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Isotopic and Geochemical Assessment of in Situ Biodegradation of Chlorinated Hydrocarbons

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Currently there is no in situ method to detect and quantify complete mineralization of chlorinated hydrocarbons (CHCs) to CO_2. Combined isotopic measurements in conjunction with traditional chemical techniques were used to assess in situ biodegradation of trichloroethylene (TCE) and carbon tetrachloride (CT). Vadose zone CHC, ethene, ethane, methane, O_2, and CO_2 concentrations were analyzed using gas chromatography over 114 days at the Savannah River Site. $\delta^{13}C$ of CHC and $\delta^{13}C$ and ^{14}C of vadose zone CO_2, sediment organic matter, and groundwater dissolved inorganic carbon (DIC) were measured. Intermediate metabolites of TCE and CT accounted for ≤10% of total CHCs. $\delta^{13}C$ of *cis*-1,2-dichloroethylene (DCE) was always heavier than TCE indicating substantial DCE biodegradation. ^{14}C-CO_2 values ranged from 84 to 128 percent modern carbon (pMC), suggesting that plant root-respired CO_2 was dominant. ^{14}C-CO_2 values decreased over time (up to 12 pMC), and contaminated groundwater ^{14}C-DIC (76 pMC) was substantially depleted relative to the control (121 pMC). ^{14}C provided a direct measure of complete CHC mineralization in vadose zone and groundwater in situ and may improve remediation strategies.

Introduction

Field monitoring of chlorinated hydrocarbon (CHC) biodegradation is an essential component of any monitored natural attenuation program. Monitoring chlorinated solvent biodegradation is particularly difficult due to low transformation rates and complex microbial pathways that can potentially form daughter products that are more toxic than the parent compound. For instance, sequential reductive dechlorination of perchloroethylene (PCE) produces trichloroethylene (TCE), *cis*-1,2-dichloroethylene (DCE), vinyl chloride (VC), and eventually ethene and ethane in anaerobic environments (*1*). Laboratory radioisotope studies have shown that CO_2 is an important end product of chlorinated ethene anaerobic oxidation under varying redox conditions (*2*−*4*). In aerobic environments, microbes may quickly utilize DCE (*5*) or VC (*6*) as a sole carbon source or utilize methane and ethene as primary substrates for VC mineralization (*7*). Reductive dechlorination of carbon tetrachloride (CT) sequentially forms chloroform (CF), methylene chloride (MC), and eventually CO_2 (*8, 9*). It is difficult to demonstrate that contaminants in situ are being biodegraded instead of attenuated by physical processes such as sorption to sediment, volatilization, or dilution. Biogeochemical indicators in groundwater (e.g., oxidation−reduction potential, terminal electron acceptors) and in vadose zone sediments (O_2 and CO_2) are capable of detecting potential microbial processes but cannot differentiate between the degradation of natural carbon or contaminant carbon.

Recently, $\delta^{13}C$ measurements of individual chlorinated ethenes have been shown to be a powerful tool for detecting CHC biodegradation in groundwater. Studies have shown that $\delta^{13}C$ ratios are largely conserved when compounds undergo physical processes such as sorption, volatilization, or dissolution (*10, 11*). However, biological reductive dechlorination may invoke large reproducible fractionation generally following a Rayleigh type model. Laboratory studies have shown that the initial dechlorination product will be depleted in ^{13}C relative to the corresponding precursor due to the microbial discrimination against ^{13}C molecules (*12*−*15*). This isotopic fractionation increases with decreasing chlorination and can provide evidence of the occurrence and relative extent of microbial reductive dechlorination of chlorinated ethenes (*12*−*15*).

Currently there is no method to detect and quantify complete mineralization of CHCs in the field. $\delta^{13}C$-CO_2 (*16*−*21*) or ^{14}C-CO_2 (*22*−*24*) measurements in the vadose zone or groundwater have been used to detect the complete aerobic and anaerobic biodegradation of petroleum hydrocarbons to CO_2 and may be equally effective in monitoring in situ CHC mineralization. Noncontaminant sources of CO_2 in the subsurface have a distinctive range of $\delta^{13}C$ values and include atmospheric CO_2 (−7.4 to −12‰ (*25*)), root-respired CO_2 from C_3 (−22 to −33‰ (*26*)) or C_4 plants (−5.6 to −18.6‰ (*27*)), or microbial degradation of plant material. Theoretically, $\delta^{13}C$-CO_2 of CHC metabolites will be depleted in ^{13}C relative to the precursor, but the $\delta^{13}C$ of the precursor can vary widely (±60‰ (*12*−*15*)). The effectiveness of $\delta^{13}C$-CO_2 to detect biodegradation is dependent on the (1) $\delta^{13}C$ of the parent compound, (2) fractionation factor associated with biochemical metabolism, and (3) proportion of contaminant-derived CO_2 versus natural CO_2. ^{14}C-CO_2 may differentiate contaminant and natural carbon biodegradation because of vast differences in ^{14}C activity between the two sources. All ^{14}C has radioactively decayed (0 percent modern carbon (pMC)) in the CO_2 and methane produced from CHCs (synthesized from petroleum) and is vastly dissimilar to plant-derived or atmospheric inputs which are modern in ^{14}C activity (>100 pMC).

The objectives of this field study were to assess biodegradation processes in (1) vadose zone sediments using traditional biogeochemical measurements (CHCs, O_2, CO_2) and isotopic measurements ($\delta^{13}C$ of individual CHCs, $\delta^{13}C$ and ^{14}C of vadose zone CO_2) and (2) in groundwater by measuring geochemical parameters, CHCs, and $\delta^{13}C$ and ^{14}C of DIC. We believe this field study is the first to report the use of combined isotopic methods (^{14}C and $\delta^{13}C$ of CO_2 and

