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Bioremediation Journal

ISSN: 1088-9868 (Print) 1547-6529 (Online) Journal homepage: https://www.tandfonline.com/loi/bbrm20

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To cite this article: Brian C. Kirtland , C. Marjorie Aelion & Peter A. Stone (2000) Monitoring Anaerobic Natural Attenuation of Petroleum Using a Novel In Situ Respiration Method in Low-Permeability Sediment, Bioremediation Journal, 4:3, 187-201, DOI: 10.1080/10588330008951109

To link to this article: https://doi.org/10.1080/10588330008951109

Monitoring Anaerobic Natural Attenuation of Petroleum Using a Novel In Situ Respiration Method in Low-Permeability Sediment

Brian C. Kirtland,[1] *C. Marjorie Aelion,*[1,2*] *and Peter A. Stone*[3]

[1]Department of Environmental Health Sciences and [2]Marine Science Program, University of South Carolina, Columbia, SC 29208; [3]Ground Water, South Carolina Department of Health and Environmental Control, Columbia, SC 29201

Abstract: Assessing petroleum biodegradation rates is an important part of predicting natural attenuation in subsurface sediments. Monitoring carbon dioxide (CO_2) and methane (CH_4) produced in situ, and their radiocarbon (^{14}C), stable carbon (^{13}C) and deuterium (D) signature provide a novel method to assess anaerobic microbial processes. Our objectives were to: (1) estimate the rate of anaerobic petroleum hydrocarbon (PH) mineralization by monitoring the production of soil gas CH_4 and CO_2 in the vadose zone of low-permeability sediment, (2) evaluate the dominant microbial processes using $\delta^{13}C$ and δD, and (3) determine the proportion of CH_4 and CO_2 attributable to anaerobic mineralization of PH using ^{14}C analysis. Argon was sparged into the subsurface to dilute existing CO_2 and CH_4 concentrations. Vadose zone CO_2, CH_4, oxygen, total combustible hydrocarbons, and argon concentrations were measured for 75 days. CO_2 and CH_4 samples were collected on day 86 and analyzed for ^{14}C, $\delta^{13}C$, and δD. Based on CH_4 soil gas production, the anaerobic biodegradation rate was estimated between 0.017 to 0.055 mg/kg soil-d. CH_4 ^{14}C (2.6 pMC), $\delta^{13}C$ (–45.64‰), and δD (–316‰) values indicated that fermentation of PH was the sole source of CH_4 in the vadose zone. CO_2 ^{14}C (62 pMC) indicated that approximately 47% of the total CO_2 was from PH mineralization and 53% from plant root respiration. Although low-permeability sediment increases the difficulty of completely replacing in situ soil gas and assuring anaerobic conditions, this novel respiration method distinguished between anaerobic processes responsible for PH degradation.

Introduction

Natural attenuation (NA) of petroleum hydrocarbons (PH) requires adequate monitoring to ensure contaminants are contained on-site or destroyed by microbial processes. Intrinsic biodegradation is one of the most important processes that occurs during NA because petroleum contaminants are mineralized to carbon dioxide (CO_2) and/or methane (CH_4). During NA of PH, anaerobic biodegradation may dominate in the source area and estimating biodegradation rates becomes difficult. Microcosm studies often are utilized to measure anaerobic rates of petroleum biodegradation in soil and to provide a decisive evaluation of the biodegradation potential of the microbial community. Microcosm studies must be carefully designed, implemented, and interpreted to ensure appropriate extrapolation of the biodegradation rate constants to in situ environments.

In situ respiration tests are a common method used to estimate the rate of aerobic petroleum biodegradation in the vadose zone. This test usually involves injecting atmospheric air mixed with a helium tracer into the vadose zone and subsequently monitoring the depletion of oxygen and production of CO_2 over time. The method provides an estimate of the rate of aerobic biodegradation occurring in the vadose zone and is a common procedure in bioventing studies (Hinchee and

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[*] Corresponding author: Tel: (803) 777-6994; Fax: (803) 777-3391; E-mail: aelionm@sc.edu

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Bioremediation Journal *4*(3):187–201 (2000)

Ong, 1992; Sayles et al., 1997). These results may be confounded by CO_2 produced from the biodegradation of natural substrates, abiotic processes, and plant root respiration such as the degradation of humus, dissolution of carbonate minerals, diffusion from the atmosphere into shallow soils, and plant respiration. To overcome these limitations, we incorporated the use of radiocarbon (^{14}C), stable carbon ($\delta^{13}C$) and deuterium (δD) measurement with CO_2 and CH_4 soil gas monitoring over time to estimate the in situ rate of anaerobic petroleum biodegradation occurring in low permeability sediment.

Application of Isotopes to Biodegradation Studies

Characteristic fractionation of $\delta^{13}C$ values in CO_2 or $\delta^{13}C$ and δ deuterium (δD) of CH_4 occurs during biodegradation. ^{13}C and D isotopic ratios (R) are reported in the standard delta (δ) notation in units of per mil (‰) deviation from the standard:

$$\delta(‰) = \frac{R_{sample} - R_{standard}}{R_{standard}} \times 1{,}000 \qquad (1)$$

Where R is $^{13}C/^{12}C$ or $^{2}H/H$. Stable carbon isotopic results are reported relative to the Vienna PeeDee belemnite (VPDB) standard ultimately based on the Peedee Formation (Cretaceous) of South Carolina. The standard has an isotopic ratio typical of marine carbonates. δD values are reported relative to Vienna Standard Mean Ocean Water (VSMOW) (Coplen, 1994). $\delta^{13}C$ values of PH range from –34‰ to –20‰ with gasoline averaging –27 ± 2.3‰ (Deines, 1980). During aerobic mineralization of PH to CO_2, little fractionation of ^{13}C occurs, thus the CO_2 produced has a similar $\delta^{13}C$ value as its source. Plants using C_3 and C_4 metabolism produce, respectively, root-respired $\delta^{13}C$-CO_2 values (and organic matter) of approximately –22 to –33‰ (Deines, 1980) and –5.6 to –18.6‰ (Smith and Epstein, 1971). Atmospheric CO_2 has $\delta^{13}C$ values of –7.4 to –12‰, shifting diurnally and depending on the amount of air pollution (Boutton, 1991). Stable carbon isotopes have been used to verify aerobic petroleum biodegradation by comparing the $\delta^{13}C$ values of CO_2 in a remediation area to an uncontaminated area (Aggarwal and Hinchee, 1992; Aggarwal et al., 1997; Aravena et al., 1996; Cifuentes et al., 1996; Jackson et al., 1996; Landmeyer et al., 1996; Van de Velde et al., 1995).

