Ground Water Monitoring&Remediation

Anaerobic Biodegradation of MTBE at a Gasoline Spill Site

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Abstract

To manage risk or to implement natural attenuation as a remedy, regulatory agencies must understand the processes that attenuate methyl-*tert*-butyl ether (MTBE) in ground water. Most case studies and laboratory studies in the literature indicate that natural biodegradation is not important; however, recent reports indicate that natural biodegradation of MTBE plays an important role under certain conditions. In an MTBE plume at a retail gasoline station in Parsippany, New Jersey, the long-term monitoring data indicated that the concentration of MTBE was slowly declining over time in the wells that were within the footprint of the plume. The ratio of *tert*-butyl alcohol (TBA) to MTBE increased with distance from the source area, and the ratio of TBA to MTBE in individual monitoring wells in the plume increased over time. This anecdotal evidence of natural biodegradation of MTBE to TBA at field scale was confirmed with a microcosm study. Core material from the interior of the plume was used to construct the microcosms. Following an initial lag period of 58 d, the concentration of MTBE decreased from more than 1460 µg/L to less than 10 µg/L within 199 d of incubation. As concentrations of MTBE declined in the microcosms, concentrations of TBA increased. The decrease in concentration of MTBE in the microcosms could be accounted for by an increase in the concentration of TBA.

Introduction

In the United States, the responsibility for managing spills of gasoline from underground storage tanks falls to the individual states. Where it has been appropriate, many states have selected monitored natural attenuation as a remedy for organic contaminants in ground water (U.S. EPA 1999; New England Interstate Water Pollution Control Commission 2000). Many states also use a formal process of risk management to select the most appropriate remedy at gasoline spill sites. Both monitored natural attenuation and risk management require an understanding of the environmental processes that control the behavior of a contaminant in ground water.

Sorption and dispersion of *tert*-butyl methyl ether (MTBE) in ground water are fairly well understood (Squillance et al. 1997); however, natural biodegradation processes for MTBE are not well understood (National Research Council 2000, 93.) The Committee on Intrinsic Remediation of the National Academy of Sciences determined that biological transformation was the dominant process responsible for attenuation of MTBE in ground water. They further determined that the current level of understanding of biological transformation of MTBE is moderate, and as a result the likelihood of success for using monitored natural attenuation as a remedy for MTBE contamination at a particular site is low (National Research Council 2000, 8).

MTBE can be degraded under a variety of anaerobic conditions. Yeh and Novak (1994) were the first to demonstrate anaerobic degradation of MTBE in natural materials. Subsequent laboratory studies demonstrated biodegradation of MTBE under methanogenic conditions in bed sediment from the Ohio River (Mormile et al. 1994), aquifer sediment contaminated by jet fuel (Wilson et al. 2000), and bed sediments of fresh water streams and lakes (Bradley et al. 2001a, 2001b). In these studies the biodegradation of MTBE under methanogenic conditions resulted in the production and accumulation of *tert*-butyl alcohol (TBA). MTBE has been shown to degrade under iron-reducing conditions in the bed sediments of a fresh water stream (Landmeyer et al. 1998) and in aquifer sediments (Finneran and Lovley 2001). Finneran and Lovley (2001) demonstrated complete mineralization of MTBE under iron-reducing conditions. Under sulfate-reducing conditions, MTBE has been shown to degrade in stream and lake sediments (Bradley et al. 2001b) and in enrichment cultures from estuary sediment (Somsamak et al. 2001). In the studies of Bradley et al. (2001b), there was substantial mineralization of MTBE. The culture of Somsamak et al. (2001) produced TBA from MTBE and failed to degrade the TBA. Under nitrate-reducing conditions, MTBE was completely mineralized in bed sediments of fresh water streams and lakes (Bradley et al. 2001b).

In an attempt to evaluate natural attenuation of MTBE at gasoline spill sites, Kolhatkar et al. (2000) collected monitoring data from MTBE plumes at 74 gasoline service stations in the Eastern United States. They were able to extract first-order rate constants for biodegradation of MTBE from only 4 of the 74 sites. These sites were not research sites; the monitoring wells were installed at the direction of the appropriate state agencies. At most sites the regulatory agencies used risk management rather than monitored natural attenuation; as a consequence the monitoring wells were installed to determine the boundary of the plumes. Wells in the interior of the plume that could be used to estimate attenuation along a flow path were sparse.

Kolhatkar et al. (2000) collected data on the geochemistry of the plumes and used this information to assign the plumes to different geochemical categories. Ground water was considered to be methanogenic if the concentration of dissolved methane was >0.5 mg/L, weakly methanogenic if methane was present <0.5 mg/L, and not methanogenic if methane was <0.001 mg/L. Sulfate was considered to be available if the concentration was >1.0 mg/L.