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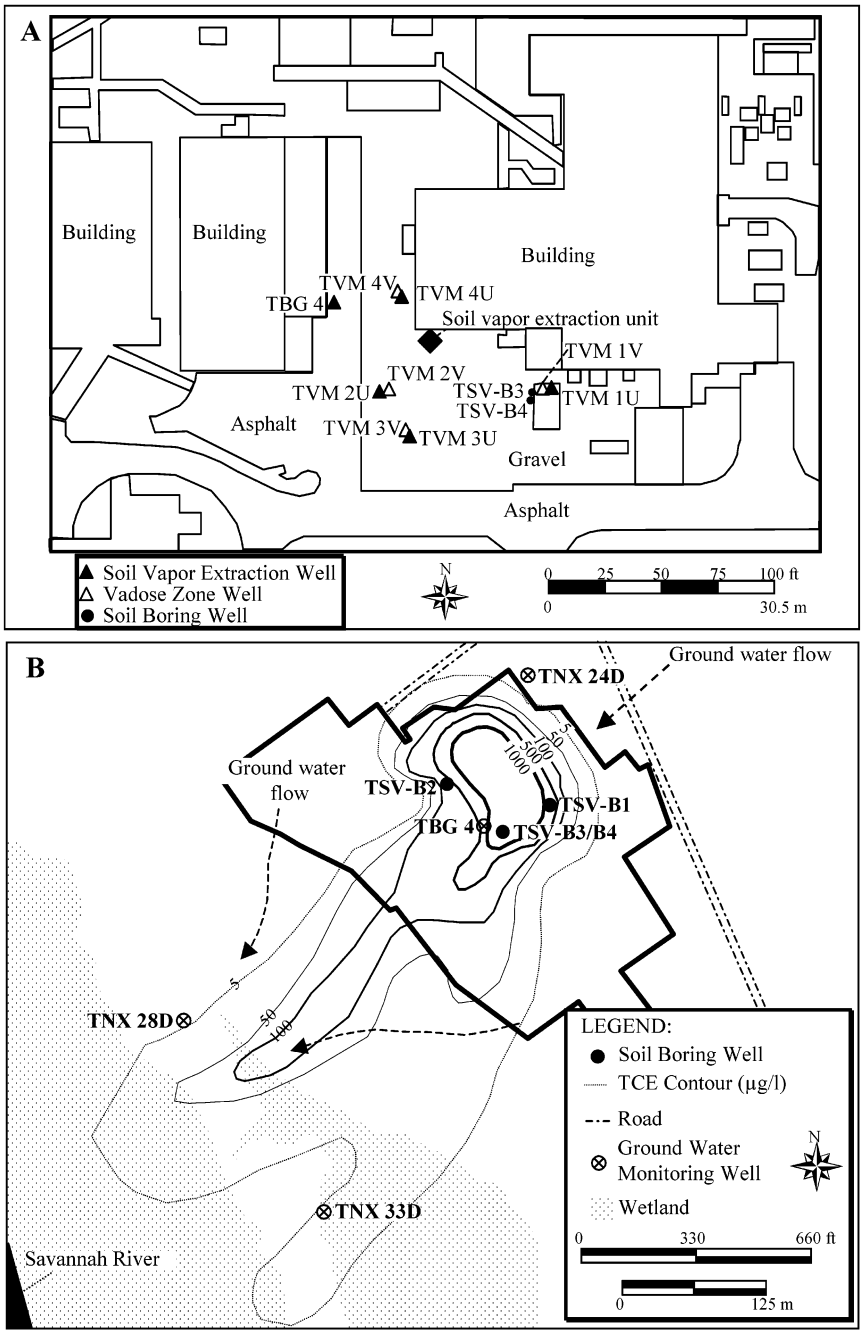

FIGURE 1. Site map of the TNX area at the Savannah River Site showing (A) the soil vapor extraction unit and well locations and (B) sediment boring locations and trichloroethylene (TCE) contours (*29*).

$\delta^{13}C$ of CHCs) to assess in situ CHC mineralization in vadose zone sediments and groundwater.

Methods

Site Characterization. The field site was located in the TNX area at the Department of Energy's Savannah River Site (SRS) near Aiken, SC. Wastewater disposed in the TNX area contaminated shallow groundwater and sediments with a complex mixture of CHCs (Figure 1). TCE and CT are the major contaminants, and PCE, DCE, MC, CF, and trichlorofluoromethane (TCFM) (CFC-11) are detectable in many of the wells at lower concentrations (Table 1). The 200-m wide and 700-m long TCE plume flows southeast and discharges into the Savannah River located one-quarter mile from the TNX facilities (Figure 1B). No petroleum hydrocarbons have been detected in the study area. The TNX facility is covered with asphalt, concrete, or gravel, and surrounding areas are a mixture of grasses and trees. The down-gradient flood plain is virtually an uncontaminated forested area containing various wetland shrubs and trees.

The effectiveness of SVE at the TNX site is being assessed as a remediation option for unsaturated sediment contamination. The SVE system utilized groundwater wells TVM-1U and 4U as vacuum extraction wells. Sediments collected by SRS personnel as part of this investigation were composed of interbedded sand, silty sand, and relatively thin clay layers (*28*). Sandy clay lenses were interspersed throughout the 21-m borings. Unsaturated sediments were comprised of densely packed stratified sands (well-sorted medium to fine grain) which exhibited relatively low intrinsic permeability ranging from 5.4 to 18.6 darcies (5.3×10^{-8} to 1.8×10^{-7} cm^2) (*29*) (Table 2). The majority of TCE contamination in sediment

TABLE 1. Groundwater Monitoring Results Collected from an Up-Gradient Control Well (TNX-24D), Source (TBG-4), and Relatively Uncontaminated Down-Gradient Wells (TNX-28D and 33D) at the TNX Area of the Savannah River Site

