Carbon Isotope Fractionation during Microbial Dechlorination of
Trichloroethene, cis-1,2-Dichloroethene, and Vinyl Chloride: Implications for
Assessment of Natural Attenuation

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Carbon isotope fractionation during dechlorination of
chlorinated ethenes was investigated using a methanogenic
microbial enrichment culture. Subcultures were amended
with trichloroethene (TCE), cis-1,2-dichloroethene (cis-
DCE), and vinyl chloride (VC), respectively. Carbon isotope
ratios and concentrations of reactants and of all
dechlorination products were monitored during two
experiments. All dechlorination steps were accompanied
by significant isotope fractionation. The isotope ratios of the
reactants were described with a Rayleigh type model,
and the following enrichment factors (ε/R) were obtained:
−6.6 and −2.5% for dechlorination of TCE, −14.1 and
−16.1% for dechlorination of cis-DCE, and −26.6 and −21.5% for
dechlorination of VC. Isotope and mass balances suggested
that ethene (ETH) was degraded. In additional experiments
with ETH as reactant, ETH became enriched in
13C as its concentration decreased indicating the cultures
were capable of degrading ETH. The average value for
the enrichment factor obtained for the degradation of ETH
was −3.0%. The large carbon isotope fractionation
observed in this study confirms that carbon isotope ratios
are a sensitive tool for monitoring dechlorination of
chlorinated ethenes to nontoxic end products.

Introduction

Trichloroethene (TCE) is the most frequently detected
groundwater contaminant at hazardous waste sites in the
United States (1). TCE is a suspected carcinogen, and
therefore its presence in the environment is of concern. Under
aerobic conditions, TCE is resistant to degradation or may
degrade slowly in the presence of a cosubstrate (2, 3) and as
a result tends to persist in groundwater for long periods of
time. However, TCE has been shown to degrade rapidly with
microbiological mediation under anaerobic conditions (4, 5).
This is usually accomplished through the activity of halorespiring bacteria, which utilize chlorinated solvents as
electron acceptors (5). During this process, TCE is reductively
dechlorinated to ethene (ETH) with cis-1,2-dichloroethene
(cis-DCE) and vinyl chloride (VC) as intermediate degradation
products (e.g. ref 5). In some cases, further end products of
TCE dechlorination have been documented, including carbon
dioxide (6) and ethene (7). TCE and its degradation products,
cis-1,2-DCE and VC, have been identified as priority pollutants
by the Environmental Protection Agency in the United States.

Microbially mediated reductive dechlorination provides
a natural attenuation mechanism for TCE contaminant
plumes. In the United States, monitored natural attenuation
is a recognized remedial method for sites with chlorinated
solvent contamination (8). Current monitoring strategies
include documentation of the presence of intermediate and
final degradation products, documentation of the loss of
reactants, and evaluation of the contaminant distribution
within the plume (8). In addition, contaminant degradation
is often confirmed using laboratory microcosms (8). However,
proving the existence of intrinsic bioremediation of TCE is
difficult. For example, the most common end product of
TCE dechlorination, ETH, can originate from degradation of
compounds other than TCE (e.g. dihaloelimination of 1,2-
DCA (9)). Furthermore, ETH may migrate to the unsaturated
zone due to its high Henry’s constant. The use of compound-
specific stable carbon isotope analysis in conjunction with
conventional methods may provide more definitive evidence
for the existence and extent of natural attenuation at sites
contaminated with chlorinated ethenes (10, 11).

The use of compound-specific isotope analysis to sub-
stantiate biodegradation relies on the fact that many biotic
processes are accompanied by isotope fractionation. Bonds
formed by light isotopes of an element are generally weaker
and more reactive than bonds formed by heavy isotopes (12).
As a result, reaction rates are often slightly faster for molecules
containing light than for molecules with heavy isotopes,
leading to a progressive enrichment of heavy isotopes in the
residual reactant and the formation of a product depleted in
heavy isotopes (13). The change in isotope ratios between
reactant and products during a unidirectional process, such
as a microbially mediated reaction, is denoted kinetic isotope
fractionation (14). The magnitude of isotope fractionation
depends on various factors including temperature, bacterial
species, structure of the organic compounds, and availability
of nutrients (14). Several microbially mediated redox reactions
in groundwater, such as methanogenesis (15), denitrification
(16, 17), and sulfate reduction (18, 19) are accompanied by
significant and characteristic isotope fractionation between
substrate and products.

