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Carbon Isotopes as a Tool To Evaluate the Origin and Fate of Vinyl Chloride: Laboratory Experiments and Modeling of Isotope Evolution

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Accumulation of vinyl chloride (VC) is often a main concern at sites contaminated with chlorinated ethenes and ethanes due to its high toxicity. Since there can be several possible sources of VC and ethene at such sites, assessing the origin and fate of VC can be complicated. Aim of this study was to evaluate carbon isotope fractionation associated with various anaerobic processes that lead to the production of VC and ethene in view of using isotopes to evaluate the origin and fate of these compounds in groundwater. Microcosms were constructed using sediments and groundwater from a contaminated site and amended with potential precursors for VC and ethene production. In the microcosms with dichloroethene isomers, sequential reductive dechlorination was observed, and isotopic enrichment factors of -19.9 ± 1.5‰ for *cis*-1,2-dichloroethene, -30.3 ± 1.9‰ for *trans*-1,2-dichloroethene, and -7.3 ± 0.4‰ for 1,1-dichloroethene were obtained. In microcosms with chlorinated ethanes, 1,2-dichloroethane (1,2-DCA) and 1,1,2-trichloroethane (1,1,2-TCA) were predominantly transformed by dichloroelimination to ethene and VC, respectively, and enrichment factors of -32.1 ± 1.1‰ for 1,2-DCA and -2.0 ± 0.2‰ for 1,1,2-TCA were observed. Except for 1,1,2-TCA, a strong ^{13}C enrichment in each of the potential precursor of VC was observed, which opens the possibility to trace the origin of VC based on the isotope ratio of potential precursors. Furthermore, it was possible to model the isotope evolution of VC present as substrate or intermediate product as a function of time. The study demonstrates that carbon isotope ratios can potentially be used for qualitative and possibly quantitative evaluation of the origin and fate of VC at sites with complex contaminant mixtures.

Introduction

Chlorinated ethenes and ethanes have been produced in large quantities since the 1950s and have been widely used as solvents and intermediates in chemical production. Due to spills, accidents, and improper disposal, they have entered the environment and are frequently detected in groundwater (*1*, *2*). Chlorinated ethenes and ethanes have a relatively high solubility compared to permissible levels in groundwater and a low tendency to sorb onto the aquifer matrix (*2*) and therefore often migrate over long distances in groundwater.

Biodegradation of chlorinated ethenes and ethanes can serve as a naturally occurring remediation mechanism, usually referred to as natural attenuation, if the compounds are transformed to innocuous products such as ethene and ethane (*3*). However, if only partial dechlorination occurs, biodegradation may increase the risk associated with the presence of chlorinated ethenes and ethanes in groundwater. The accumulation of vinyl chloride (VC), a confirmed carcinogenic compound, is usually of particular concern. Understanding the origin and fate of VC at sites contaminated with mixtures of chlorinated ethenes and ethanes is often a key question when evaluating the effectiveness of natural attenuation. Since there can be several possible sources of VC, ethene, and ethane, assessing the origins of VC and predicting the long-term effectiveness of natural attenuation can be complicated.

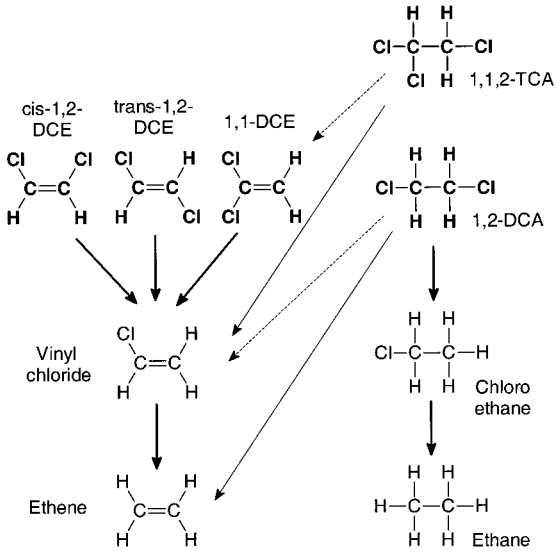

FIGURE 1. Degradation pathways for dichloroethenes, 1,2-dichloroethane, and 1,1,2-trichloroethane (see text). Bold arrow: reductive dechlorination; fine arrow: dichloroelimination; dashed arrow: dehydrochlorination.

VC can be produced by microbially mediated processes (Figure 1) including reductive dechlorination of *cis*-1,2-dichloroethene (*cis*-1,2-DCE), *trans*-1,2-dichloroethene (*trans*-1,2-DCE), or 1,1-dichloroethene (1,1-DCE) (*4*−*9*) and dichlo-roelimination of 1,1,2-trichloroethane (1,1,2-TCA (*10*, *11*)). Furthermore, it may be produced abiotically by dehydro-chlorination of 1,2-dichloroethane (1,2-DCA (*12*)) or may be present as a primary contaminant. Several of the potential precursors of VC may be transformed to other compounds than VC as well (Figure 1). 1,1,2-TCA can be transformed to 1,1-DCE by dehydrochlorination (*13*) and 1,2-DCA to chlo-roethane and ethane by reductive dechlorination (*14*). Assessment of biodegradation of VC can be difficult since the product of reductive dechlorination of VC, ethene, may be further degraded to ethane (*7*), methane, and/or CO_2 and since ethene and ethane are very volatile and may degass. Therefore, ethane and ethene usually cannot be considered as a conservative indicator of VC biodegradation (*3*). Fur-thermore, at sites with mixtures of chlorinated ethenes and chlorinated ethanes, ethene may be produced by dihaloe-

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limination of 1,2-DCA (*15*−*17*) rather than reductive dechlorination of VC making ethene an inconclusive indicator as to whether anaerobic biodegradation of VC occurs.