Fractionation effects are more pronounced during anaerobic petroleum biodegradation. Landmeyer et al. (1996) found that sulfate reduction produced dissolved inorganic carbon (DIC) with $\delta^{13}C$ values of –18‰ and methanogenesis highly fractionated residual CO_2 to values between +2.6 to +11.9‰. During methanogenesis, CO_2 becomes enriched with ^{13}C, while CH_4 is depleted to between –40 and –65‰ for the fermentation process (i.e., acetate reduction) and between –60 to –110‰ for CO_2 reduction (Coleman et al., 1996; Whiticar et al., 1986). In contrast, microbial oxidation by methanotrophs in aerobic environments produces CO_2 approximately 5.0 to 29.6‰ lighter than the CH_4 substrate consumed (Barker and Fritz, 1981).

Deuterium analysis of CH_4 may further elucidate the microbial pathway by which CH_4 gas was formed because δD values distinguish between fermentation processes, CO_2 reduction, and thermogenic CH_4. δD values of CH_4 are between –400 to –250‰ and –250 to –170‰ for microbial fermentation and CO_2 reduction pathways, respectively (Coleman et al., 1996; Whiticar et al., 1986). Thermogenic CH_4, where present, can diffuse into shallower depths and generally has a δD range of –250 to –90‰, depending on the maturity of the substrate (Whiticar et al., 1986). ^{14}C is virtually absent from thermogenic CH_4 and biogenic CH_4 produced from PH. Thus, a combination of ^{13}C, ^{14}C, and δD analysis of CH_4 may be used to determine the dominant methanogenic process and the proportion of PH used to produce the CH_4.

^{14}C is formed in the upper atmosphere and is oxidized to form atmospheric $^{14}CO_2$. ^{14}C activities are reported as percent modern carbon (pMC) (Stuiver and Polach, 1977), which is the ratio of the activity of the sample (corrected for fractionation by normalizing the $\delta^{13}C$ = -25‰) to the activity of standard as follows:

$$^{14}C\,(pMC) = \left(\left[\frac{\delta^{14}C}{1{,}000}\right]+1\right)\left(1-2\left[\frac{25+\delta^{13}C}{1{,}000}\right]\right)\times 100\% \qquad (2)$$

The standard is defined as 95% activity of the National Bureau of Standards (NBS) oxalic acid and represents the age-corrected activity of wood grown between 1840 and 1860 AD. Living plants (and animals) are in ^{14}C-equilibrium with the atmosphere due to CO_2 exchange during photosynthesis. However, after death exchange stops and ^{14}C incorporated into tissues decays with a half-life of 5730 years. Incorporation of ^{14}C into plant organic matter, vadose zone CO_2, and CH_4 permits the use of radiocarbon as a tracer of petroleum biodegradation in soil and groundwater because PH are devoid of ^{14}C due to their ancient origin. Therefore, CO_2 or CH_4 produced from the aerobic or anaerobic mineralization of PH will also be devoid of ^{14}C, a signal that contrasts strongly with that derived from modern plants via root respiration and the degradation of recent humus found in most soils (ca. ≥100 pMC).

Radiocarbon has been employed recently for in situ biodegradation investigations of chlorinated solvents (Suchomel et al., 1990) and verified aerobic PH mineralization in zones where the $\delta^{13}C$ values of endogenous plant matter overlapped those of gasoline, producing nondefinitive results (Aelion et al., 1997; Conrad et al., 1997). Methanogenesis also produced isotopically heavy $\delta^{13}C$ values in CO_2 that made interpretation of $\delta^{13}C$ values difficult, but depleted ^{14}C values between 14 to 19 pMC confirmed that the majority of the CO_2 was from gasoline mineralization (Conrad et al., 1997).

To our knowledge, there are no published studies that combine CO_2 and CH_4 monitoring with isotopic measurements to assess the in situ rate of anaerobic petroleum biodegradation in the vadose zone. The objectives of this investigation were to evaluate a novel in situ anaerobic respiration test to (1) estimate the rate of petroleum mineralization in the source area by monitoring the production of CH_4 and CO_2 in the vadose zone of low permeability sediment, (2) evaluate the dominant microbial processes using stable carbon and deuterium analyses, and (3) estimate the proportion of CH_4 and CO_2 attributable to anaerobic PH mineralization using radiocarbon analysis.

Methods

Site Description

The field site is located in Columbia, SC, at a former gasoline station contaminated by gasoline from leaky underground storage tanks (USTs). In 1993, an air sparging/soil vapor extraction (AS/SVE) system, monitoring wells, and soil vapor probes were installed in the vadose zone as part of a pilot study of AS/SVE effectiveness in low permeability sediment (Figure 1) (Aelion et al., 1995; Widdowson et al., 1997). As with the AS/SVE tests, the current study was expected to be complicated by the low permeability of the sediments and represented a "worse case" scenario. Our study was conducted between January 13 and March 13, 1998 after AS/SVE was shut down and the site was approved for final remediation by NA.

The site is located in the transition zone between the Atlantic Upper Coastal Plain and the Appalachian Piedmont in low-permeability clayey soil. It contains three general soil strata: (1) clayey upper stratum (0 to