Several studies have been published on carbon isotope
fractionation during biotic and abiotic transformation of TCE
and tetrachloroethylene (PCE). Negligible carbon isotope
fractionation was observed by Ertl et al. (21) during reductive
dechlorination of TCE to cis-DCE under reducing conditions.
Sherwood-Lollar et al. (11) determined an enrichment factor
(ε/R) of −7.1% for the reductive dechlorination of TCE to
cis-DCE under sulfate reducing conditions. Hunkeler et al.
(10) performed microcosm and field studies to evaluate
isotope fractionation during reductive dechlorination of PCE
under methanogenic conditions. Their study indicated that
isotope analysis is particularly useful to monitor the last two
steps of TCE or PCE dechlorination (cis-DCE to VC, VC to
ETH), which frequently control the rate of complete dechlo-
rination. In the study, enrichment factors (ε/R) were estimated
based on the initial isotopic separation between precursor
and product. The following values were found: 2% for the
PCE to TCE step, 4% for the TCE to cis-DCE step, 12% for
the cis-DCE to VC step, and 26% for the VC to ETH step. Significant isotope fractionation was also observed during abiotic dechlorination of chlorinated compounds by zero-valent iron (21, 22).

The main objective of the work presented in this paper was to identify the extent of carbon isotope fractionation during reductive dechlorination of TCE and the intermediate dechlorination products, cis-DCE and VC, to ETH by a methanogenic microbial consortium. In contrast to previous studies, separate experiments were performed with TCE, cis-DCE, and VC, respectively, as initial reactant. This approach makes it possible to evaluate and quantify isotope fractionation during each dechlorination step using a Rayleigh type model.

Materials and Methods

Enrichment Cultures. The enrichment cultures KB-1 utilized in this study were derived from sediment samples collected at a site contaminated with TCE in southern Ontario. The site is located over 100 km away from that described by Hunkeler et al. (10). The sediment samples were used in an earlier study to create microbial microcosms to confirm the presence of intrinsic biodegradation of TCE at the site, and the microbial consortium was shown to be efficient at reductively dechlorinating TCE and intermediate degradation products to ETH (23). The cultures were enriched in the bacteria capable of performing reductive dechlorination using TCE, cis-DCE, and VC, respectively, as initial reactant. Three subcultures were taken for the isotope experiments. The cultures contained little original sediment and were maintained in a defined mineral medium (24). The cultures were sustained in 250 mL glass bottles with 200 mL of liquid and 50 mL of headspace, which were sealed with Mininert caps and electrical tape and incubated in an anaerobic glovebox.

Two experiments were performed with each of the three subcultures. Prior to each biodegradation experiment, the three cultures were purged for approximately 1 h with a 5% CO₂–95% N₂ mixture to remove volatile organic compounds from the system. To minimize disturbance of the microbial consortium, the medium was not changed between the two experiments. Once the removal of volatile organic compounds was confirmed with headspace analysis, the chlorinated ethenes were added. The first subculture (TCE culture) was injected with 30 μL of stock solution (9:1 methanol:TCE) containing 33.4 μmol TCE (22 ppm). The second subculture (DCE culture) was injected with 30 μL of stock solution (9:1 methanol: cis-DCE) containing 39.8 μmol cis-DCE (20 ppm). The third subculture (VC culture) was injected with 50 μL gaseous VC and 10 μL HPLC grade methanol, the equivalent of 39.3 μmol VC (43 ppm).

Sampling and Analysis. Duplicate headspace samples ranging from 0.2 and 0.5 mL were taken using gastight syringes. For both experiments, samples were initially taken at 12-h intervals; however, sampling became less frequent as concentration changes with time became smaller. The total amount of mass removed for carbon isotope analysis during the experiments was calculated to be 5–12%. No controls were run since the enrichment cultures were previously shown to reductively dechlorinate TCE, cis-1,2-DCE, and VC in a study designed to confirm biodegradation of TCE by the microbial community (23).