The distribution of sites into different categories is shown in Table 1. Most of the MTBE plumes were methanogenic and most were depleted of sulfate. One of the four sites where it was possible to extract a biodegradation rate constant for MTBE was a retail gasoline station in Parsippany, New Jersey. The site had a good record of long-term monitoring and had a useful number of monitoring wells that were inside the plume. The site at Parsippany, New Jersey, belonged to the most abundant geochemical category; the concentrations of methane in the contaminated wells were near 5 mg/L, and sulfate was depleted in most of the contaminated wells. Kolhatkar et al. (2000) used the approach of Buscheck and Alcantar (1995) to extract a first-order biodegradation rate constant for biodegradation of MTBE in the plume at Parsippany, New Jersey. This approach assumes uniform flow of ground water in the aquifer and requires an estimate of longitudinal dispersivity to extract the rate of biodegradation. To provide an additional line of evidence that MTBE was biologically degraded at this site, a microcosm study was constructed with sediment from the interior of the MTBE plume.

Site Background

In 1990, a release of gasoline was detected from an underground storage tank at a retail service station in Parsippany, New Jersey. The tank was removed, and monitoring wells were installed. In 2000, sediment samples were acquired for the microcosm study. Figure 1 presents the location of the underground tanks and the monitoring wells that had been installed by 2000. Figure 2 depicts a generalized stratigraphic cross section of the site.

Table 1
Geochemistry of Selected MTBE Plumes in the Eastern United States (from Kolhatkar et al. 2000)

Geochemistry of Plume		Number of Sites
Methanogenic	Sulfate depleted	43
Methanogenic	Sulfate available	5
Weakly methanogenic	Sulfate depleted	8
Weakly methanogenic	Sulfate available	5
Not methanogenic	Sulfate available	13

The site is underlain by alternating layers of medium to coarse sand extending down to 0.9 m below ground surface, then a layer of silty clay with traces of coarse sand extending from 0.9 to 2.1 m below ground surface, then a layer of coarse sand extending 2.1 to 3.3 m below ground surface, and then a clay layer that extends from 3.3 to 4.6 m below land surface. Monitoring wells were screened from 0.6 to 4.6 m below land surface. The depth to ground water varied from 0.30 to 1.22 m below land surface. Sediment samples to construct microcosms were acquired from 0.61 to 1.22 m below land surface, and from 1.22 to 1.83 m below land surface. At the time the sediment samples were acquired, the water table was at 1.22 m below land surface.

At the time they were installed, four wells at the site produced water with relatively high concentrations of BTEX (benzene, toluene, ethylbenzene, and xylenes) compounds as well as MTBE and TBA (MW-2, MW-5, MW-6, and MW-11). The area containing these four wells in Figure 1 is shaded gray; compare Table 2 for data on concentrations of contaminants. Two wells to the east produced water with lower concentrations of BTEX compounds and MTBE (MW-3 and MW-4). Six wells to the west, north, and further to the east produced water with lower concentrations of MTBE and TBA and essentially no BTEX compounds.

In the conceptual model of the site developed by Kolhatkar et al. (2000, 2002), the source of the plume was near MW-5 (Figure 1), and the predominant direction of ground water flow was to the north toward wells MW-6, MW-10, and MW-11. The network of monitoring wells was resurveyed in January 2003. Twenty-three rounds of water elevation data (corrected by the new survey) were available for wells MW-1 through MW-12 for a period between May 1996 and September 2002.

At this site, the variation in the local hydraulic gradient in any round of sampling was controlled by variations in the regional gradient. The local water table could be approximated as a plane. Regression analysis (Srinivasan et al. 2004) was used to fit a plane to the water table elevations in each round of sampling. For 20 of the regressions, the correlation coefficient (r^2) of the regression was 0.63 or greater, with a median value of 0.75. The results of the 20 regressions are presented in Figure 1. The direction of the arrow is the direction of ground water flow, and the length of the arrow is proportional to the hydraulic gradient. In 14 of the 20 regressions, the predominant direction of ground water flow was to the east.

The mean of the hydraulic gradient in the 14 rounds of sampling was 0.026 m/m, with a standard deviation of 0.0074 m/m. Based on slug tests of monitoring wells, the average hydraulic conductivity of the depth interval sampled by the monitoring wells varied from 0.37 to 0.91 m/d, with a mean of 0.57 m/d. Based on the range in hydraulic conductivity and the standard deviation of the hydraulic gradient, at an assumed porosity of 0.25, the average seepage velocity toward the east should be in the range of 10