parameter	units	TBG-4	TNX-24D	TNX-28D	TNX-33D
date of collection		6/7/2001	6/28/2001	6/28/2001	6/28/2001
dissolved O_2	mg/L	2.4	9.2	7.1	7.7
dissolved inorganic carbon (DIC)	mg CO_2/L	43	65	52	81
alkalinity	mg/L-$CaCO_3$	0	12	8	5
ammonia	mg/L (NH_4-N)	0.02	0.00	0.00	0.01
nitrate	mg/L (NO_3-N)	21.70	1.60	0.56	3.81
nitrite	mg/L (NO_2-N)	0.005	0	0.001	0.001
total iron	mg/L	0.01	0.11	0	0.05
ferrous iron	mg/L	0	0	0	0
ferric iron	mg/L	0.01	0.11	0	0.05
sulfate	mg/L	0	39.2	34.1	29.9
sulfide	mg/L	0.008	0	0	0
chloride	mg/L	3.07	11.48	1.95	9.24
conductivity	μS/cm	305	99	44	112
pH		4.5	5.4	5.3	5.3
pE[a]	mV	210	158	151	157
temperature	°C	25.0	25.1	19.8	19.2
PCE	μg/L	18.70 ± 1.4	0.00	0.02 ± 0.03	0.00
TCE	μg/L	149.80 ± 17.5	0.02 ± 0.01	0.10 ± 0.01	10.10 ± 0.2
cis−1,2-DCE	μg/L	22.30 ± 3.5	0.00	0.00	0.08 ± 0.02
1,1-DCE	μg/L	0.17 ± 0.01	0.00	0.00	0.00
trans−1,2-DCE	μg/L	0.39 ± 0.05	0.00	0.00	0.00
VC	μg/L	0.11 ± 0.01	0.00	0.00	0.00
CT	μg/L	33.8 ± 1.8	0.00	0.00	0.013 ± 0.004
CF	μg/L	0.00	0.00	0.00	0.00
MC	μg/L	0.00	0.00	0.00	0.00
ethene	μg/L	0.00	0.00	0.00	0.00
ethane	μg/L	0.01	0.00	0.00	0.00
methane	μg/L	0.045 ± 0.0035	0.10	0.00	0.00
^{14}C-DIC	pMC	76	121	106	113
δ^{13}C-DIC	‰	−20.1	−21.1	−21.3	−22.8

[a] Measured >24 h after collection.

TABLE 2. Subsurface Hydrogeological Parameters at the Savannah River Site (*28, 29*)

parameter	units	value
Shallow Groundwater		
horizontal hydraulic conductivity (K_h)	cm/s	2.3×10^{-2}
vertical hydraulic conductivity (K_v)	cm/s	1.1×10^{-2}
specific yield (S_y)		0.29
storativity (S)		0.067
effective porosity		0.15
pore velocity	cm/s	1.1×10^{-3}
horizontal gradient	ft/ft	0.007
sand:silt:clay	%	89:00:11
soil texture		sand
bulk density	g/mL	1.56
particle density	g/mL	2.65
pore space	%	41
Unsaturated Sediment		
intrinsic permeability (average)	Darcies	8.42
vertical permeability (K_z)	Darcies	1.68
transmissivity	ft^2/min	0.023−0.076
SVE radius of influence	m	12−33
water table	m	13
sand:silt:clay	%	90:00:10
soil texture		sand
bulk density	g/mL	1.35
particle density	g/mL	2.63
pore space	%	49

was 14−18 m below ground surface (bgs) (data not shown). Sedimentary carbonates that can influence ^{14}C values were absent in these shallow sediments (*30*).

Sample Collection and Analysis. Vadose zone soil gas samples were collected between 12/18/00 and 4/11/01 and coincided with SVE shut-down. Samples were collected from source wells TVM-1V, 1U, 2V, 2U, 3U, 4V, 4U, and TNX-28D and 33D twice weekly initially, followed by at least twice monthly for 114 days (Figure 1A). TVM-2U data were excluded due to a leaking well cap discovered on day 30. The groundwater monitoring wells designated "U" have 3-m screens with approximately 1.5 m in the vadose zone at 12-m bgs and 1.5 m in the saturated zone. Vadose zone monitoring wells labeled "V" have 0.3-m screens located approximately 10-m bgs. Flood plain groundwater wells TNX-28D and 33D were installed at a total depth of 4.6 m with a screened section between 1.5- and 4.6-m bgs. An increase in groundwater elevation prevented vadose zone sampling of the flood plain wells after day 58, and TVM-3U was only periodically sampled due to processing time constraints.

Vadose zone air samples were collected by attaching a PVC cap fitted with a 1/4-in. Swagelok fitting to the top of the well. A small vacuum pump was attached to the PVC cap, and at least three well volumes of air were purged before collection of the sample into 1- or 5-L Tedlar bags. Soil air samples were analyzed for O_2, CO_2, methane, ethene, ethane, PCE, TCE, DCEs, VC, CT, CF, MC, and TCFM or processed for δ^{13}C and ^{14}C of CO_2 in our laboratory within 12 h after collection. Also, gas samples were screened at each time point for petroleum hydrocarbons that may interfere with carbon isotopic ratios, but no petroleum hydrocarbons were detected in the TNX area. Detection limits for benzene, toluene, ethylene benzene, and xylene were 1 ppb (v/v).

O_2 and CO_2 concentrations were measured using a Varian CP-3800 gas chromatograph (GC) equipped with a thermal conductivity detector (TCD) and a 60/80 Carboxen 1000 column (15 ft × 1/8 in. ss, Supelco). Detection limits were approximately 0.1 and 0.03% (v/v), respectively. Methane, ethane, and ethene concentrations were measured using a Varian 3700 GC equipped with a flame ionization detector (FID) and a 60/80 Carbopack B column packed with 1% SP 1000 (8 ft × 1/8 in. ss, Supleco). The analysis of chlorinated

and petroleum hydrocarbons was performed on a Varian 3800 GC/Saturn 2000 mass spectrometer (MS) with a Varian CP-8200 autosampler equipped for solid-phase microextraction (SPME) with a SPME fiber coated with 75-μm Carboxen/poly(dimethylsiloxane) (Supelco, Bellefonte, PA). Details of all analyses are reported in Supporting Information.

Groundwater samples were collected by SRS personnel from TBG 4 located in the source area on 6/07/01 (Figure 1A). Control well TNX-24D and down-gradient wells TNX-28D and 33D were sampled on 6/27/01 (Figure 1B). Field measurements by SRS personnel included dissolved O_2, pH, conductivity, and temperature. The following day in our laboratory, groundwater DIC was extracted and purified for isotopic analysis, and geochemical parameters DIC, nitrate, nitrite, sulfate, sulfide, total iron, iron(II), iron(III), chloride, and ammonia were measured using colorimetric analysis (Hack Company; Loveland, CO).