The main aim of this study was to evaluate if carbon isotopes can be used as a tool to trace the origin and fate of VC at anaerobic field sites with multiple potential sources for VC production. The use of isotopes to trace biodegradation is based on the frequent occurrence of a kinetic isotope effect during biodegradation of organic compounds, which leads to a depletion of heavy isotopes in the product and an enrichment of heavy isotopes in the remaining substrate (denoted as isotope fractionation). The occurrence of isotope fractionation may be used to assess the progress of biodegradation and/or to relate contaminants to degradation products. Recently, it has been revealed that for reductive dechlorination of chlorinated ethenes, a particularly large carbon isotope fractionation occurs during the steps from *cis*-1,2-DCE to VC and VC to ethene under laboratory and field conditions (*18*). As a result, strong shifts in the $\delta^{13}C$ of *cis*-1,2-DCE and VC to values >0‰ occur (*18*), providing unambiguous evidence for reductive dechlorination of *cis*-1,2-DCE and VC. Carbon isotope fractionation has also been observed during reductive dechlorination of tetrachloroethene (PCE) and trichloroethene (TCE) (*18*−*20*), oxidation of dichloromethane (*21*), and oxidation of 1,2-dichloroethane (*22*). The focus of this study was to evaluate if a strong carbon isotope fractionation also occurs during production of VC and/or ethene from *trans*-1,2-DCE, 1,1-DCE, 1,1,2-TCA, and 1,2-DCA. For comparison, *cis*-1,2-DCE and VC were included as primary compounds, too. Carbon isotope fractionation was investigated using microcosms that were prepared with sediment and groundwater from a site that had been contaminated with a range of chlorinated ethenes and chlorinated ethanes. Isotope fractionation was quantified using the Rayleigh evolution model. In addition, for selected experiments, the temporal evolution of isotope ratios was modeled using a one-step and a newly developed two-step isotope fractionation model.

Material and Methods

Preparation of Microcosms. Aquifer material and groundwater for preparation of the microcosms was obtained from a site in Louisiana, U.S.A. that had been used for the disposal of liquid chlorinated hydrocarbons from the late 1950s to 1972 (*23*). The chlorinated hydrocarbons were primarily disposed in surface impoundments, resulting in contamination of underlying sandy aquifers to a depth of some 70 m. A plume of dissolved chlorinated hydrocarbons, consisting of various chlorinated ethanes, ethenes and methanes, has migrated to distances in excess of 300 m from the former impoundments. Soil and groundwater samples for the microcosms were obtained from a location about 180 m downgradient from the former impoundments. In March 1999, aquifer material was collected at a depth between 15 and 21 m below ground surface. Groundwater for the microcosms was collected from a monitoring well adjacent to the soil sampling location and contained (all in mg/L): 1,1,2-TCA (260), 1,2-DCA (200), 1,1-DCA (9), PCE (85), TCE (11), *cis*-1,2-DCE (11), *trans*-1,2-DCE (12), 1,1-DCE (44), VC (49), and chloroform (47). Additional groundwater samples were taken in July and December 1999 to characterize redox and geochemical conditions at the sampling location. The groundwater contained 0.6 mg/L of dissolved oxygen, <0.5 mg/L of nitrate, 2.05 mg/L of dissolved manganese, 7.03 mg/L of dissolved iron, 136 mg/L of sulfate, and 0.09 mg/L of methane and had a oxidation−reduction potential of −126 mV.

Microcosms were constructed using 250 mL sterile glass bottles, filled with 60 g of homogenized aquifer material and 150 mL of groundwater, which had been purged with a gas mixture containing 20% CO_2 and 80% N_2 to remove volatile organic compounds. The bottles were closed with Mininert caps. Separate bottles were amended with either chlorinated ethenes or chlorinated ethanes to concentrations simulating field conditions. The microcosms were initially prepared to evaluate the biodegradation potential at the site and amended with mixtures of compounds twice (see below). For the isotope studies, the microcosms were purged and amended with one compound per microcosm to be able to investigate how isotope ratios of substrate and degradation products are related. A mixture of methanol, ethanol, acetate, and lactate at a final concentration of 50 mg/L each was added as electron donor to all microcosms including controls each time when chlorinated compounds were added. Two control microcosms were prepared by autoclaving aquifer material at 121 °C for 60 min on 3 consecutive days and poisoning with mercuric chloride (1 mL of a 5% solution) and sodium azide (0.5 mL of a 5% solution) to ensure the absence of microbial activity. One of the control microcosm was amended with 1,2-DCA and 1,1,2-TCA, the other with 1,1-DCE, *trans*-1,2-DCE, and *cis*-1,2-DCE, the compounds used in the isotope studies at concentrations similar to the active microcosms. Variations in $\delta^{13}C$ in control microcosms were <0.8‰, and changes in concentration were <15% for all compounds.