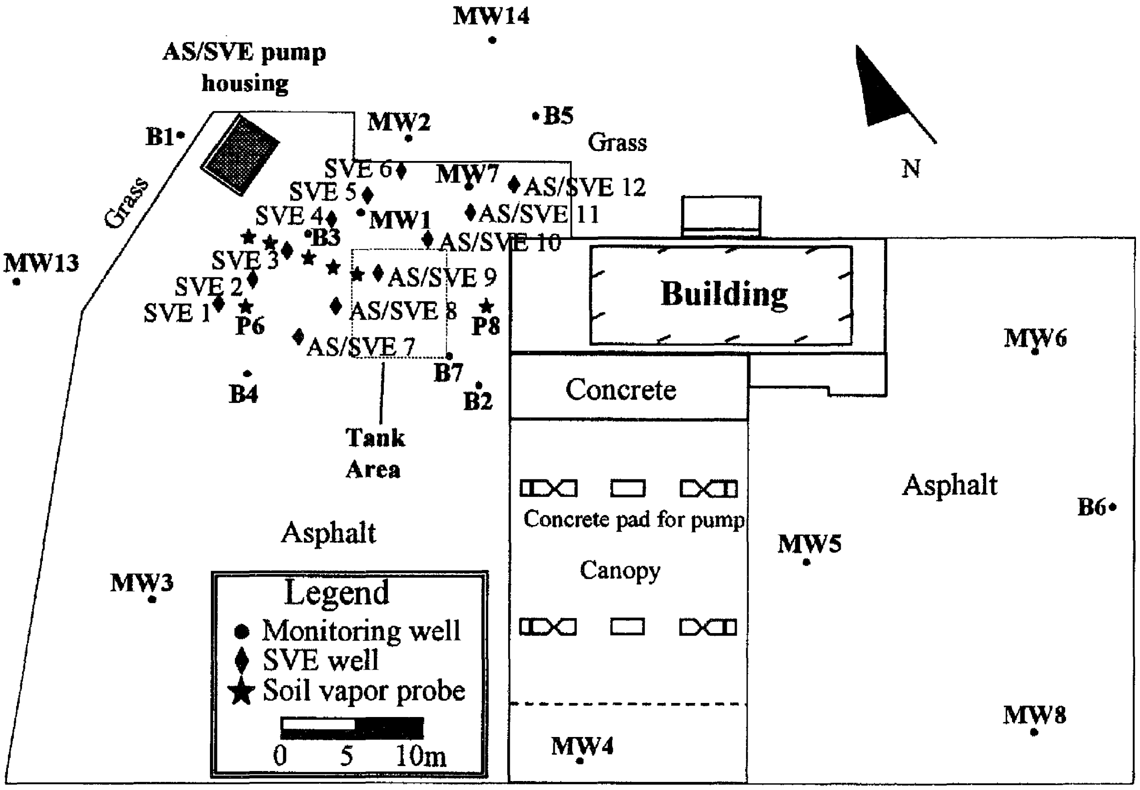

Figure 1. Site map showing the locations of the tank area, air sparging (AS) and soil vapor extraction (SVE) wells, soil vapor probes (P), and groundwater monitoring wells (MW, B).

4.6 m), (2) a sandy clay middle stratum (4.6 to 6.7 m), and (3) a lower stratum (6.7 to 10.7 m) that is predominantly sandy clay loam with intermittent quartz pebbles (Figure 2). Air permeability increases from 10^{-9} to 10^{-8} cm^2 in the upper zone to 1 to 3×10^{-7} in the lower strata (Widdowson et al., 1997). The saturated conductivity varies between 1.8×10^{-6} to 3.5×10^{-4} cm/sec from the upper to lower stratum, respectively. The water table is at approximately 6.7 m and groundwater flow is estimated between 1.0 to 4.8×10^{-6} cm/sec. Groundwater characteristics are listed in Table 1 and were collected after the AS/SVE system had been turned off for over 4 months.

The monitored area and much of the surrounding area are covered with asphalt, with some surrounding landscaped areas. A mixture of C_3 trees and C_4 grasses exists in the surrounding area that can contribute to vadose zone CO_2. Sedimentary carbonates that can influence ^{14}C values are considered to be low to essentially absent from these acidic geologic materials. Total organic carbon is low (316 mg/kg; S&ME, 1998) and is not expected be a significant contributor to CO_2 or CH_4 production in the vadose zone.

In Situ Respiration

An in situ anaerobic respiration test was conducted by injecting a large volume of industrial grade argon (Ar), an inert gas, into the subsurface to replace CO_2 and CH_4 concentrations in the vadose zone, followed by monitoring the production of CO_2 and CH_4 for 75 days. Ar was sparged into the subsurface at 2.4 psi through AS wells 7, 8, and 9 on January 14, 1998 (Figure 1). A total of 43 m^3 of Ar was injected over a 4-day period below the water table to flush the vadose zone. Injection into the groundwater limited bias from the diffusion of CO_2 or CH_4 from groundwater over the subsequent test period.

Sampling and Analysis

Vadose zone air samples were collected from SVE wells 3, 8, and 9 before Ar injection and for 75 days afterward. SVE 8 and 9 were located in the same borehole as AS 8 and 9, while SVE 3 was located approximately 6 m away. Gas exchange was not expected to be as complete for SVE 3 as for SVE 8 and 9. Samples were analyzed for oxygen, CO_2, total combustible hydrocarbons (TCH), Ar, and CH_4 concentration using portable meters and gas chromatography (GC) within 24 h of collection. At least three well volumes of vadose zone air were removed prior to sample collection into 5-L Tedlar™ bags. Samples were analyzed using a CO_2 meter (GasTech RA411A; range: 0 to 4.975%) and a hydrocarbon/O_2 meter (GasTech GT201; range 0 to 10,000 ppm, 0 to 100% lower explosive limit [LEL]). Both hand meters were calibrated at each sampling time. The CO_2 hand meter was calibrated using a 2.5% CO_2 gas standard and ultra-high purity (UHP) nitrogen. The oxygen hand meter was calibrated using UHP nitrogen (GasTech) and zero-grade air (National Welders Supply Co.). The hydrocarbon sensor was calibrated using 2.5% CH_4/balance air (50% LEL) (GasTech). However, oxygen and CO_2 values were verified in the laboratory using a Varian 3700 GC equipped with a thermal conductivity detector (TCD) and 60/80 Carboxen 1000 column (15 ft × 1/8 inch ss, Supleco) (Aelion et al., 1996).

CH_4 concentrations were measured using a Varian 3700 GC equipped with a flame ionization detector (FID) and a megabore DB-1 capillary column (30 m × 0.53 μ, J&W Scientific). Off-scale gas samples were diluted with atmospheric air using a small vacuum pump and flow meter into a new Tedlar™ bag. A 500 μL gas-tight syringe (Gas-Lock) was used to remove gas samples from the Tedlar™ bags. Both GCs were equipped with a gas sampling valve (Valco Instruments, Inc.) for precise gas injection volumes.

Ar was measured indirectly using GC because oxygen and Ar co-elute. However, Ar gas does not interfere with the electrochemical oxygen sensor in the hand meter. GC area counts due to oxygen concentration (measured using the hand meter) were subtracted from total GC area counts (Ar + O_2). This method was effective with estimated errors < 10% because oxygen concentrations were always low (0.2 to 1.6%) in the SVE wells, thus the majority of GC area counts were due to Ar concentration. The biodegradation rate was calculated from the first 25 days of CH_4 production during which oxygen levels were below 1% in the SVE wells, Ar concentrations were relatively stable, and both CO_2 and CH_4 rates were more linear.

Carbon and Deuterium Isotope Analysis

$\delta^{13}C$ was measured from free product (weathered unleaded gasoline) collected from groundwater monitoring well B4 (Figure 1) on February 18, 1997. 20 μL of free product was sealed under vacuum in a quartz tube and heated to 900°C for 1 h with copper oxide as an oxidant (Boutton et al., 1983). The resulting CO_2 was cryogenically purified using liquid nitrogen and ethanol slush traps and sealed in a 6-mm glass ampule and sent to an analytical laboratory for $\delta^{13}C$ analysis.