Sediment samples were processed for carbon isotopic analysis using quartz tubes and copper oxide wire (*31, 32*). The resulting CO_2 from sediment combustion, vadose zone CO_2, and CO_2 stripped from groundwater samples was released into a purification line in a He stream and purified cryogenically and chemically using liquid nitrogen (−196 °C), pentane slush (−129 °C), ethyl alcohol slush traps (−117 °C), and magnesium perchlorate. The purified CO_2 was measured using a Baratron (MKS Instruments) capacitance manometer and sealed under vacuum in 6-mm Pyrex ampules and sent to an analytical isotope laboratory for $\delta^{13}C$ or ^{14}C analysis. The mean $\delta^{13}C$ value for a known CO_2 isotopic standard (−44.06 ± 0.04‰) processed identically as the vadose zone samples was −43.99 ± 0.07 (relative percent recovery = 99.85 ± 0.16; n = 6) and verified that limited fractionation occurred during sample processing. Stable carbon isotope ratios were reported in the standard δ notation in per mil (‰) as

$$\delta(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1000 \qquad (1)$$

where R is the ratio of ^{13}C to ^{12}C and the $\delta^{13}C$ standard is the belemnite fossil of the Cretaceous Peedee formation of South Carolina (PDB). The ^{14}C activity was expressed as pMC and is the ratio of the activity of the sample to the corrected activity of the oxalic acid standard **(***33***)**. ^{14}C precision was ± 0.9 pMC.

Vadose zone air samples collected from wells TVM-1U, 2V, 2U, and 4V on day 70 and TVM-1V and 4V collected on day 80 were sent to the University of Waterloo, Canada, for $\delta^{13}C$ analysis of PCE, TCE, DCE, and CT. A combination of SPME-GC-isotope ratio MS allowed $\delta^{13}C$ analysis of low concentrations of CHCs in vadose zone air (Supporting Information).

Statistical Analysis. All statistical analyses were performed using SAS (*34*), with 0.05 significance level. A mixed model was utilized to perform an analysis of covariance (ANACOVA) to determine if there were significant differences in the rate of CO_2 production and O_2 depletion between source wells. The mixed model is a general linear model modified to account for repeated sampling from each well. A general linear model also was utilized to determine the regression coefficients for O_2 depletion and CO_2 production. All correlations were calculated using Pearson's correlation.

Results and Discussion

Sediment Characterization. Geochemical and isotopic analysis of sediment organic matter (SOM) suggested that SOM mineralization to vadose zone CO_2 would be insignificant at the monitored depth (Table 3). Total organic carbon was low (8−136 mg/kg) and averaged 22 ± 3 mg/kg at 9-m bgs. $\delta^{13}C$ of SOM averaged −24.6 ± 1.7‰ which is typical of C_3 vegetation. ^{14}C values of SOM in the vadose zone were significantly higher than SOM in the saturated zone at 18 m-bgs (Table 3). SOM-^{14}C values at 9-m bgs (168−174 pMC) resembled atmospheric levels from the 1960s when atmospheric CO_2 was highly contaminated with artificial ^{14}C from above-ground nuclear weapons tests. Maximum increases in atmospheric ^{14}C content occurred in 1961 and 1962 (*35*) and ^{14}C concentrations in plant tissue reached a peak (up to ca. 200 pMC) in 1964 and declined thereafter (*36*). It also is possible that these elevated ^{14}C values reflect releases of radiocarbon from SRS during its period of nuclear materials production. The ^{14}C value of SOM in the saturated zone (88 pMC) corresponded to an age of 1060 ± 45 yrs old and suggested that the limited amount of sediment organic carbon present was recalcitrant and not a significant contributor to DIC.

TABLE 3. Geochemical Analysis of Vadose Zone and Saturated (>13 m-bgs) Sediment Collected in the Source Area

well	depth (m-bgs)	total organic carbon (mg/kg)	$\delta^{13}C$ (‰)	^{14}C (pMC)
TSV-B1	9	20	−24.5	
TSV-B2	3	136	−24.7	
TSV-B2	6	66	−25.3	
TSV-B2	9	21 ± 3	−23.5	174
TSV-B2	12	43	−25.3	
TSV-B2	15	14	−25.7	
TSV-B2	18	8 ± 1	−25.1	88
TSV-B3	3	14	−21.1	
TSV-B3	6	2	−21.3	
TSV-B3	9	23 ± 6	−24.8	168
TSV-B3	12	27	−26.1	
TSV-B3	15	99	−25.3	
TSV-B4	9	8 ± 9	−26.8	

Groundwater Analysis. Geochemical, contaminant, and carbon isotopic analysis suggested that reductive dechlorination of chlorinated solvent and complete mineralization to CO_2 was occurring in groundwater located in the source area (Table 1). Available electron acceptors were O_2 and nitrate. Groundwater DO in the source area (TGB-4) was significantly depleted (2.4 mg/L) compared to control well TNX-24D which was near saturation (9.2 mg/L). Nitrate, a reported contaminant at this site (*28*), was elevated in TBG-4 (21.7 mg/L), and nitrite was measurable compared to control well TNX-24. Chloride was not a good indicator of reductive dechlorination with greater chloride concentrations in the control well (11.5 mg/L) than the source or down-gradient wells (1.9−9.2 mg/L).

DCEs accounted for 10.2% of CHC concentration, and trace levels of ethane in TBG-4 suggested complete but limited reductive dechlorination. CT concentrations in all wells were relatively low (0.01−33.8 μg/L) compared to vadose zone concentrations (up to 3.3 ppm in TVM-1V), and no CT metabolites were detected.

A depleted ^{14}C-DIC value of 76 pMC in TBG-4 compared to 121 pMC for the control well DIC indicated that CHC mineralization was occurring in groundwater within the source area. $\delta^{13}C$ was also enriched by 1.0 ‰ in TBG-4 compared to TNX-24D, but more data from groundwater wells in the source area are necessary to assess the variation of $\delta^{13}C$-DIC values.