The microcosms with chlorinated ethenes were first amended with a mixture of PCE, TCE, *cis*-1,2-DCE, *trans*-1,2-DCE, 1,1-DCE, and VC at a concentration of 10 ppm each in July 1999. At the beginning of May 2000, they were purged for 3 h with 5% CO_2/95% N_2 and amended with a mixture of *cis*-1,2-DCE, *trans*-1,2-DCE, and 1,1-DCE (40 ppm each), the chlorinated ethenes selected for this study. For the isotope study, the microcosms were purged for 3 h with a gas mixture of 5% CO_2/95% N_2 at the end of July 2000. The absence of chlorinated ethenes and degradation products was verified using the GC method described below. Afterward, three microcosms were amended with one of the three dichloroethene (DCE) isomers in each bottle. The final concentrations in the aqueous phase were 70.0 ppm for *cis*-1,2-DCE, 68.3 ppm for *trans*-1,2-DCE, and 68.0 ppm for 1,1-DCE. The microcosm with *trans*-1,2-DCE was purged again with 5% CO_2/95% N_2 at the end of the experiment and amended with VC at a concentration of 39 ppm.

The microcosms with chlorinated ethanes were first amended with a mixture of 1,1,2-TCA (200 ppm), 1,2-DCA (200 ppm), and 1,1-DCA (10 ppm) in July 1999. At the beginning of May 2000, they were purged with 5% CO_2/95% N_2 for 3 h and amendment with a mixture of 1,1,2-TCA and 1,2-DCA (100 ppm each). For the isotope study, the microcosms were purged with a gas mixture of 5% CO_2/95% N_2 for 3 h and amended with 1,1,2-TCA and 1,2-DCA, respectively. The final concentrations were 103.4 ppm for 1,2-DCA and 104.4 ppm for 1,1,2-TCA. One microcosm was prepared for each substrate, and possible variations in isotope fractionation between identical microcosms were not investigated. However, previous studies have shown that variations in isotope fractionation between identical microcosms are small (*20*).

Analysis of Concentration of Chlorinated Ethenes and Ethanes. Concentrations of chlorinated ethenes, 1,2-DCA, ethene, ethane, and methane were analyzed using a Hewlett-Packard 6890 gas chromatograph equipped with a J&W Scientific GS-GasPro (30 m × 0.32 mm) column and a flame ionization detector. Gas samples of 0.2 mL volume from the headspace of the microcosms were injected using gastight syringes. Headspace concentrations were calculated based on peak areas of samples and external standards. To be able to compare concentrations of substrate and degradation products with different Henry's coefficients, relative concentrations are reported, which were obtained by calculating

the total amount of each compound in the microcosm at a given time and dividing it by the amount of primary compound initially added to the microcosm. For 1,1,2-TCA, aqueous samples of 2 mL volume were taken, and dissolved compounds were extracted using headspace solid-phase microextraction (SPME) and analyzed using a Hewlett-Packard 6890 gas chromatograph equipped with a Rtx-5 column (60 m × 0.25 mm) and a flame ionization detector. Detection limits were between 6 and 200 ppb depending on the Henry's coefficient of the compound. The standard uncertainty of the method was 6% for chlorinated hydrocarbons and 9% for ethene.

Compound-Specific Isotope Analysis. Carbon isotope ratios of chlorinated ethenes, 1,2-DCA, and ethene were determined using gas samples from the headspace of the microcosms as described in ref *18*. Carbon isotope ratios of 1,1,2-TCA were determined using a SPME method similar as described in ref *24*. The standard uncertainty of both methods is 0.5‰ (*24*). The lower concentration limit in ppm for isotope analysis was 3.3 (*cis*-1,2-DCE), 1.4 (*trans*-1,2-DCE), 0.6 (1,1-DCE), 0.3 (VC), 0.03 (ethene), 12.5 (1,2-DCA), and 0.5 (1,1,2-TCA). All isotope ratios are reported in the δ-notation referenced to VPDB.

Quantification of Isotope Fractionation. Isotope fractionation during initial transformation of a compound can be evaluated using a Rayleigh-type evolution model (*25*). The Rayleigh model makes it possible to determine if the fractionation factor remains constant over the course of the experiment and to quantify its value. According to the Rayleigh model, the isotopic composition of the substrate is given by

$$R_S = R_{So} f^{(\alpha-1)} \tag{1}$$

where f is the fraction of substrate remaining, R_S is the isotope ratio of substrate at a remaining fraction of substrate f, R_{So} is the initial isotope ratio of the substrate, and α is the fractionation factor. The fractionation factor is defined by

$$\alpha = \frac{dP_{13}/dP_{12}}{S_{13}/S_{12}} \tag{2}$$

where dP_{13} and dP_{12} are increments of product containing ^{13}C and ^{12}C, respectively, which appear at an infinitely short time (instantaneous product), and S_{13} and S_{12} are the concentration of substrate with ^{13}C and ^{12}C, respectively.