CO_2 and CH_4 samples were collected from SVE 8 on day 86 of the study. Soil air samples were routed through a flow meter then to a CO_2 trap that consisted of a gas-washing bottle containing 500 mL of repurified

Groundwater Data	
Total Petroleum Hydrocarbons	< 1 - 370 mg/l
BTEX[a] levels	ND[b] - 160 mg/l
Water table	6.1 - 7.6 m
pH	3.8 - 6.3
Dissolved oxygen	0.24 - 7.32 μg/l
Hydraulic conductivity (K_{sat})	1.8×10^{-6} to 3.5×10^{-4} cm/s
Average temperature	24.3 °C
Soil Data	
Total Petroleum Hydrocarbons[c]	n/d - 7,400 mg/kg
BTEX[c] levels	20,000 - 50,000 mg/m^3
Total Petroleum Hydrocarbons[d]	102 mg/kg (diesel fraction)
BTEX[d] levels	95 mg/kg
PAH[d,e] levels	18 mg/kg
Air-phase permeability (K_{air})	1.6 to 2.2×10^{-7} cm^2
Bulk density	1.431 g/cm^3
Particle density	4.8 g/cm^3
Porosity	0.3
Total organic carbon	316 mg/kg
Soil moisture content	0.1

[a] Benzene, toluene, ethylbenzene, *m,o,p*-xylene; [b] Non-detect; [c] Soil samples collected in 1991 from MW1-12 and B1-7; [d] Soil sample collected in 1997 from B7 at 6.1 m (S&ME, 1998); [e] Polyaromatic hydrocarbons (EPA Method 8100)

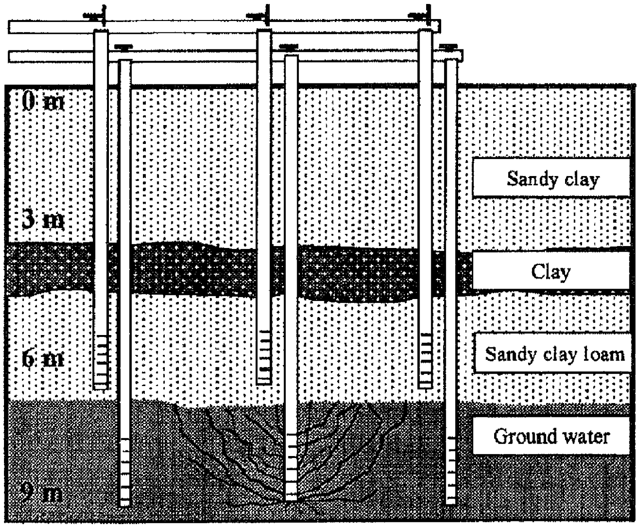

Figure 2. Subsurface characteristics and a schematic of the subsurface layers.

Table 1. Groundwater characteristics from samples collected in December 1996

	Water level	Dissolved O_2	Temperature	Conductivity		BTEX[b]	Sulfide	Sulfate	Nitrate	Total iron	Ferrous iron	Ferric iron	Ammonia
Well	m-bgs[a]	mg/L	°C	μΩcm	pH	mg/L	mg/L	mg/L	mg/L-N	mg/L	mg/L	mg/L	mg/L (NH_3-N)
MW1	8.0	ND[c]	NA[d]	140	4.97	160	0.073	BD[e]	0.23	2.28	0.96	1.32	0.53
MW2	8.0	ND	NA	110	5.23	83	0.013	BD	0.01	2.95	2.61	0.34	0.38
MW3	7.6	0.2	22.4	50	4.79	14	0.086	BD	0.02	22.9	1.53	21.37	0.08
MW5	7.9	0.2	22.7	70	4.90	83	0.024	BD	BD	2.95	1.41	1.54	0.03
MW7	NA	ND	NA	20	5.57	3	0.017	BD	0.3	0.06	BD	0.06	BD
MW9	6.5	0.11	24.9	220	4.57	BD	0.014	BD	5.8	BD	BD	BD	1.62
MW10	6.6	0.41	26.5	120	4.90	1	BD	BD	4.7	BD	BD	BD	0.01
MW14	7.8	0.97	23.6	100	4.47	27	0.042	BD	5.8	BD	BD	BD	3.22

[a] *Meters below ground surface.*

[b] *Benzene, toluene, ethylbenzene, xylenes.*

[c] *Non-detect.*

[d] *Not analyzed.*

[e] *Below detection.*

5-N sodium hydroxide as a CO_2 trap (Aelion et al., 1997). The CO_2 samples were sent to an analytical laboratory where the ^{14}C activity ratio was determined using an accelerator mass spectrometer (AMS). A small aliquot of the sample was used for ^{13}C analysis.

A 1-L Tedlar™ bag sample of vadose zone air containing approximately 2% CH_4 also was collected from SVE 8 and sent to an analytical laboratory for ^{14}C, $\delta^{13}C$ and δD analysis of CH_4. For ^{14}C determination, the CH_4 sample was combusted online to CO_2, cryogenically purified, and analyzed by an AMS.

In quantifying the percent of total vadose zone CO_2 attributable to the anaerobic mineralization of gasoline, a two end-member mixing equation was used as described by Aelion et al. (1997):

$$CO_{2pet} = \left(1 - \frac{pMC_{sample}}{pMC_{unc.}}\right) \times 100\% \tag{3}$$

Where $CO_{2\ pet.}$ is the proportional contribution from petroleum mineralization, $pMC_{unc.}$ is the measured pMC value from the uncontaminated site (117.9 pMC; Aelion et al., 1997), and pMC_{sample} is the measured pMC value of the sample.

Biodegradation Calculations

The anaerobic biodegradation rate of PH was estimated using toluene as the representative hydrocarbon and equations modified from Hinchee and Ong (1992). Studies have shown that toluene is readily mineralized to CO_2 and CH_4 under methanogenic conditions (Edwards and Grbić-Galić, 1994; Grbić-Galić, 1990; Grbić-Galić and Vogel, 1987; Hunt et al., 1997). The stoichiometric relation for the methanogenic mineralization of toluene is given as follows:

$$C_7H_8 + 5H_2O \rightarrow 2.5CO_2 + 4.5CH_4 \tag{4}$$

Utilizing the mass-mole relation and the measured rate of CH_4 production in the vadose zone, the estimated anaerobic biodegradation rate is:

$$K = \frac{K_{CH_4} A\, D_{CH_4}\, C_{TOL}}{100} \tag{5}$$

Where K is the biodegradation rate (mg-toluene/kg soil-d), K_{CH4} is the measured CH_4 production rate (% (v/v)/d) in the vadose zone, A is the air volume per mass of soil (L/kg), D_{CH4} is the density of CH_4 at 10°C (690 mg/L), and C_{TOL} is the toluene/CH_4 mass ratio (1.28). Site-specific soil parameters used in these calculations were bulk density (1431 kg/m^3), porosity (0.3), and the air-filled pore space (0.21 L/kg). The incorporation of carbon into biomass was excluded because biomass yields of anaerobic microbial cultures are usually low. Biomass yields of 5% have been reported for toluene degradation under methanogenic conditions (Edwards and Grbić-Galić, 1994). A General Linear Model (SAS Institute, 1996) of percent CO_2 and CH_4 over 25 days was utilized to estimate the production rate of each gas.