Vadose Zone Geochemical Analysis. *O_2 and CO_2.* Vadose zone O_2 and CO_2 were measured after SVE shut-down in an effort to detect microbial chlorinated solvent mineralization. The vadose zone within the source area was oxygenated to near atmospheric levels either naturally or induced by previous SVE operation (Figure 1, Supporting Information). CO_2 concentrations at day 0 were elevated between 2.0 and

TABLE 4. Regression and Correlation Results of Vadose Zone O_2, CO_2, ^{14}C, and $\delta^{13}C$ Production and Consumption Rates Measured over Time in the TNX Area Source Wells and Relatively Uncontaminated Peripheral Flood Plain Wells at the Savannah River Site

well ID	parameter	CO_2 or O_2 (% d^{-1})	CO_2 or O_2 reg. (r)	O_2-CO_2 corr. (r)	^{14}C (pMC d^{-1})	^{14}C reg. (r)	$\delta^{13}C$ (‰ d^{-1})	$\delta^{13}C$ reg. (r)	$\delta^{13}C$-CO_2 corr. (r)
				Source Wells					
TVM-1V	O_2	−0.007	−0.29						
	CO_2	0.003	0.09	−0.94[a]	−0.13	−0.90	0.010	0.71	0.69[a]
TVM-1U	O_2	−0.006	−0.55						
	CO_2	0.003	0.40	−0.73[a]	−0.07	−0.89	0.001	0.09	0.84[a]
TVM-2V	O_2	−0.007	−0.51						
	CO_2	0.004	0.76	−0.71[a]	−0.23	−0.99	−0.015	−0.77	−0.60
TVM-3U	O_2	−0.002	−0.11						
	CO_2	0.005	0.56	−0.73	−0.28	1.00	−0.011	−0.57	0.92
TVM-4V	O_2	0.003	0.24						
	CO_2	−0.006	−0.64	−0.60[a]	−0.09	−0.45	0.003	0.33	−0.02
TVM-4U	O_2	−0.003	−0.46						
	CO_2	−0.001	0.32	−0.94[a]	−0.15	−0.60	−0.0003	−0.24	0.53
				Flood Plain Wells					
TNX-28D	O_2	0.006	0.40						
	CO_2	0.002	0.63	−0.45	−0.10	−0.98	−0.001	−0.44	−0.32
TNX-33D	O_2	−0.069	−0.92						
	CO_2	0.013	0.68	−0.90[a]	−0.05	−0.73	−0.024	−0.59	−0.49

[a] p-value < 0.05.

4.1% in the source wells and suggested that the surrounding vadose zone air that was pulled into the monitored area via SVE was elevated in CO_2 concentration (Figure 1A−C, Supporting Information). CO_2 concentrations and $\delta^{13}C$ values (discussed below) were typical of plant root respiration (*37*, *38*) and probably the main source of CO_2 within these sandy sediments with limited organic matter.

CO_2 production and a concurrent depletion of O_2 were statistically measured in all but three wells (TVM-4V, TVM-4U, and TNX-28D) (Table 4). A statistical difference in the production of CO_2 was not detected between individual wells (p-value = 0.45) or wells grouped by depth (p-value = 0.83). CO_2 production rates were low (0 to 0.013% (v/v) d^{-1}) and suggested aerobic biodegradation of contaminants was either limited or that contaminant concentrations were too low for this GC monitoring technique to effectively measure their oxidation to CO_2.

Contaminant Monitoring. TCE metabolites (DCE, VC, ethene, and ethane) and CT metabolites (CF and MC) were detected in low concentration in the majority of source wells and suggested that the indigenous microbial community was capable of anaerobically metabolizing the CHC mixture via reductive dechlorination under bulk aerobic conditions (Figure 2 and Figures 2 and 3, Supporting Information). This field site was not ideal for reductive dechlorination with low organic matter and oxygenated sandy sediments. Reductive dechlorination probably only occurred within anaerobic microenvironments of sediment pores. Ellis et al. (*39*) also described a field study where anaerobic processes occurred in dominantly aerobic groundwater, and they concluded that the anaerobic processes potentially occurred at the micropore level. Minimal methanogenesis was detected at this site with the highest average methane concentration in TVM-2V of 4.5 ppm (v/v) (data not shown).

The highest concentrations of contaminants were measured in source wells TVM-1U, 4V, and 4U with maximum TCE and CT concentrations of 12.7 ppm in TVM-1U (Figure 2B, Supporting Information) and 3.3 ppm in TVM-4V (Figure 3C, Supporting Information), respectively. A strong positive correlation between TCE, DCE, and CT concentrations over time in TVM-1V and 1U (r^2 = 0.61−0.93, p-values < 0.0015) suggested these compounds were revolatilizing into the soil air after SVE shut-down at day 0 (Figure 2, Supporting Information). This physical diffusion process, termed contaminant "rebound", is common during SVE shut-down at petroleum contaminated sites (*40*). Similar positive statistical correlations between TCE, DCE, and CT were detected in all source wells (data not shown). Thus, measurement of the reduction of CHC concentrations over time due to biodegradation was inhibited due to the physical diffusion of contaminants.

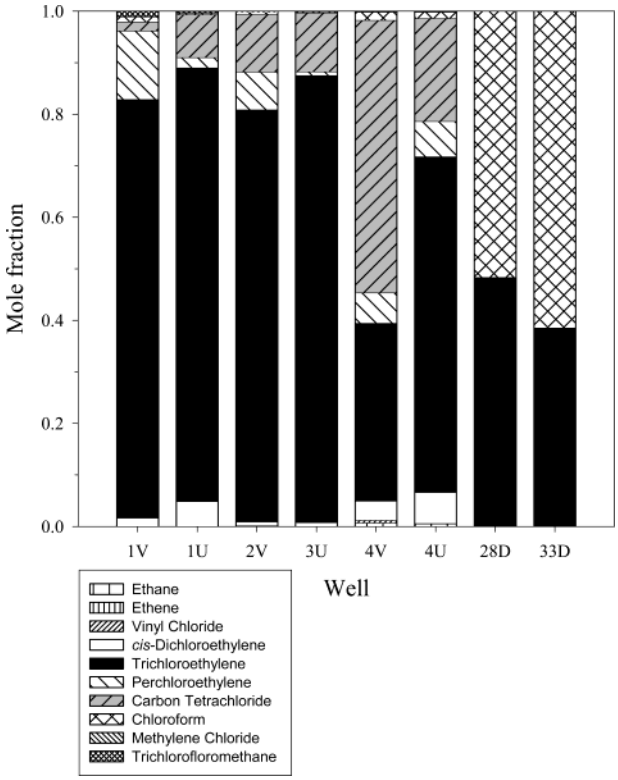

FIGURE 2. Molar fractions of CHC and metabolite concentrations within each vadose zone well on day 114. The mole fractions were calculated by dividing the concentration of each compound by the total concentration of chlorinated hydrocarbons and metabolites.