By using the $\delta^{13}C$ notation for carbon isotope ratios and the relationship that links the fractionation factor α to the isotopic enrichment factor ϵ, $\epsilon = (\alpha-1) \times 1000$, eq 1 transforms to

$$1000 \cdot \ln \frac{\delta^{13}C_S + 1000}{\delta^{13}C_{So} + 1000} = \epsilon \ln f \tag{3}$$

where $\delta^{13}C_S$ is the carbon isotope ratio of the substrate at a remaining fraction f, and $\delta^{13}C_{So}$ is the initial carbon isotope ratio of the substrate. Equation 3 can be approximated by

$$\delta^{13}C_S = \delta^{13}C_{So} + \epsilon \ln f \tag{4}$$

While the Rayleigh model is a useful tool to quantify isotope fractionation during initial substrate transformation, it cannot be used to evaluate isotope fractionation during formation and consumption of an intermediate unless substrate transformation is completed before transformation of the intermediate starts. Because several of the reactions investigated in this study involve two transformation steps, and because VC, the compound of main concern at many sites, often occurs as intermediate rather than as primary compound, a model was developed that makes it possible to evaluate isotope fractionation for two-step processes. The model is based on the assumption that the rate of each transformation step can be approximated by first-order kinetics. Furthermore, analogous to the Rayleigh model, the model relies on the assumption that the fractionation factor remains constant and that the overall reaction progress can be approximated by the progress of transformation of the dominant isotope, ^{12}C. The concentration of substrate C_1, intermediate C_2, and final product C_3 as a function of time is given by the following equations

$$C_1 = C_{1,0} e^{-k_1 t} \tag{5}$$

$$C_2 = \frac{k_1 C_{1,0}(e^{-k_2 t} - e^{-k_1 t})}{k_1 - k_2} \tag{6}$$

$$C_3 = \frac{k_1 k_2 C_{1,0}\left(\frac{e^{-tk_1}}{k_1} - \frac{e^{-tk_2}}{k_2}\right)}{k_1 - k_2} + C_{1,0} \tag{7}$$

where $k_1$and k_2 are the first-order rate constants of the first and second transformation step, respectively, and $C_{1,0}$ is the initial substrate concentration. Approximate expressions for isotope ratios of substrate, intermediate, and final product as a function of time can be obtained by dividing eqs 5−7 for ^{13}C by analogous equations for ^{12}C. The reaction rates for the equations with ^{12}C ($^{12}k_1$ and $^{12}k_2$) were derived from the concentration data, and the reaction rates for ^{13}C ($^{13}k_1$ and $^{13}k_2$) were calculated using the following equations that relate first-order reaction rates for ^{12}C and ^{13}C (*25*)

$$\alpha_1 = \frac{^{13}k_1}{^{12}k_1} \tag{8}$$

$$\alpha_2 = \frac{^{13}k_2}{^{12}k_2} \tag{9}$$

where α_1 and α_2 are the fractionation factors for the first and second transformation step, respectively.

Results and Discussion

Chlorinated Ethenes. In all microcosms, dissolved Fe was detected at final concentrations between 31.5 and 66.9 ppm, sulfate had been completely consumed, and no methane was detected indicating that the microcosms were under Fe-reducing and sulfate-reducing conditions. Transformation of chlorinated ethenes was fastest for 1,1-DCE which was completely dechlorinated within about 8 days (Figure 2B), followed by *cis*-1,2-DCE (25 days, Figure 2A), *trans*-1,2-DCE (90 days, Figure 2C), and VC (>100 days, Figure 2D). In the microcosms with 1,1-DCE or *cis*-1,2-DCE as parent compounds, VC accumulated to a concentration of about 90% of the initial substrate concentration, and substantial transformation of VC only started after the DCE had completely disappeared (Figure 2A,B). In the *trans*-1,2-DCE microcosm, the maximum VC concentration amounted to only about 50% of the initial substrate concentration due to the relatively low rate of *trans*-1,2-DCE transformation to VC. The relative rates of dichloroethene degradation and product patterns are similar to those observed in previous studies. For example, the same order of the reaction rates for dichloroethene transformation (1,1-DCE > *cis*-1,2-DCE > *trans*-1,2-DCE) was previously reported in studies with an enrichment culture (*8*) and methanogenic sludge (*9*). In the study by Tandoi et al. 1994 (*8*), similarly as in this study, less VC accumulation was observed in the experiment with *trans*-1,2-DCE than in