Results and Discussion

Soil Gas

Before Ar was injected into the subsurface, preliminary soil gas analyses suggested that anaerobic petroleum biodegradation was occurring in the vadose zone (Figures 3 and 4). CO_2 levels were elevated to between 5.7 and 9.6% in SVE wells 3, 8, and 9 (Figure 3A), and CH_4 was also present at concentrations between 0.01 and 0.4% (Figure 3B). Oxygen levels were between 0.3 and 1.9% (Figure 4A). All SVE wells were heavily contaminated with TCH ranging from approximately 32,000 to 39,500 ppm (Figure 4B).

Ar was effectively delivered to a wide area in the vadose zone, ranging between 48% in SVE 3 to 69% in SVE 8 after four days of Ar sparging (Figure 4C). As expected, the low permeability of the subsurface prevented complete Ar saturation. CO_2 and CH_4 concentrations were effectively reduced 3.8- to 1.5-fold, respectively, in SVE 8. CO_2 was reduced 1.2-fold in SVE 3, and no reduction was measured in SVE 9 (Figures 3A and B). In previous AS/SVE studies at this site, SVE 8 had approximately twice the flow rate of SVE 3 or 9, indicating greater air permeability in this region that may be responsible for the larger measured Ar concentrations and reduction in CO_2, CH_4, and TCH than the other SVE wells.

Significant increases in CO_2 and CH_4 concentrations after Ar injection in SVE 8 suggested that anaerobic petroleum biodegradation was occurring in the vadose zone. Ar levels were stable during the first 25 days, suggesting that the observed increases in CO_2 and CH_4 were biological and not attributable to diffusion (Figure 4C). The maximum calculated CO_2 production rate was 0.08%/d (Figure 3A), and the concurrent CH_4 production rate was 0.03%/d (Figure 3B), or approximately one-third that of CO_2. There was a significant positive correlation ($r^2 = 0.63$; $\alpha < 0.05$) between CO_2 and CH_4 production in SVE 8, suggesting that methanogenesis was occurring in this area.

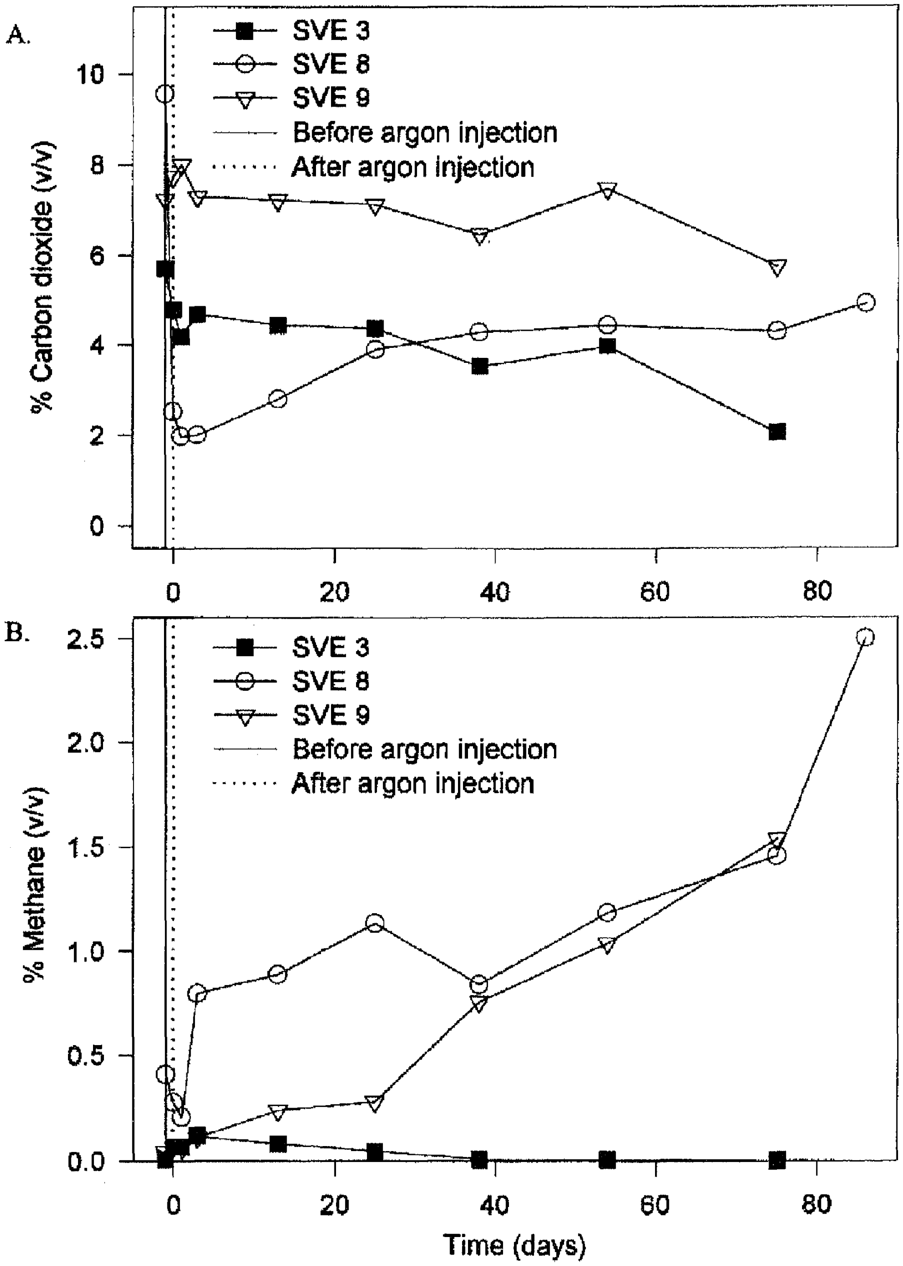

Figure 3. (A) CO_2 and (B) CH_4 concentrations in soil vapor extraction (SVE) wells 3, 8, and 9 over time. Solid line denotes time prior to Ar injection. Dotted line denotes time Ar injection stopped.