The extent of TCE and CT reductive dechlorination was examined by comparing the ratio of each contaminant to the total CHC concentration of its known biodegradative

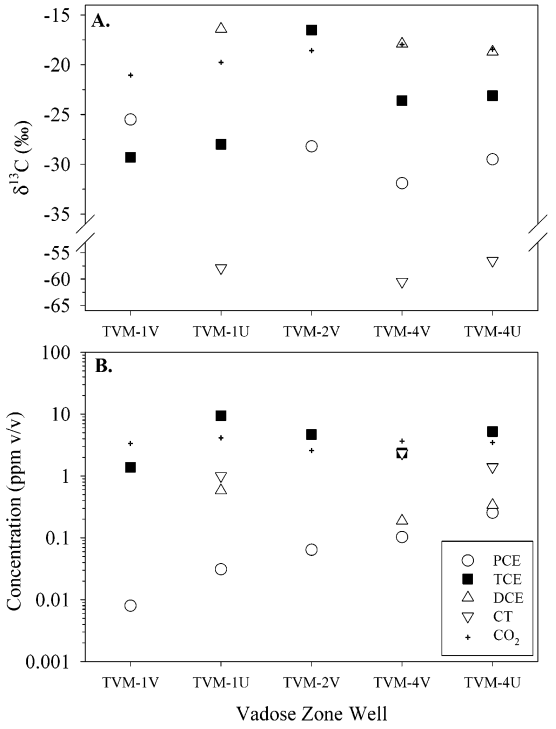

FIGURE 3. (A) $\delta^{13}C$ values of perchloroethylene (PCE), trichloroethylene (TCE), *cis*-1,2-dichloroethylene (DCE), carbon tetrachloride (CT), and carbon dioxide (CO_2) and (B) PCE, TCE, DCE, CT, and CO_2 concentrations in vadose zone wells measured on day 70 or 80 (TVM-1V and TVM-4V) at the Savannah River Site.

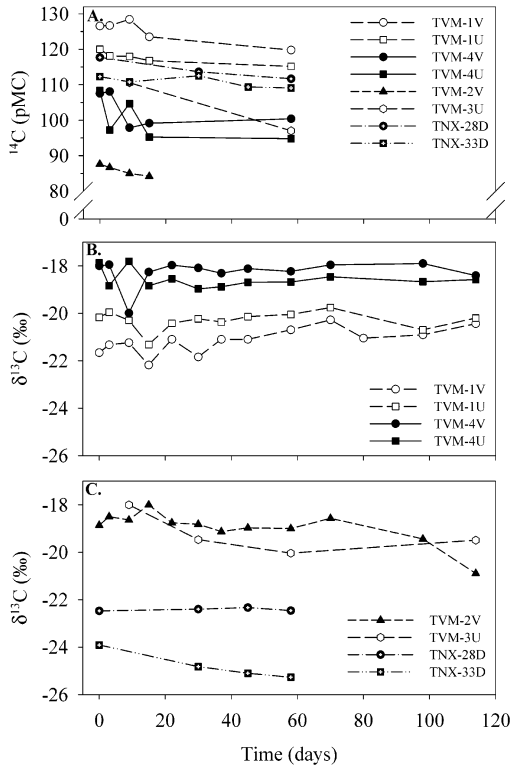

FIGURE 4. Vadose zone CO_2 values of (A) ^{14}C (in percent modern carbon (pMC)), and (B) and (C) $\delta^{13}C$ measured over 58 or 114 days in source (TVM) and flood plain (TNX) wells at the Savannah River Site.

pathway. Reductive dechlorination of TCE and CT was minimal with the metabolites accounting for less than 10% of total CHC concentration (Figure 2). CF was the main metabolite of CT reductive dechlorination and accounted for 4−5% of the total CT, CF, plus MC concentration, whereas MC accounted for only 0.04−0.07%. Similar TCE dechlorination ratios were measured with DCE accounting for 5−8% of the total TCE, DCE, VC, ethene, plus ethane concentration, whereas VC accounted for 0.01−0.8%. VC was detected on day 37 and complete transformation to ethene and ethane was not detected until day 98 when contaminant concentrations were highest after contaminant revolatilization (data not shown). Undetectable or low VC concentrations suggested either limited anaerobic transformation after DCE was produced, or rapid oxidation of VC to CO_2, both of which have been reported in laboratory studies (*1*).

Vadose Zone Isotope Analysis. *$\delta^{13}C$ of Contaminants.* Assessment of in situ reductive dechlorination was accomplished by measuring the $\delta^{13}C$ of individual chlorinated compounds in the vadose zone. Monitoring contaminant concentration alone was successful in detecting reductive dechlorination of TCE and CT but was limited in assessing the extent of biodegradation. $\delta^{13}C$ measurement of contaminants showed that DCE was always heavier than TCE by approximately 4−12‰ (Figure 3A). This suggested that a substantial amount of DCE had been degraded either via oxidation in the bulk aerobic vadose zone or via reductive dechlorination in the anaerobic microniches followed by oxidation of VC, ethene, and ethane in the bulk aerobic vadose zone. Microcosm studies have shown the initial DCE produced is depleted in ^{13}C relative to its parent compound (*13*−*15*). Over time, the carbon of DCE and metabolites becomes enriched in ^{13}C during extensive reductive dechlorination. Thus, extensive reductive dechlorination of DCE was indicated by $\delta^{13}C$ analysis, and DCE concentration may not be a good quantitative indicator for the progress of biodegradation.

$\delta^{13}C$ of PCE ranged from −31.9 to −25.5‰ and was inversely correlated with concentration ($r = -0.53$) with higher $\delta^{13}C$ values measured in wells with lower PCE concentrations (Figure 3A,B). PCE is a "parent"compound in that no other compound is being degraded to produce PCE, similar to CT at this site. Thus, as microbes discriminate against the ^{13}C-PCE molecule during reductive dechlorination, the residual PCE becomes enriched in ^{13}C (*13*−*15*). The measured $\delta^{13}C$ enrichment of PCE suggested that substantial reductive dechlorination of PCE was occurring at this site.