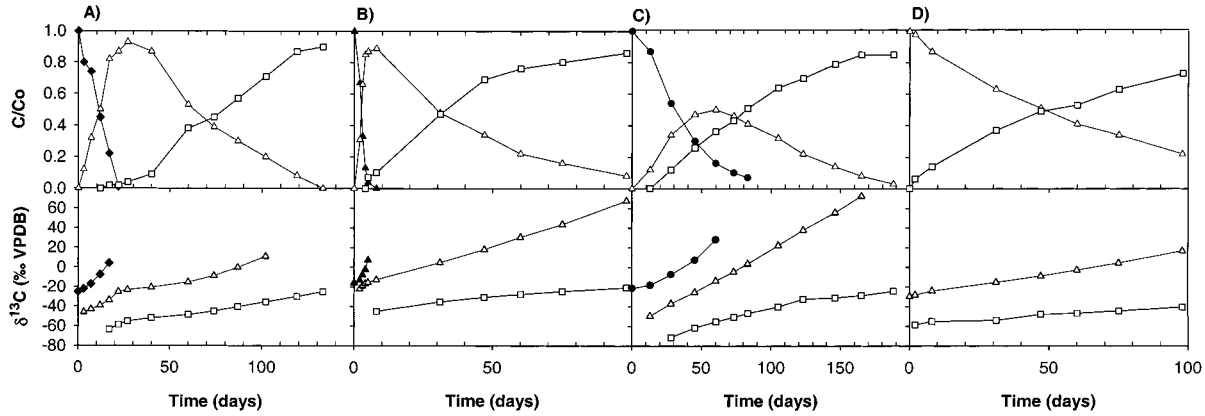

FIGURE 2. Reductive dechlorination of *cis*-1,2-dichloroethene (A), 1,1-dichloroethene (B), *trans*-1,2-dichloroethene (C), and vinyl chloride (D). Relative concentrations and carbon isotope ratio of dichloroethene (filled markers), vinyl chloride (open triangle), and ethene (open square).

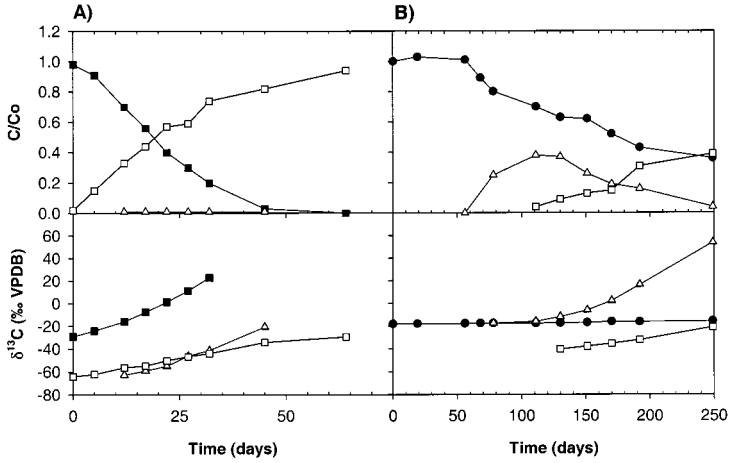

FIGURE 3. Anaerobic transformation of 1,2-dichloroethane (A) and 1,1,2-trichloroethane (B). Relative concentration and carbon isotope ratio of 1,2-dichloroethane (filled square), 1,1,2-trichloroethane (filled circle), vinyl chloride (open triangle), and ethene (open square).

those with *cis*-1,2-DCE or 1,1-DCE, and in the *cis*-1,2-DCE experiment, degradation of VC only started after *cis*-1,2-DCE had completely been consumed.

In all three microcosms with DCE, a substantial increase in the $\delta^{13}C$ of the substrate occurred (Figure 2), while no significant change in $\delta^{13}C$ was observed in control microcosms (data not shown). Furthermore, in all microcosms, VC was depleted in ^{13}C compared to the various DCE isomers, and ethene was depleted in ^{13}C compared to VC. A similar strong depletion of ^{13}C in ethene compared to VC has previously been observed in studies with TCE and PCE as primary compounds (*18*−*20*). In all microcosms, the $\delta^{13}C$ of ethene tended to approach the $\delta^{13}C$ of the initially added substrate.

Chlorinated Ethanes. In the microcosm with 1,2-DCA, this compound was consumed within about 50 days, accompanied by the accumulation of ethene (Figure 3A). Furthermore, VC accumulated transiently at low concentrations, while no ethane was detected. 1,2-DCA can be transformed to ethene by dichloroelimination or by abiotic dehydrochlorination to VC (*12*) followed by reductive dechlorination of VC. In this study, 1,2-DCA appeared to be directly transformed to ethene by dichloroelimination since reported rates for dehydrochlorination of 1,2-DCA are low (*12*) compared to the observed 1,2-DCA-transformation rate. Furthermore, if the pathway via VC were important and assuming that VC was dechlorinated at a similar rate as in the experiments with chlorinated ethenes, then substantial accumulation of VC would have been expected. In control microcosms, the 1,2-DCA concentrations remained within 10% of the initial concentration and the $\delta^{13}C$ of 1,2-DCA within 0.5 ‰ confirming that abiotic 1,2-DCA transformation was unimportant. Dichloroelimination of 1,2-DCA was previously observed in studies with methanogenic bacteria (*15, 16*), microcosm studies (*17*), and a study with a PCE degrading enrichment culture (*8*).