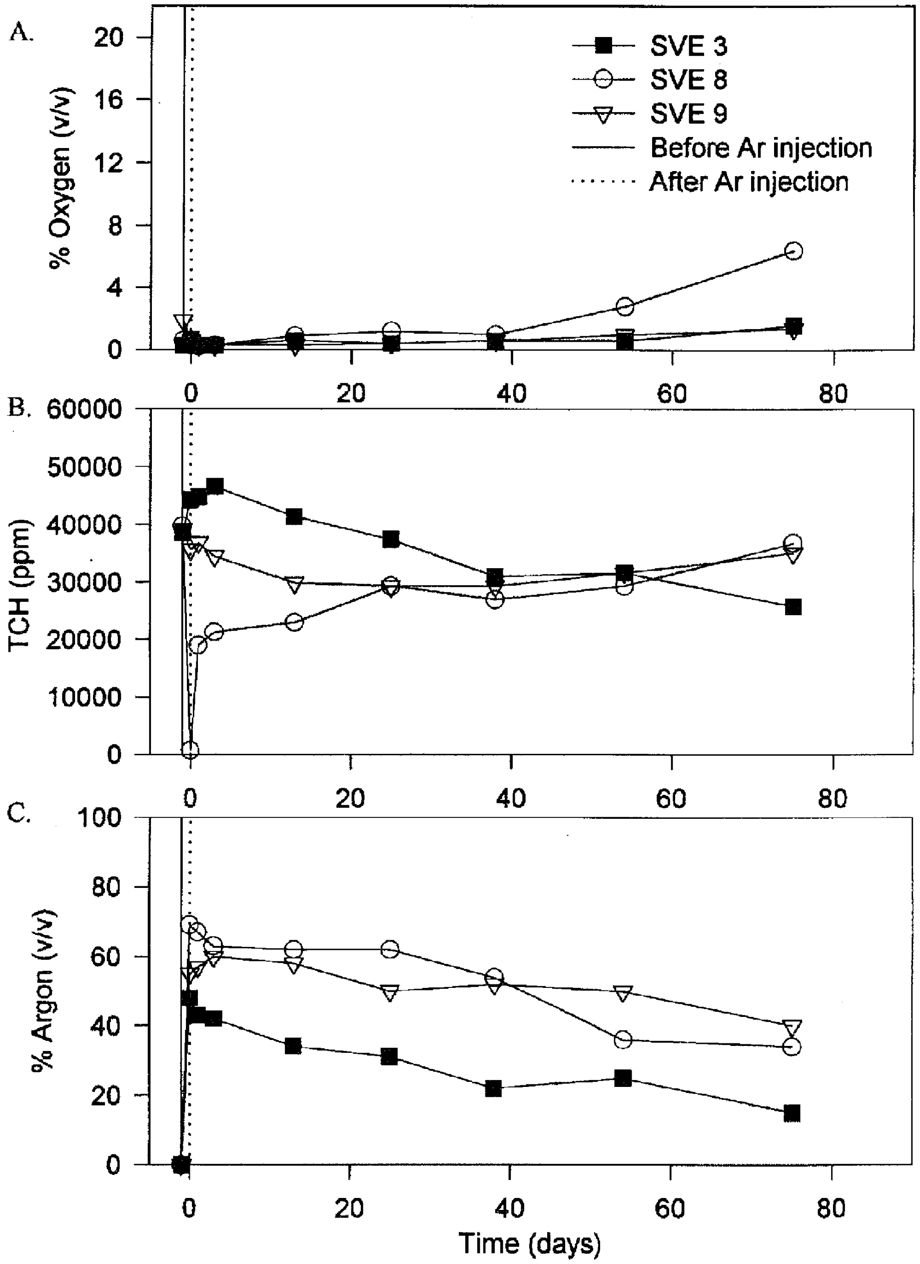

Figure 4. (A) O_2, (B) Total combustible hydrocarbons (TCH), and (C) Ar concentrations in soil vapor extraction (SVE) wells 3, 8, and 9 over time. Solid line denotes time prior to Ar injection. Dotted line denotes time Ar injection stopped.

On day 25, four times more CO_2 was produced in SVE 8 than expected based only on methanogenic toluene mineralization (Equation 4). By day 86, 2.95% CO_2 and 2.29% CH_4 were produced in SVE 8 (subtracting concentrations at day 1, which represent CO_2 not flushed out by Ar, from day 86), which was 2.3 times more CO_2 produced than predicted by toluene biodegradation stoichiometry. This suggested that plant root respired CO_2 from uncontaminated adjacent areas or from contaminated aerobic areas may have diffused into the source area as shown by a decrease in Ar concentration from 62 to 34% from day 25 to 75 in SVE 8 for which more gas was injected but for which gas diffused out more readily. Alternatively, other anaerobic processes were occurring simultaneously in the vadose zone, or alternative PH was being mineralized. Depleted nitrate and iron (III) concentrations in groundwater in MW1 and MW2 suggested that nitrate and iron (III) reduction may have occurred in the source area (Table 1), thus it is possible that these metabolic processes were contributing to CO_2 production in the vadose zone. Finally, using toluene as a model PH did not account for oxygenated compounds which would affect the mass mole relation (Equation 4).

CH_4 was produced in SVE 9 at a calculated rate of 0.02%/d based on the first 58 days of monitoring in which the Ar concentration was relatively steady and oxygen levels were $\leq$ 0.6%. This rate was about 1.6 times less than the calculated rate in SVE 8. When calculated over the same 25 days, CH_4 was produced at a rate of 0.01%/d, about 3.6 times less than the rate in SVE 8. In contrast to SVE 8, CO_2 levels in SVE 9 did not increase appreciably during the 75-day monitoring period. However, there was no initial drop in CO_2 concentrations due to Ar injection. Thus, results of soil gas monitoring of CO_2 could not discern the accompanying small increases in CO_2 concentration, or be used to calculate a CO_2 production rate.

Soil gas in SVE 3 did not indicate active anaerobic biodegradation (Figure 3). Initial CO_2 levels were reduced by Ar sparging only from 6 to 4%, and CH_4 was low (undetectable to 0.17%). Although SVE 3 remained anaerobic throughout the 75-day period with oxygen concentrations of approximately 0.5%, CO_2 and CH_4 concentrations decreased during this period. There was a significant positive correlation ($r^2 = 0.98$; $\alpha = 0.05$) between Ar and TCH, suggesting that diffusion of soil air may have been responsible for the decrease observed in both gases. SVE 3 was not coupled to an AS well and Ar was not sparged directly into it. SVE 3 had the least Ar measured (48%) at the beginning of the study, was located farthest from the sparge point SVE 8, and served as an indicator of the effective radius of influence (ROI) of Ar injection. Therefore, SVE 3 results were not included in the biodegradation rate estimate. Results from SVE 3 demonstrated that while this method is appropriate in low permeability sediments, application is well specific and limited by the ROI for Ar injection, as would be expected.