Stable carbon isotope analysis was performed with a gas chromatograph combustion isotope-ratio mass spectrometry system (GC-C-IRMS) (10, 25). The system was used to perform compound specific ¹³C/¹²C analysis and to determine concentrations in headspace samples. The GC-C-IRMS system consists of an Agilent GC (Agilent, Palo Alto, CA, U.S.) connected to a Micromass Isocrom isotope-ratio mass-spectrometer (Micromass, Manchester, U.K.) via a combustion interface. The GC was equipped with a split/splitless injector set at a 10:1 split ratio and a GS-GasPro column (J&W, Folsom, CA, U.S.). The temperature program used for the GC was 45 °C for 3 min, 15 °C/min to 75 °C, 25 °C/min to 200 °C, 200 °C for 5 min. The analytical precision for the isotope measurements was ±0.3% (10). Carbon isotope values are reported in the usual delta notation relative to the VPDB (Vienna Peedee Belemnite) standard. The δ¹³C value is defined as δ¹³C = (Rsample/Rstandard) – 1)·1000, where Rs and Rstandard are the ¹³C/¹²C ratios of the sample and reference (VPDB), respectively (14). The δ¹³C values were not corrected for isotope fractionation between compounds in solution and in the headspace since the degree of isotope fractionation is small (26, 27) in comparison with the isotope fractionation observed during dechlorination.

The concentration of individual chlorinated ethenes in the headspace was calculated with the peak area of the mass-44 ion trace in combination with external standards. The total concentration of individual compounds in each bottle was determined using Henry’s law. Dimensionless Henry’s law constants at 25 °C of 0.386 (TCE), 0.154 (cis-DCE), 1.077 (VC) (28), and 8.758 (ETH) (29) were used. Through comparison of replicate analyses the precision was estimated to be ±3.5 μmol/L for the two experiments.

Calculations. Isotope fractionation between substrate and product can be expressed in terms of a fractionation factor (α) which is defined by (14)

\[ \alpha_{PR} = \frac{13P/12P}{13R/12R} \]

where 13P/12P is the isotope ratio of instantaneous product and 13R/12R is the isotope ratio of the reactant. Isotope fractionation can also be expressed in terms of an enrichment factor (εPR/α), which is defined by (14)

\[ \varepsilon_{PR} = 1000(\alpha_{PR} - 1) \]

The definition of α according to eq 2 yields fractionation factors less than 1 and negative enrichment factors for normal kinetic isotope effects (i.e. the reaction rate is faster for ¹²C than for ¹³C), which is compatible with the notation used by Mariotti (17). In some studies (e.g. Hunkeler et al. (10)), the fractionation factor is defined by the inverse of the ratio given in eq 1.

The evolution of isotopic ratios of a reactant and a product for one-step processes can often be described with the Rayleigh eq 17 which is given by

\[ 10^3 \cdot \ln \left( \frac{10^{-3} \delta^{13}C_R + 1}{10^{-3} \delta^{13}C_{R0} + 1} \right) = \varepsilon_{PR} \cdot \ln f_R \]

where δ¹³C_R is the isotopic ratio of the reactant, δ¹³C_{R0} is the initial isotope ratio of the reactant, and f_R is the remaining fraction of reactant (17). The reactant reservoir must be finite homogeneous and must not re-react with the product reservoir (14, 30). The Rayleigh equation can be used to evaluate whether the enrichment factor is constant and to quantify the enrichment factor. For that purpose, 10³ln((10⁻³δ¹³C_{R1} + 1)/(10⁻³δ¹³C_{R0} + 1)) was plotted versus the natural log of f_R. The value of ε (eq 3) was quantified using linear regression (17).

Since the experiment was performed in a closed system, an isotope balance (δ¹³C_R) was calculated. For each sampling event, the δ¹³C values of the individual compounds were multiplied by their mole fractions and the obtained values were summed. In a closed system, the isotope balance is expected to remain at the initial value of the added compound, and the final δ¹³C value of accumulated product
is expected to equal the $\delta^{13}C$ of the initially added compound if the mass balance covers all carbon compounds (10).

**Results**

Two biodegradation experiments, A and B, were carried out for each substrate (Figures 1–3). In the second experiment, the time required for reductive dechlorination of the different initial compounds was longer than in the first experiment, in particular for VC (Figures 1–3). A large range of $\delta^{13}C$ values, reaching from $-58.8$ to $+54.1\%$, was observed for precursors and products of reductive dechlorination (Figures 1–3). The lowest degree of isotopic separation between precursors and products was detected between TCE and cis-DCE, whereas the highest isotopic separation occurred between VC and ETH. A similar degree of isotopic separation was observed in all experiments for each of the individual dechlorination steps, regardless of the initial compound (Figures 1–3).