Transformation of 1,1,2-TCA started after a lag period of about 40 days and was very slow (Figure 3B). After 250 days, when the experiment was terminated, about 64% of 1,1,2-TCA had been transformed. 1,1,2-TCA was degraded to VC and ethene. VC accumulated at a smaller maximal concentration than in the experiments with chlorinated ethenes. This can be explained by a slow rate of VC production compared to VC consumption. Biotic transformation of 1,1,2-TCA to VC and small amounts of 1,2-DCA was previously observed in a study with methanogenic microcosms (*10*).

Concerning the isotope trends, the $\delta^{13}C$ of 1,2-DCA strongly increased as the reaction proceeded (Figure 3A), while only a small increase of the $\delta^{13}C$ of 1,1,2-TCA from −18.1‰ to −15.9‰ was observed during degradation of this compound (Figure 3B). The isotope patterns for VC and ethene are remarkably different than for the microcosms with chlorinated ethenes. For the 1,2-DCA microcosm, ethene and VC show similar $\delta^{13}C$ values (Figure 3A), while in microcosms with chlorinated ethenes, ethene was strongly depleted compared to VC. The absence of a ^{13}C depletion of ethene relative to VC confirms that dechlorination of VC to ethene is not an important process and that ethene was

TABLE 1. Isotopic Enrichment Factors for Transformation of Chlorinated Ethenes and Ethanes[a]

	isotopic enrichment factor ϵ (‰)	R^2
cis-1,2-DCE	-19.9 ± 1.5	0.984
trans-1,2-DCE	-30.3 ± 1.9	0.984
1,1-DCE	-7.3 ± 0.4	0.993
VC	-31.1 ± 0.4	0.999
1,2-DCA	-32.1 ± 1.1	0.994
1,1,2-TCA	-2.0 ± 0.2	0.919

[a] The intervals represent the uncertainty of the regression calculations.

mainly produced by dechloroelimination of 1,2-DCA. In the 1,1,2-TCA microcosms, VC becomes enriched in ^{13}C compared to the substrate (Figure 3B), while in all other microcosms the products were at each point in time depleted in ^{13}C compared to the substrate. The increase of the $\delta^{13}C$ of VC above that of 1,1,2-TCA can be explained by the small degradation rate and smaller isotope fractionation for the step from 1,1,2-TCA to VC compared to the step from VC to ethene. Similar to the result from the chlorinated ethene microcosms, a strong depletion of ^{13}C in ethene compared to VC was observed (Figure 3B), and the $\delta^{13}C$ of ethene tended to approach the $\delta^{13}C$ of 1,1,2-TCA.

Quantification of Isotopic Enrichment Factors. Isotope fractionation during transformation of DCE isomers, 1,2-DCA and 1,1,2-TCA, was quantified using the Rayleigh model. The isotope evolution during degradation of *cis*-1,2-DCE, *trans*-1,2-DCE, 1,1-DCE, 1,2-DCA, and 1,1,2-TCA can be described well using the Rayleigh model as indicated by squared correlation coefficients >0.919 (Table 1). The isotopic enrichment factors for the three DCE isomers are substantially different with values of -7.3 ± 0.4‰ for 1,1-DCE, -19.9 ± 1.5‰ for *cis*-1,2-DCE, and -30.3 ± 1.9‰ for *trans*-1,2-DCE. The value for *cis*-1,2-DCE is in a similar range as observed in previous studies which gave ϵ values between -14.1 and -20.4‰ (*19*, *20*). It is interesting to note that 1,1-DCE has a ϵ value in a similar range as observed for reductive dechlorination of TCE and PCE (-2.5‰ to -13.8‰, (*19*, *20*)), while ϵ values for the other DCE isomers are larger. The three compounds 1,1-DCE, TCE, and PCE have in common that during dechlorination, the chlorine is removed from a carbon with two chlorine atoms, while for *trans*-1,2-DCE, *cis*-1,2-DCE, and VC, which have higher ϵ values, the chlorine is removed from a carbon atom with one chlorine atom. The presence of one or two chlorine atoms, respectively, may influence the magnitude of isotope fractionation by influencing the energy differences for the two isotopes between reactant and transition state. Alternatively, the magnitude of the enrichment factors may indirectly be affected by differences in reaction rates, with 1,1-DCE, TCE, and PCE being transformed faster than *trans*-1,2-DCE, *cis*-1,2-DCE, and VC. Further studies are warranted to elucidate the connection between structure of chlorinated ethenes and the magnitude of isotope fractionation.

The enrichment factor for 1,2-DCA is much larger than that for 1,1,2-TCA (Table 1) even though both compounds seem to be predominately transformed by a similar process, dichloroelimination. The reason for this difference is not known yet.