Carbon and Deuterium Isotopic Results

CO_2 Analysis. The $\delta^{13}C$ value of CO_2 in SVE 8 (–29.5‰) was less than a near-by uncontaminated CO_2 value of -23.9‰ collected from a grassy, wooded area from a similar depth (4 to 6 m) (Aelion et al., 1997). This depleted value suggested that the CO_2 was derived from the mineralization of PH (Table 2) because the contaminated site $\delta^{13}C$ value was similar to the measured $\delta^{13}C$ value of gasoline at this site (–28.2‰). It is unclear which bacterial metabolic process produced the CO_2 by examining the $\delta^{13}C$ value alone. The isotopically heavy $\delta^{13}C$-CO_2 produced from methanogenesis may have been masked by a mixture of CO_2 produced by other potential sources such as nitrate or iron (III) reduction, CH_4 oxidation, root respiration, or aerobic respiration. For example, SVE 8 was anaerobic until day 75, at which time oxygen levels increased to 6.4%. This increase in oxygen levels may have stimulated aerobic respiration of PH that would have produced lower $\delta^{13}C$-CO_2 values typical of the measured value (–29.5‰). If oxygen diffused naturally at this site and was not drawn in during well purging immediately prior to sample collection, then the rate of petroleum mineralization due to aerobic respiration could be significantly higher than methanogenesis.

The ^{14}C value of the CO_2 in SVE 8 measured on day 86 was 62 pMC. ^{14}C was depleted relative to CO_2 from root respiration or from atmospheric CO_2 (> 100 pMC) and demonstrated that gasoline biodegradation was occurring in the vadose zone. Using Equation 3, it was estimated that approximately 47% of the total CO_2 was from petroleum hydrocarbon mineralization regardless of the specific metabolic process that was responsible for its generation. In the current study, much less CO_2 was generated by anaerobic respiration than in previous aerobic studies. Thus, it represented a much smaller proportion of the total CO_2 generated. A CO_2 concentration of 2.6% out of the total CO_2 concentration in SVE 8 on day 86 (5.0%) was due to plant root respiration, and 2.3% was from petroleum hydrocarbon mineralization. The remaining proportion of CO_2 was assumed to be from root respiration because it was the only available modern carbon source that could produce significant levels of CO_2 at this site.

Table 2. Carbon and deuterium isotopic values for CO_2 and methane

Well	Date Sampled	Screen Depth (m)	Lab # (^{14}C-CO_2)	^{13}C-CO_2 ‰	^{14}C-CO_2 pMC	^{13}C-CH_4 ‰	^{2}H-CH_4 ‰	^{14}C-CH_4 pMC	Lab # (^{14}C-CH_4)
P9[a,b]	03/08/1996	4.0-6.1	Beta-94059[c]	-23.9	117.9 ± 1.0				
SVE 8[b]	05/20/1996	4.6-7.6	Beta-94050	-27.9	24.6 ± 0.2				
SVE 8	04/13/1998	4.6-7.6	Beta-117259	-29.5	62.0 ± 0.4	–45.6	–316.0	2.6 ± 0.2	I-18914[d]
Gasoline	02/18/1997			-28.2					

[a] Uncontaminated well.
[b] Aelion et al., 1997.
[c] Beta = Beta Analytic.
[d] I = Isotech Laboratories.

In previous studies, vadose zone CO_2 levels measured from a nearby uncontaminated site were elevated between 2.0 and 4.6% due to plant respiration and radiocarbon results of aerobic respiration in the source area suggested that between 1.3 and 1.9% CO_2 was due to plant root respiration (Aelion et al., 1997). Atmospheric diffusion of CO_2 is unlikely to occur at the monitored depth (6.7 m) in low permeability sediments over the time frame of this study.

CH_4 Analysis. Unlike the CO_2 produced, which may have come from multiple sources, radiocarbon values definitively determined that essentially all the CH_4 was derived from the fermentation of PH. The very low ^{14}C value of 2.6 ± 0.2 pMC may be overestimated as slight contamination from carbon of modern activity (etc., atmospheric CO_2) during sample preparation and analysis could account for this slight increase in ^{14}C. It is unlikely that approximately 2% of the CH_4 was from the fermentation of natural organic matter or reduction of modern CO_2. Natural organic matter was low (316 mg/kg) and the $\delta^{13}C$ and δD values did not support the occurrence of CO_2 reduction. By day 86, methanogenesis produced a CO_2 concentration of approximately 1.3% out of the 3.0% CO_2 (43%) measured in SVE 8 based on toluene biodegradation stoichiometry (Equation 4). It is unclear what proportion methanogenesis contributed to the total petroleum-derived CO_2 because the ^{14}C value of CO_2 on day 1 was not measured and petroleum-derived CO_2 accounted for approximately 47% of the CO_2 measured on day 86.

The metabolic pathway of CH_4 production in freshwater and marine environments has been investigated extensively utilizing the $\delta^{13}C$ and δD ratios of CH_4 to define CO_2 reduction, acetate fermentation, and thermogenic CH_4 (Coleman et al., 1996; Schoell, 1980; Whiticar et al., 1986) (Figure 5). These regions have been mapped from over 1000 methane samples collected from all over the world. During acetate fermentation, three-fourths of the hydrogen is derived from the methyl group of acetate and the remaining hydrogen from the associated water (Pine and Barker, 1956). In contrast, CO_2 reduction derives all the hydrogen from the surrounding water that is less depleted in δD.

No ethane and low propane concentrations (0.9 ppb) were measured from the day 86 soil gas sample, which suggested that the observed methane at this site

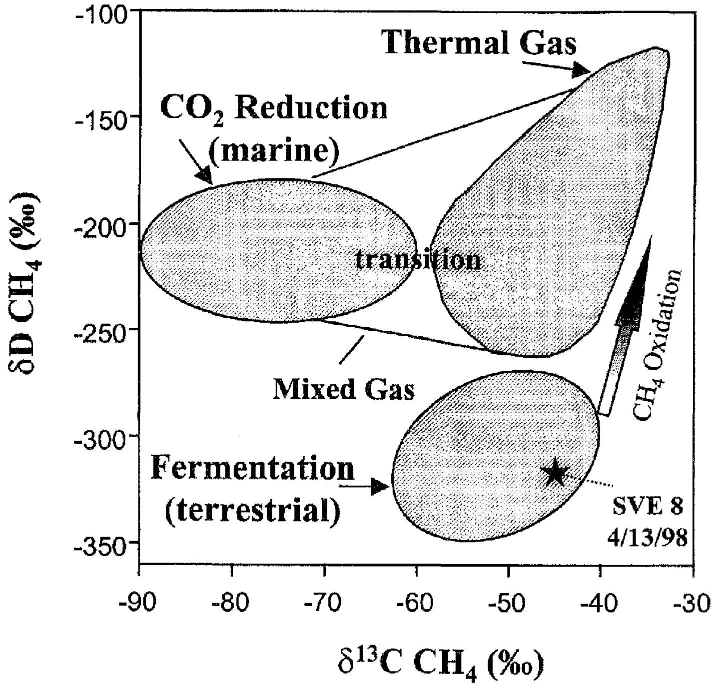

Figure 5. Typical carbon and hydrogen isotopic composition of CH_4 from fermentation, CO_2 reduction, and thermogenic gas. (Modified from Coleman et al., 1996; Whiticar et al., 1986.)