**TCE Culture**

The first subculture was amended with TCE with an initial $\delta^{13}C$ of $-29.5\%$. In experiment A (Figure 1), TCE became enriched in $^{13}C$ and reached a $\delta^{13}C$ value of $-15.3\%$ during dechlorination of TCE to cis-DCE. Initially formed cis-DCE exhibited a $\delta^{13}C$ value of $-29.0\%$. As cis-DCE was converted to VC, the remaining cis-DCE became enriched in $^{13}C$ reaching a $\delta^{13}C$ value of $-10.3\%$. The produced VC displayed an initial $\delta^{13}C$ value of $-36.6\%$ and became enriched in $^{13}C$ reaching a value of up to $+13.3\%$ as it was dechlorinated. Initially produced ETH showed a $\delta^{13}C$ value of $-58.8\%$ and became enriched in $^{13}C$ reaching a value of $-5.0\%$ as dechlorination of VC reached completion. Similar isotope patterns were observed during experiment B (Figure 1). At the end of both experiments, the isotope balance ($\delta^{13}C_T$) became more positive than the $\delta^{13}C$ of the added substrate (Figure 1).

**DCE Culture**

The carbon isotope composition of cis-DCE added to the second subculture was $-23.4\%$. cis-DCE and its dechlorination products became enriched in $^{13}C$ as the dechlorination reaction proceeded. In experiment A, the $\delta^{13}C$ of cis-DCE increased from $-23.4$ to $-7.1\%$, the $\delta^{13}C$ of VC increased from $-34.3\%$ to $+11.6\%$, and the $\delta^{13}C$ of ETH increased from $-48.9\%$ to $-21.7\%$ (Figure 2). Experiment B required a longer period of time to reach completion than experiment A (Figure 2); however, a similar isotope pattern was observed. Towards the end of experiment B, the isotope balance ($\delta^{13}C_T$) became more positive in comparison to the $\delta^{13}C$ of the initial VC (Figure 1) at the end of experiment B.

**VC Culture**

The third subculture was amended with VC with a $\delta^{13}C$ value of $-29.0\%$. In experiment A (Figure 3), residual VC became enriched in $^{13}C$ reaching a $\delta^{13}C$ value of $-1.6\%$, while the $\delta^{13}C$ of ETH increased from $-46.1\%$ to $-24.2\%$. In experiment B, a similar isotope pattern was observed. Again, the isotope balance became more positive compared to the $\delta^{13}C$ of the initial VC (Figure 3) at the end of experiment B.

**Rayleigh Model.** The model was applied to the initial dechlorination step in each culture (Figure 4) since, for the initial step, the isotope ratio of residual reactant is controlled only by degradation of the reactant. In contrast, the isotope ratios of the intermediate degradation products are affected simultaneously by both production and degradation of the compound and a Rayleigh model cannot be applied directly. The smallest enrichment factors were obtained for the TCE...
to cis-DCE dechlorination step. The $e_{PR}$ values calculated for the two experiments were $-6.6$ and $-2.5\%_0$, respectively. The cis-DCE to VC step had a larger enrichment factor than the previous step with $e_{PR}$ values of $-16.1$ and $-14.1\%_0$ for the two experiments. The VC to ETH dechlorination step showed the largest degree of isotope fractionation with values for the enrichment factor ($e_{PR}$) of $-21.5$ and $-26.6\%_0$, respectively.

**Isotope Pattern for Ethene.** At the end of the experiments, ETH was enriched in $^{13}$C by 1.5 to 24.0 relative to the added substrate. Furthermore, ETH was found at lower concentrations than the initial substrate concentration at the end of most of the experiments (Figures 1–3). To examine the fate of ETH in more detail and to evaluate the cause of the $^{13}$C enrichment in ETH, the cultures were amended with 500 L ETH (TCE culture), 250 L ETH (DCE and VC cultures), and 10 L HPLC grade methanol. In all three microcosms, the ETH concentration decreased, while the $^{13}$C of ETH increased (Figure 5). Enrichment factors were quantified utilizing the Rayleigh equation, and $e_{PR}$ values of $-4.0$, $-2.6$, and $-2.3\%_0$ were obtained. The average $e_{PR}$ value for the degradation of ETH by the three cultures was $-3.0\%_0$ (Figure 5).

**Discussion**

In general, the product of each dechlorination step was depleted in $^{13}$C relative to the corresponding precursor, with the exception of two cis-DCE measurements during experiment B (TCE culture). It is possible that, in these cases, the TCE to cis-DCE reaction occurred more slowly than the cis-DCE to VC reaction allowing cis-DCE to become enriched in $^{13}$C compared to TCE. The detection of VC in this experiment immediately after cis-DCE was produced supports this interpretation. A second significant feature of the isotope data is that each compound became progressively enriched in $^{13}$C during the dechlorination reaction. This trend confirms differences in the reaction rates between molecules containing $^{12}$C and $^{13}$C, respectively. Since $^{12}$C–Cl bonds react slightly faster than $^{13}$C–Cl bonds, the newly formed product is depleted in $^{13}$C, while the residual substrate becomes enriched in $^{13}$C. These trends are similar to those identified by Hunkeler et al. (10) for PCE and intermediate dechlorination products under methanogenic conditions and by Sherwood-Lollar et al. (11) for the dechlorination of TCE to cis-DCE under sulfate reducing conditions.