Modeling Isotope Evolution as a Function of Time. One of the most striking features of the isotope data for chlorinated ethenes is that the $\delta^{13}C$ of VC changes in a nearly linear trend over a period of up to 150 days in three of the four microcosms (Figure 2). This pattern can be observed for the microcosm with 1,1-DCE for the period > 5 days (Figure 2B), the microcosm with *trans*-1,2-DCE (Figure 2C), and the microcosm with VC (Figure 2D). Modeling of the isotope evolution

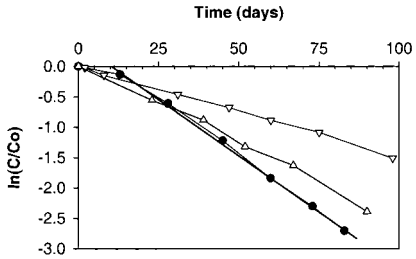

FIGURE 4. Natural logarithm of concentration relative to initial concentration as a function of time for *trans*-1,2-dichloroethene (filled circle), VC in experiment with 1,1-dichloroethene as initial compound (triangle up, points > 5 days), and VC in experiment with VC as initial compound (triangle down).

as a function of time was performed to better understand the reason for this linear trend. The three microcosms represent two different scenarios: For the VC microcosm and for the 1,1-DCE microcosm after day > 5, VC is only subject to consumption, while in the experiment with *trans*-1,2-DCE, VC is continuously produced and consumed over the entire period of the experiment. For a compound that is consumed only, the temporal evolution of the isotope ratio depends on whether the isotopic enrichment factor remains constant over time, on the magnitude of the isotopic enrichment factor and on the kinetics of substrate transformation. For the experiment with VC, it has been shown above that the isotopic enrichment factor remains constant. Regarding the kinetics of VC transformation, when the natural logarithm of the relative VC concentration is plotted versus time, an approximately straight line is obtained which indicates that transformation occurs according to first-order kinetics (Figure 4). Similarly, a straight line is obtained for VC in the 1,1-DCE microcosm for time points after day 5 (Figure 4). Rate constants were obtained using linear regression (Table 2). For first-order reactions with constant isotopic enrichment factor, a simple expression for $\delta^{13}C$ as a function of time can be obtained by combining the simplified Rayleigh expression (eq 4) with the equation for first-order kinetics given by

$$f = \frac{C}{C_o} = e^{-kt} \tag{10}$$

which leads to the following equation

$$\delta^{13}C_S = \delta^{13}C_{So} - \epsilon kt \tag{11}$$

From eq 11, it becomes immediately evident that a linear change in $\delta^{13}C$ with time is expected with a slope corresponding to the product of the first-order rate constant k times the isotopic enrichment factor ϵ. The validity of eq 11 was tested by comparison of the slope of the $\delta^{13}C$ versus time curves for VC (Figure 2) to the product ϵ times k. For the experiment with 1,1-DCE, the enrichment factor for VC degradation was calculated from data points >5 days using eq 3. A good agreement between the slope and the product of ϵ times k is found (Table 2).

While eq 11 successfully describes the temporal evolution of the $\delta^{13}C$ of VC in the microcosm with VC and 1,1-DCE as initial compound and explains the linear trend in $\delta^{13}C$, it cannot be applied to the VC isotope data in the microcosm with *trans*-1,2-DCE since VC is simultaneously consumed and produced. Therefore, the isotope data was simulated using the two-step model described in the method section. The model relies on the assumption that first-order kinetics occurs, which has been demonstrated above for VC transformation. To evaluate if *trans*-1,2-DCE transformation is also governed by first-order kinetics, the natural logarithm

TABLE 2. First-Order Rate Constant and Isotopic Enrichment Factor for VC Dechlorination in Microcosm with VC, 1,1-DCE, and *trans*-1,2-DCE as Initial Compound[a]

	k (d^{-1})	ϵ (‰)	r (‰ d^{-1})	$k^*\epsilon$ (‰ d^{-1})
VC microcosm	-0.0150 ± 0.0003 $R^2 = 0.997$	-31.1 ± 0.4 $R^2 = 0.999$	0.463 ± 0.014 $R^2 = 0.994$	0.467 ± 0.012
1,1-DCE microcosm	-0.0264 ± 0.0006 $R^2 = 0.998$	-32.6 ± 1.7 $R^2 = 0.998$	0.885 ± 0.026 $R^2 = 0.997$	0.862 ± 0.027
trans-1,2-DCE microcosm	-0.0188 ± 0.0021	-33.7 ± 0.6		

[a] Rate of change of the $\delta^{13}C$ of VC (r) in microcosm with VC and 1,1-DCE as initial compound. The intervals represent the uncertainty of the regression calculations except for $k^*\epsilon$ where it represents the propagated uncertainty.

