphatic compounds by *Xanthobacter autotrophicus* GJ10. Appl. Envir. Microbiol. 49: 673-677.

34. Hartsman, S., J. A. M. de Bont, J. Tramper and K. C. A. M. Luyben. 1985. Bacterial degradation of vinyl chloride. Biotechnol. Lett. 7: 383-388.

35. Pignatello, J. J. 1987. microbial degradation of 1,2-dibromoethane in shallow aquifer materials. J. Environ. Quality. 16: 307-312.

36. Klier, N. J., R. J. West, and P. A. Donberg. 1997. Aerobic biodegradation of dichlorethylenes in surface and subsurface soils. Chemosphere. In Press.

37. Janssen, D. B., G. Grobben, R. Hoekstra, R. Oldenhuis and B. Witholt. 1988. Degradation of trans-1,2-dichloroethene by mixed and pure cultures of methanatrophic bacteria. Appl. Envir. Microbiol. 29: 392-399.

38. Federle, T. W., R. M. Ventullo and D. C. White. 1990. Spatial distribution of microbial biomass, activity, community structure, and the biodegradation of linear alkylbenzene sulfonate (LAS) and linear alcohol ethoxylate (LAE) in the subsurface. Microbial Ecology. 20: 297-313.

DOW CONFIDENTIAL - Do not share without permission

- DOW CONFIDENTIAL -

39. Pignatello, J. J., M. M. Martinson, J. G. Steiert, R. E. Carlson and R. L. Crawford. 1983. Biodegradation and photolysis of pentachlorophenol in artificial freshwater streams. Appl. Environ. Microbiol. 46: 1024-1031.

40. Stanlake, G. J. and R. K. Finn. 1982. Isolation and characterization of a pentachlorophenol-degrading bacterium. Appl. Environ. Microbiol. 44: 1421-1427.

41. Saber, D. L. and R. L. Crawford. 1985. Isolation and characterization of *Flavobacterium* strains that degrade pentachlorophenol. Appl. Environ. Microbiol. 50: 1512-1518.

42. Spain, J. C. and P. A. Van Veld. 1983. Adaptation of natural microbial communities to degradation of xenobiotic compounds: Effects of concentration, exposure time, inoculum, and chemical structure. Appl. Environ. Microbiol. 45: 428-435.

43. Nishino, S. F., J. C. Spain and C. A. Pettigrew. 1994. Biodegradation of chlorobenzene by indigenous bacteria. Environ. Tox. Chem. 13: 871-877.

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Table 1. Chemical and physical properties of the soil samples.

PARAMETER	KINGSVILLE	NORMAN
Major Cations/Anions (ppm)		
Calcium	1454	1256
Sodium	16	93
Potassium	71	32
Magnesium	244	99
Nitrate	18	21
Sulfur	16	29
Iron	60	16
Phosphorous	11	6
Organic Matter % (by combustion)	4.8	0.26
Soil Ph	6.7	8.6
Cation Exchange Capacity (meq/100g)	9.6	7.6
Bacterial Population (CFU/g)	3×10^{7}	1×10^{5}
Texture		
% Sand	74	94
% Silt	18	4
% Clay	8	2

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Figure 1. Disappearance of 1 ppm (w/w) 1,1-DCE in viable (o) Kingsville soil microcosms and in controls treated with 2% formaldehyde (G), 0.023% $HgCl_2$ (x), and a combination of autoclaving and 0.023% $HgCl_2$ (Δ).