CT was highly depleted in ^{13}C relative to PCE, TCE, and DCE and measured between −51.4 and −60.5‰ (Figure 3A). $\delta^{13}C$ increased as CT concentration decreased ($r = -0.80$) suggesting the concentration decrease may be attributed to biodegradation. No previous measurement of $\delta^{13}C$-CT has been reported in the literature to the authors' knowledge. It was unclear whether microbial processes were solely responsible for the overall depletion of ^{13}C or if depletion was also due to the manufacturing process. We measured the $\delta^{13}C$ value of commercial CT (Supelco, Bellefonte, PA) and found relatively depleted values (−39.1 ± 0.3‰) compared to reported TCE (−31 to −27‰) and PCE ratios (−37 to −23‰ (*41*)) which suggested that a proportion of ^{13}C-CT depletion may be attributed to the manufacturing process.

^{14}C and $\delta^{13}C$ of Vadose Zone CO_2. ^{14}C values of vadose zone CO_2 decreased over time in all contaminated source and flood plain wells (Figure 4A). This measured depletion in ^{14}C suggested chlorinated solvents were completely aerobically or anaerobically biodegraded to CO_2 over time because no other significant depleted carbon source, such as carbonate minerals or petroleum hydrocarbons, was detected at this site. Also, fractionation due to carbon isotopic exchange or equilibrium processes would be minimal in these acidic sediments (*42*) and thus not account for measured ^{14}C reductions over time (up to 12 pMC in TVM-3U). To our

knowledge, these results are the first to provide qualitative evidence of complete in situ biodegradation of CHCs to CO_2.

^{14}C values varied between well locations and ranged from 84 to 128 pMC in TVM-2V and TVM-1V, respectively (Figure 4A). Overall, ^{14}C values were near or above modern activity and suggested that the majority of vadose zone CO_2 was from natural background inputs, particularly plant root respiration as also suggested by SOM geochemical and isotopic analysis. ^{14}C-CO_2 values in TVM-1V and 1U were initially elevated above measured atmospheric levels at this site (108 pMC). The source of elevated ^{14}C activity was unclear with numerous natural and anthropogenic possibilities. Ideally, several nearby control wells installed in uncontaminated areas could provide further insight into CO_2 dynamics, but a relatively large contaminant plume and strict drilling and sampling restrictions at this DOE facility limited available control sites.

^{14}C-CO_2 measurement was effective in detecting chlorinated solvent mineralization even though CO_2 was naturally elevated which may have diluted CO_2 derived from contaminant mineralization. TVM-2V and 3U had markedly lower CO_2 concentrations (Figure 1B, Supporting Information) and had between 1.5 and 4 times higher rates of ^{14}C depletion over time compared to other source wells (Table 4). Small inputs of contaminant-derived CO_2 (0 pMC) produced during biodegradation would have a greater effect in TVM-2V and 3U compared to the other source wells which have greater CO_2 concentrations.

$\delta^{13}C$-CO_2 trends over time were less pronounced compared to ^{14}C values (Figure 4B,C). $\delta^{13}C$-CO_2 values from natural contributions were elevated at this site and may have masked contaminant-derived CO_2. $\delta^{13}C$ of TCE (−16 to −29‰) and DCE (−16 to −19‰) was similar to the range of $\delta^{13}C$-CO_2 from root-respired plants (−5.6 to −33‰ (*26*, *27*)). The clearest indication of contaminant mineralization was measured in TVM-1V and 1U with a statistically significant, positive correlation between $\delta^{13}C$ values and CO_2 production over time ($r = 0.69$ and 0.84, respectively) (Table 4). This $\delta^{13}C$-CO_2 correlation corresponded to the measured depletion of ^{14}C over the same time scale indicating that a ^{14}C depleted carbon source with a relatively enriched ^{13}C value was being mineralized. Laboratory studies have demonstrated DCE and VC may become enriched in $\delta^{13}C$ through extensive microbial reductive dechlorination processes producing $\delta^{13}C$ values of these metabolites near or greater than 0‰ (*12*−*15*). $\delta^{13}C$ measurement of DCE in TVM-1U (−16‰) confirmed ^{13}C enrichment relative to CO_2 (Figure 3A) and thus DCE oxidation would match this scenario assuming limited DCE fractionation during mineralization.

^{14}C-CO_2 measurement over time identified in situ biodegradation of chlorinated solvents to CO_2 which had previously only been detected in laboratory studies. ^{14}C analysis was more effective than geochemical measurements (CO_2 and O_2) in detecting biodegradation and was successful even though CO_2 was naturally elevated which may dilute CO_2 derived from contaminant mineralization. $\delta^{13}C$ measurement of contaminants provided further insight into the extent of biodegradation over that of traditional contaminant monitoring alone. Monitoring ^{14}C and the $\delta^{13}C$ of contaminants worked effectively in vadose zone wells with varying contaminant and CO_2 concentrations demonstrating that it was a valuable field monitoring technique for microbial degradation of chlorinated solvents. The continued development of these field monitoring techniques may provide direct measures of microbial degradation of chlorinated pollutants in groundwater and subsurface sediments and improve remediation strategies at contaminated facilities.

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Supporting Information Available