was of microbial rather than thermogenic origin. Highly depleted values of δ^{13}C-CH_4 (–45.6‰) and δD-CH_4 (–316.0‰) confirmed that the CH_4 was produced biologically (Table 2). The measured δ^{13}C value of -45.6‰ was similar to δ^{13}C values for CH_4 (–50.3 and –52.3‰) determined by Van de Velde et al. (1995) produced from the anaerobic biodegradation of Number 2 fuel oil. CH_4 in SVE 8 was highly depleted with δD at –316.0‰, typical of CH_4 produced by fermentation (Coleman et al., 1996; Schoell, 1980; Whiticar et al., 1986). Methane oxidation also was not evident as indicated by the δ^{13}C and δD values of CH_4 and δ^{13}C of CO_2 even though low levels of oxygen were present from day 75 to 86. Thus, methanogenesis was the dominant intrinsic biodegradation process occurring during the NA of gasoline, which is consistent with results from other in situ studies (Chiang et al., 1989; Kampbell et al., 1996).

Biodegradation Rate Results

The anaerobic biodegradation rate was calculated using the CH_4 production rate measured in soil gas in SVE 9 and SVE 8 from day 1 to 25 (0.01 and 0.03%/d, respectively). Using Equation 5, we calculated a biodegradation rate range in the source area of 0.02 to 0.06 mg toluene/kg soil-d. The rate estimates only included methanogenesis, and thus are underestimates if other anaerobic processes (nitrate or iron (III) reduction) are occurring or if oxygen is naturally diffusing into the source area, which occurred at longer time periods. Total soil BTEX levels in the former tank area (B7) measured 95 mg/kg in August 1997 and assuming these constituents were degraded at our calculated rate, approximately 4.7 to 15.3 years would be required for remediation of BTEX by NA occurring at a constant rate. Methanogenic rates that dominate during NA are much lower than the reported range of in situ aerobic petroleum respiration rates (0.2 to 100 mg/kg-d; Hinchee and Ong, 1992) when oxygen is introduced during active remediation.

Considerations

Although extremely useful under many instances, there are several considerations to using this type of anaerobic respiration test combined with isotopic analysis (Table 3). In situ respiration tests require injection of large quantities of inert gas into the subsurface to effectively reduce vadose zone gas concentrations, particularly in low permeability sediments. Ar may not be as evenly distributed in the subsurface in low-permeability sediments; thus, an estimate of the ROI is helpful in determining the extent of Ar sparging. In more permeable sediments, replacement of natural soil gases with Ar may improve, but diffusion of gases over time also may increase. The sampling times should be appropriately selected in accordance with site-specific characteristics (shorter study periods for more permeable sediments). Our results suggested that sparging directly into each monitoring well may improve soil gas dilution, reduce diffusion bias during relatively long soil gas monitoring times, and a biologically active study can be accomplished in 2 to 4 weeks.

Stable carbon and hydrogen isotopic analyses are relatively inexpensive ($40 to $85) and can be applied at each sampling time to expand the detail of in situ microbial processes occurring through time. A relatively large amount of carbon is required for standard radiometric analysis by decay counting (4 g desired with a minimum of 500 mg final carbon). AMS re-

Table 3. Advantages and disadvantages of anaerobic in situ respiration tests combined with isotopic measurements

Advantages

- Provides in situ quantitative estimate of anaerobic petroleum hydrocarbon mineralization
- Both respiration tests and isotopic analysis can be applied to numerous petroleum-derived contaminants and most sediment types
- Isotopic analysis verifies petroleum biodegradation, differentiates between biotic and abiotic degradation processes, and distinguishes between bacterial metabolic pathways
- Isotopic analysis is applicable to varying and low contaminant and CO_2 concentrations found under in situ conditions
- Accelerator mass spectrometry (AMS) technology expands test to sites with low biologic activity

Disadvantages

- A large amount of inert gas must be evenly distributed throughout the subsurface
- Diffusion of gas may occur over the long test period
- Radiocarbon is subject to interferences from atmospheric modern carbon, dissolution of carbonate minerals, oxidation of ancient organic matter, and thermogenic CH_4
- ^{14}C, δ^{13}C, and δD analysis of CH_4 is relatively complex and may be expensive if AMS measurement is required
- Overlap between d^{13}C values of petroleum hydrocarbons and C_3 plant root respiration and microbial degradation complicates interpretation of results

duces the minimum amount of carbon needed to 1 mg, which extends the application of this method to sites with limited biological activity, but doubles the cost of analysis. Radiocarbon analysis of CH_4 is considerably more complex than that of CO_2 and usually requires AMS technology. In addition, radiocarbon interferences from atmospheric modern carbon, dissolution of carbonate minerals, and oxidation of ancient organic matter (peaty and liginitic matter), must be adequately accounted for and addressed.

Application of radiocarbon and stable carbon isotopes in conjunction with soil gas monitoring offered several advantages over current practices for estimating biodegradation rates in soil during natural attenuation using soil gas alone. While soil gas production was used to calculate biodegradation rates, ^{14}C, $\delta^{13}C$, and δD isotopic data verified petroleum biodegradation in the vadose zone, distinguished between biotic and abiotic degradation processes, and distinguished between bacterial metabolic pathways. In addition, isotopic data are direct field measures and are applicable to varying and low contaminant and CO_2 concentrations found under in situ conditions. Measuring ^{14}C-CO_2 at the start and at the end would of soil gas monitoring expand quantitative estimate of petroleum mineralization by providing more detail of other anaerobic mineralization processes besides methanogenesis. ^{14}C, $\delta^{13}C$, and δD analyses can be used effectively during natural attenuation or active remediation investigation not only for soil gas, but also for analysis of groundwater DIC and dissolved CH_4 to verify and quantify the biodegradation of the many petroleum-synthesized contaminants in aerobic or anaerobic environments.

Acknowledgments

We thank Matt Addler for his assistance in sample preparation for isotopic analysis. Funding was provided by the South Carolina Hazardous Waste Management Research Fund and the National Science Foundation (Grant No. 93-50314; Environmental Engineering).

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