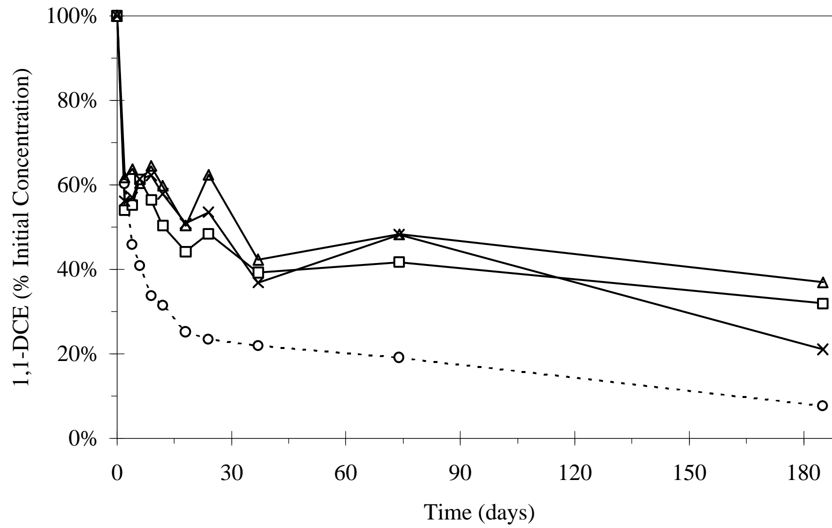

Figure 2. Disappearance of 10 ppm (w/w) 1,1-DCE in viable (o) Kingsville soil microcosms and in controls treated with 2% formaldehyde (G), 0.023% $HgCl_2$ (x), and a combination of autoclaving and 0.023% $HgCl_2$ (Δ).

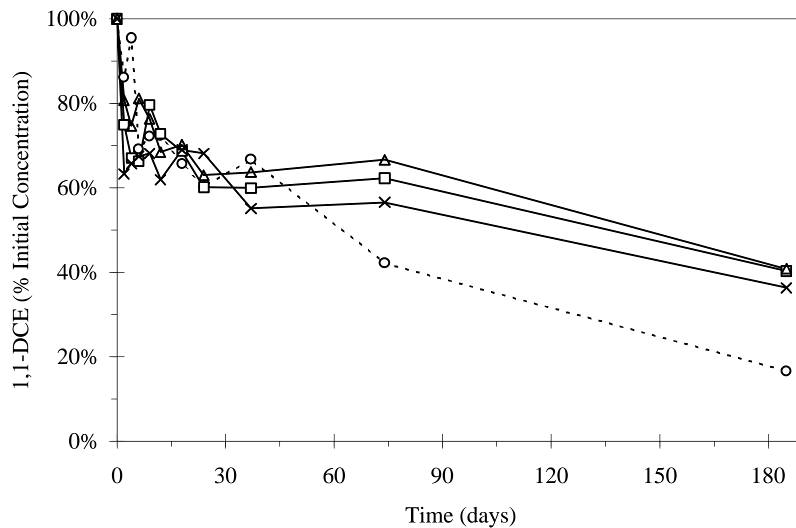

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Figure 3. Disappearance of 1 ppm (w/w) 1,1-DCE in viable (o) Norman soil microcosms and in controls prepared with autoclaved (Δ) and gamma-irradiated (x) soil.

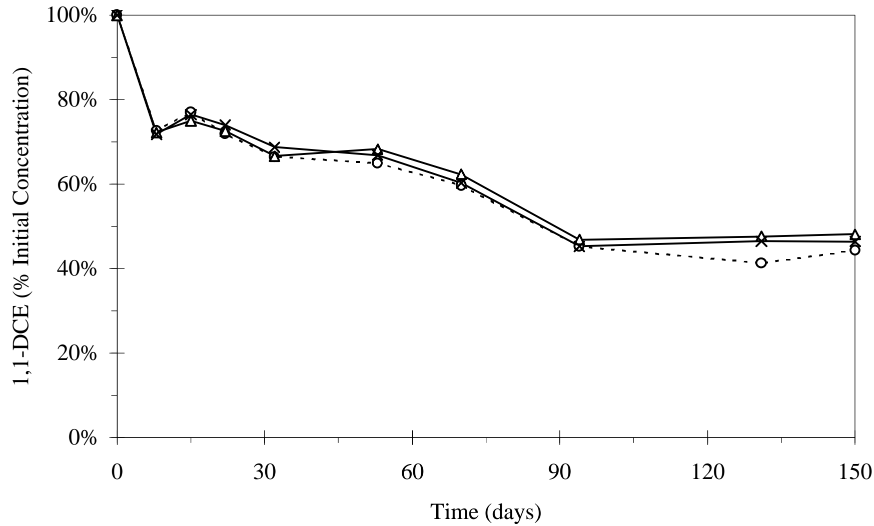

Figure 4. Disappearance of 10 ppm (w/w) 1,1-DCE in viable (o) Norman soil microcosms and in controls prepared with autoclaved (Δ) and gamma-irradiated (x) soil.