Method details for analysis of O_2, CO_2, methane, ethene, ethane, CHC, and $\delta^{13}C$ of CHCs; O_2 and CO_2 concentration over time (Figure 1 SI), CHC concentration measured over time in vadose zone wells TVM-1V and 1U and TVM-4V and 4U (Figures 2 and 3 SI, respectively). This material is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) *Technical Protocol for Evaluating Natural Attenuation of Chlorinated Solvents in Groundwater;* EPA/600/R-98/128; U.S. Environmental Protection Agency, Office of Research and Development: Washington, DC, 1998.
(2) Bradley, P. M.; Chapelle, F. H. *Appl. Environ. Microbiol.* **1998**, *64*, 1560−1562.
(3) Bradley, P. M.; Chapelle, F. H. *Environ. Sci. Technol.* **1997**, *31*, 2692−2696.
(4) Bradley, P. M.; Chapelle, F. H. *Environ. Sci. Technol.* **1996**, *30*, 2084−2086.
(5) Bradley, P. M.; Chapelle, F. H. *Environ. Sci. Technol.* **2000**, *34*, 221−223.
(6) Hartmans, S.; de Bont, J. A. M. *Appl. Environ. Microbiol.* **1992**, *58*, 1220−1226.
(7) Freedman, D. L.; Danko, A. S.; Verce, M. F. *Water Sci. Technol.* **2001**, *43*, 333−340.
(8) Bouwer, E. J.; McCarty, P. L. *Appl. Environ. Microbiol.* **1983**, *45*, 1286−1294.
(9) Criddle, C. S.; DeWitt, J. T.; Grbic-Galic, D.; McCarty, P. L. *Appl. Environ. Microbiol.* **1990**, *56*, 3240−3246.
(10) Slater, G. F.; Ahad, J. M. E.; Lollar, B. S.; Allen-King, R.; Sleep, B. *Anal. Chem*. **2000**, *72*, 5669−5672.
(11) Harrington, R. R.; Poulson, S. R.; Denver, J. I.; Colberg, P. J. S.; Kelly, E. F. *Org. Geochem*. **1999**, *30*, 765−776.
(12) Hunkeler, D.; Aravena, R.; Cox, E. *Environ. Sci. Technol.* **2002**, *36*, 3378−3384.
(13) Slater, G. F.; Lollar, B. S.; Sleep, B. E.; Edwards, E. A. *Environ. Sci. Technol.* **2001**, *35*, 901−907.
(14) Bloom, Y.; Aravena, R.; Hunkeler, D.; Edwards, E.; Frape, S. K. *Environ. Sci. Technol.* **2000**, *34*, 2768−2722.
(15) Hunkeler, D.; Aravena, R.; Butler, B. J. *Environ. Sci. Technol.* **1999**, *33*, 2733−2738.
(16) Aggarwal, P. K.; Hinchee, R. E. *Environ. Sci. Technol*. **1992**, *25*, 1178−1180.
(17) Van de Velde, K. D.; Marley, M. C.; Studer, J.; Wagner, D. M. In *Monitoring and Verification of Bioremediation-Volume 5;* Hinchee, R. E., Douglas, G. S., Ong, S. K., Eds.; Battelle Press: Columbus, OH, 1995; pp 241−257.
(18) Aravena, R.; Frape, S. K.; Van Warmerdam, E. M.; Daimmie, R. J.; Moore, B. J. In *Isotopes in Water Research Management: Proceedings of a Symposium on Isotopes in Water Resources Management*; IAEA-SM-336/3; Vienna, Austria, 1996, p 32−42.
(19) Jackson, A. W.; Pardue, J. H.; Araujo, R. *Environ. Sci. Technol.* **1996**, *30*, 1139−1144.
(20) Landmeyer, J. E.; Vroblesky, D. A.; Chapelle, F. H. *Environ. Sci. Technol*. **1996**, *30*, 1120−1128.
(21) Aggarwal, P. K.; Fuller, M. E.; Gurgas, M. M.; Manning, J. F. Dillon, M. A. *Environ. Sci. Technol*. **1997**, *31*, 590−596.
(22) Aelion, C. M.; Kirtland, B. C.; Stone, P. A. *Environ. Sci. Technol*. **1997**, *31*, 3363−3370.

(23) Conrad, M. E.; Daley, P. F.; Fischer, M. L.; Buchanan, B. B.; Leighton, T.; Kashgarian, M. *Environ. Sci. Technol.* **1997**, *31*, 1463−1469.
(24) Kirtland, B. C.; Aelion, C. M.; Stone, P. A. *Biorem. J.* **2000**, *4*, 187−201.
(25) Boutton, T. W. In *Carbon Isotope Techniques*; Coleman, D. C., Fry, B., Eds.; Academic Press: San Diego, CA, 1991.
(26) Deines, P. In *Handbook of Environmental Isotope Geochemistry, Volume 1, the terrestrial environment*; Fritz, P., Fontes, J., Eds.; Elsevier Scientific Publishing Company: New York, 1980.
(27) Smith, B. N.; Epstein, S. *Plant Physiol.* **1971**, *47*, 380.
(28) *TNX Geosiphon Cell (TGSC-1) Phase I Deployment/Demonstration Final Report (U)*; WSRC-TR-00023, Rev. 0; Westinghouse Savannah River Company: Aiken, SC, 1998.
(29) *TNX Area Phase II Soil Vapor Extraction Test Treatability Study Report*; WSRC-TR-99-00051, Rev. 2; Westinghouse Savannah River Company: Aiken, SC, 2000.
(30) Fallaw, W. C.; Price, V. *Southeast. Geol.* **1995**, *35*, 21−58.
(31) Boutton, T. W. In *Carbon Isotope Techniques*; Coleman, D. C., Fry, B., Eds.; Academic Press: San Diego, CA, 1991.
(32) Sofer, Z. *Anal. Chem.* **1980**, *52*, 1389−1391.
(33) Stuiver, M.; Polach, H. A. *Radiocarbon* **1977**, *19*, 355−363.
(34) Statistical Analysis System, Version 6.12, Statistical Analysis System Institute, Cary, NC, 1998.
(35) Goh, K. M. In *Carbon Isotope Techniques*; Coleman, D. C., Fry, B., Eds.; Academic Press: San Diego, CA, 1991.
(36) Mazor, E. In *Applied Chemical and Isotopic Groundwater Hydrology*; Halsted Press: New York, 1991.
(37) Boynton, D.; Compton, O. C. *Soil Sci.* **1944**, *57*, 107−117.
(38) Rightmire, C. T. *Water Resour. Res.* **1978**, *14*, 691−692.
(39) Ellis, D. E.; Lutz, E. J.; Klecka, G. M.; Pardieck, D. L.; Salvo, J. J.; Heitkamp, M. A.; Gannon, D. J.; Mikula, C. C.; Vogel, C. M.; Sayles, G. D.; Kampbell, D. H.; Wilson, J. T.; Maiers, D. T. *Symposium on Natural Attenuation of Chlorinated Organics in Groundwater*; EPA/540/R-96/509; U.S. Environmental Protection Agency, Office of Research and Development: Washington, DC, 1997; pp 95−99.
(40) Aelion, C. M.; Kirtland, B. C. *Environ. Sci. Technol.* **2000**, *34*, 3167−3173.
(41) Warmerdam, E. M.; Frape, S. K.; Aravena, R.; Drimmie, R. J.; Flatt, H.; Cherry, J. A. *Appl. Geochem.* **1995**, *10*, 547−552.
(42) Mook, W. G.; Bommerson, J. C.; Staverman, W. H. *Earth Planet. Sci. Lett.* **1974**, *22*, 169−176.

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