The good correlation between $10^{\ln((10^{-3}^{13}C_R+1)/(10^{-3}^{13}C_{Ro}+1))}$ and In $f_0$ for each experiment with each culture (Figure 4) indicates that the isotope ratios of TCE, cis-DCE, and VC followed a Rayleigh type evolution. In other words, the enrichment factors remained constant throughout the experiment. This is the first study to quantify enrichment factors for the reductive dechlorination of TCE, cis-DCE, and VC by a microbial consortium under methanogenic conditions with the Rayleigh model. The enrichment factors obtained for the dechlorination steps TCE to cis-DCE, cis-DCE to VC, and VC to ETH lie within the same range as those estimated for dechlorination of PCE by a methanogenic culture (10). In addition, the $e$ value for the TCE to cis-DCE dechlorination step is similar to that determined by Sherwood-Lollar et al. (11), $-7.1\%_0$.

There were some differences in $e$ values between the two experiments for the reductive dechlorination of TCE ($e_{PR} = -6.5, -2.5\%_0$), cis-DCE ($e_{PR} = -16.1, -14.1\%_0$), and VC ($e_{PR} = -21.5, -26.6\%_0$). These differences may be due to the observed variation in microbial activity in the two experiments. The time required for complete dechlorination was significantly different in the two experiments, especially for VC. The variations in the microbial activity may have resulted from nutrient limitations since the mineral medium was not changed between the two experiments. Similar variability of
enrichment factors associated to biomass activity have been reported for other microbial systems (e.g. sulfate reduction (31)).

One of the striking findings of the experiment was associated with the isotope pattern of ETH. This compound is frequently the final product of dechlorination of chlorinated ethenes, and, in this case, it was expected that the isotope ratio of accumulated ETH would equal that of the initially added compound, as observed in a previous study with PCE (10). However, in this study ETH became enriched in 13C compared to the initial isotope ratio of the substrate. This, in large measure, accounted for the fact that the isotope balance (δ13C) became more positive than the δ13C of the initial reactant toward the end of the experiments. The enrichment of 13C in ETH, the increase of δ13C above that of the added substrate, and the decrease of the ETH concentration at the end of the experiment all suggest that ETH was either transformed or incorporated into biomass. The fact that the δ13C of ETH and δ13C increased in the presence of chlorinated ethenes indicates that ETH consumption started before the dechlorination reaction reached completion. The isotope data obtained in the three subcultures amended with ETH follow the typical trend associated to biodegradation whereby an enrichment in 13C with decreasing concentration of ETH occurred. The experiment with ETH confirmed that the microbial consortium was capable of degrading ETH and that the degradation process resulted in significant fractionation of stable carbon isotopes. In future studies, the products of ETH degradation will be evaluated using isotopically labeled ETH.

Implications for Assessment of Natural Attenuation of Chlorinated Ethenes. The data presented in this paper confirm that significant carbon isotope fractionation occurs during microbial dechlorination of chlorinated ethenes. The enrichment factors obtained in this study were in the same range as those obtained by Hunkeler et al. (10) and Sherwood-Lollar et al. (11). This suggests that a similar level of carbon isotope fractionation may occur during dechlorination of chlorinated ethenes by bacteria indigenous to a variety of field sites. Furthermore, the study demonstrates that dechlorination of cis-DCE and VC can be described by a Rayleigh model, which provides isotope enrichment factors for these steps. Dechlorination of cis-DCE and VC are usually the slowest steps in the dechlorination of PCE and TCE. Therefore, the success of natural attenuation depends on the rate with which these dechlorination steps occur. The results of the study indicate that the extent to which these two dechlorination steps have proceeded can be substantiated using compound-specific isotope ratios. A mathematical model will be developed that links carbon isotope ratios of intermediate cis-DCE and VC to the extent of dechlorination of these compounds. In addition, the influence of biomass activity and environmental conditions such as temperature on the degree of carbon isotope fractionation of TCE and intermediate degradation products warrants further attention.

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