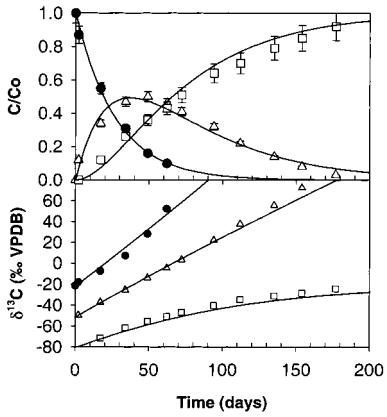

FIGURE 5. Relative concentrations and isotope ratios calculated using a two-step isotope fractionation model for microcosm with *trans*-1,2-dichloroethene as initial compound. Measured concentrations and isotope ratios of *trans*-1,2-dichloroethene (filled circles), vinyl chloride (triangle), and ethene (square). The uncertainty of the $\delta^{13}C$ is ±0.5‰.

of C/C_0 was plotted versus time. All points except for the first one lay on an approximately straight line (Figure 4). The deviation of the first point may be due to a short lag period. To account for this lag period, the model calculations were started at the time where the regression line calculated for all points except time zero (Figure 4) intercepts the x-axis, which corresponds to a time of 11 days. The first-order rate constant for *trans*-1,2-DCE transformation was obtained from the regression line displayed in Figure 4, and the first-order rate constant for VC transformation was obtained by fitting eq 6 to the VC concentration data using nonlinear regression. The isotopic enrichment factor for *trans*-1,2-DCE transformation was taken from Table 1, the isotopic enrichment factor for VC transformation corresponds to the one from the experiment with VC as initial substrate. The concentration curves and the curve of $\delta^{13}C$ for *trans*-1,2-DCE are expected to agree well with the data since the model parameters where derived from the curves. The true test of the model is how well it reproduces the $\delta^{13}C$ curves for VC and ethene, which are modeled using an independently determined enrichment factor for the VC to ethene step. As can be seen in Figure 5, a good agreement between the calculated and measured $\delta^{13}C$ values for VC and ethene is obtained indicating that the isotopic enrichment factor for VC dechlorination was similar when VC is present as intermediate compound and when VC is present as initial compound. To verify this conclusion, the isotopic enrichment factor for VC dechlorination in the *trans*-1,2-DCE experiment was calculated using nonlinear regression. As expected, a similar value as for the experiment with VC as initial compound was obtained (Table 2). The calculations demonstrates that if the reaction kinetics and isotopic enrichment factors are known, it is possible to model isotope data of multistep processes. The model developed in this study can easily be expanded to reactions with more than two steps. Furthermore, analogous models could be developed for multistep reactions with a more complex kinetics using numerical rather than analytical methods.

Implications

The main aim of this study was to evaluate if carbon isotope ratios can potentially be used to identify the origin and fate of VC at sites with multiple precursors. Regarding the origin of VC, shifts in the isotope ratios of precursors and/or characteristic isotope ratios of VC may help to identify VC sources. This study demonstrates that during dechlorination of all DCE isomers substantial carbon isotope fractionation occurs, i.e., that each of the isomers is expected to become enriched in ^{13}C if it is subject to reductive dechlorination. Thus, carbon isotope ratios of each of the DCE isomers can serve as an indicator as to whether it is a source of VC. This is also true for situations in which DCE itself is a degradation product, since under such conditions, DCE frequently accumulates and the $\delta^{13}C$ of DCE only increased above that of the parent compound (e.g. TCE or PCE) if transformation of DCE occurs (*18*). Due to the strong carbon isotope fractionation during reductive dechlorination of *trans*-1,2-DCE and *cis*-1,2-DCE, VC formed from these precursor is expected to be initially depleted in ^{13}C compared to VC present as a primary compound. For example in this study, initially formed VC exhibits a $\delta^{13}C$ of −46.0‰ for *cis*-1,2-DCE as precursor and −49.6‰ for *trans*-1,2-DCE as precursor, while in a previous study, synthetic VC was found to have a $\delta^{13}C$ of −28.6‰ (*24*). If 1,1-DCE is a precursor to VC production, the $\delta^{13}C$ may be in a similar range as synthetic VC due to the smaller carbon isotope fractionation associated with 1,1-DCE dechlorination. It may also be possible to distinguish between VC produced from DCE versus 1,1,2-TCA since, as indicated by this study, VC from 1,1,2-TCA is expected to have a similar $\delta^{13}C$ as the parent substrate and is expected to change little if no VC consumption occurs. Furthermore, the study has shown that a strong enrichment of ^{13}C in VC occurs independent of the initial substrate leading to VC with a positive $\delta^{13}C$, which can be used to trace anaerobic degradation of VC.

Regarding the model development, the study demonstrates that it is possible to model isotope data of single and multistep biodegradation processes as a function of time if reaction kinetics and isotopic enrichment factors are known, which has implications for potential field applications. In field situations, there is usually an uncertainty regarding degradation rates and regarding the contribution of biological versus physical processes to concentration reduction. In such a situation, it may be possible to verify the rate of biodegradation by calculating the isotope ratio of contaminants based on available enrichment factors and comparing it to measured isotope data. Alternatively, isotope data may help to constrain rates using inverse modeling. Isotope data of inorganic compounds have successfully been used in geochemical models (e.g. NETPATH) to constrain reactions during forward and inverse modeling (*26*). In conclusion, this study demonstrates the potential of isotopes as a qualitative and possibly quantitative tool to evaluate the origin

and fate of VC in groundwater. The results of the microcosm study presented in this paper are currently being tested at a field site characterized by a complex mixture of potential VC precursors.

Acknowledgments

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