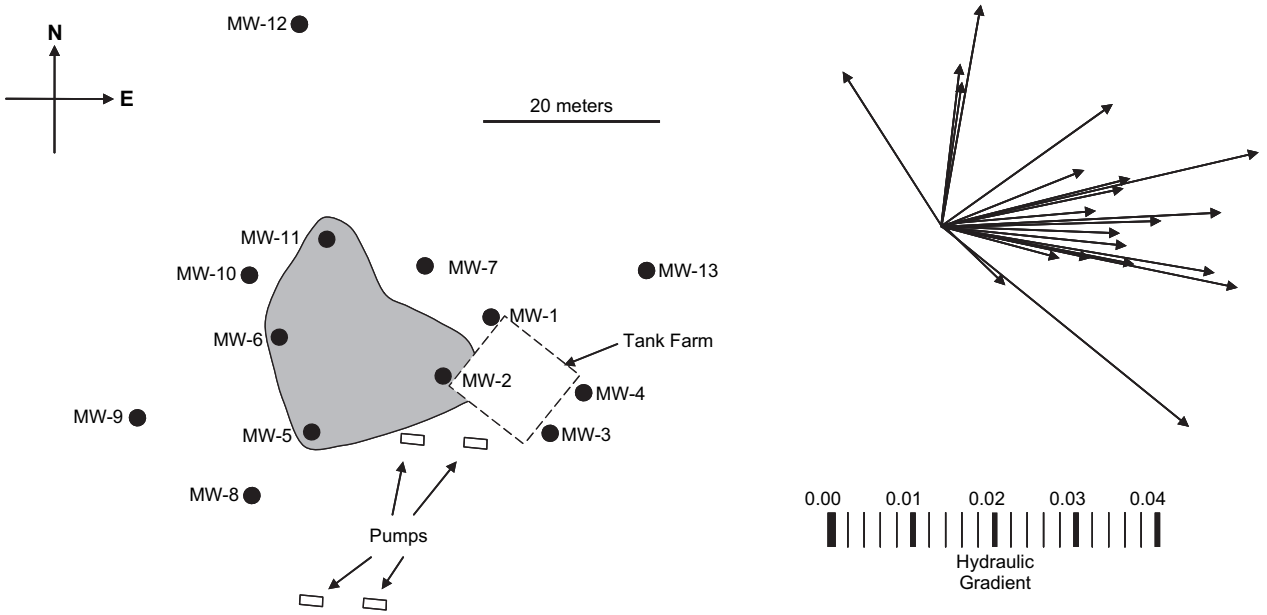

Figure 1. Distribution of monitoring wells and the direction of ground water flow. The gray shade encloses wells that are influenced by residual-phase gasoline. The cluster of arrows represents the direction of ground water flow on each of 20 separate monitoring events. The length of the arrow is proportional to the hydraulic gradient.

to 44 m/year. Notice from Figure 2 that the tested interval included major units of clay and silty clay. The velocity of the MTBE plume in the depth intervals composed of coarse sand and medium to coarse sand may be higher than the average ground water seepage velocity across the entire screened interval.

Changes in Concentrations of MTBE and TBA in the Plume over Time

Concentrations of BTEX compounds were persistent over time in wells MW-5, MW-6, and MW-11. Of the alkylbenzenes, toluene is the most labile to anaerobic biodegradation. The behavior of toluene in these wells is presented in Figure 3. Although toluene is readily biodegraded in ground water, it was persistent in MW-5 and MW-6 and to a lesser extent in MW-11. This implies that residual gasoline in the proximity of these wells acted as a continuing source of BTEX compounds to ground water. The reduction in concentration of toluene was first order on time, as would be expected if the concentration of toluene were controlled by the dissolution and mass transfer of toluene from residual gasoline to the flowing ground water.

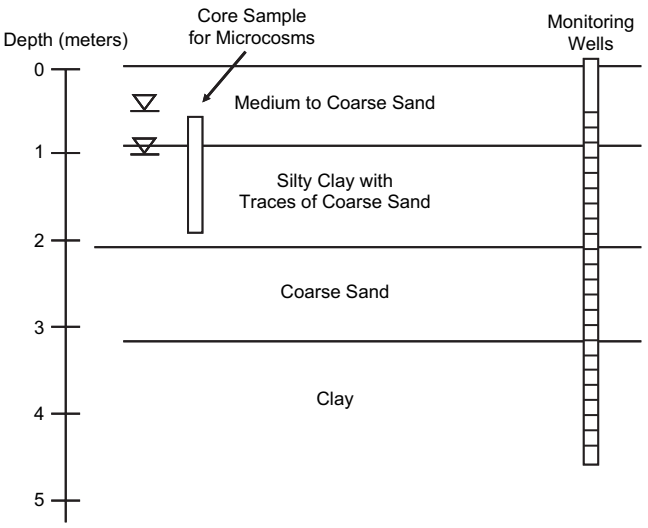

Figure 2. Generalized stratigraphic cross section of the site.

Figure 4 compares changes in the concentrations of MTBE and TBA over time in wells MW-5, MW-6, and MW-11 in the source area, and wells MW-1, MW-4, and MW-3 downgradient.

Apparently, the residual gasoline in proximity to MW-5 also acted as a continuing source of MTBE. The concentrations of TBA in MW-5 were very similar to the concentrations of MTBE, and there was little change in the proportions of MTBE and TBA over time. In MW-6, the initial concentrations of TBA were lower than the concentrations of MTBE. However, in samples taken in 1996, the concentrations of TBA were higher. In the period around 2000, the concentration of TBA was between four and five times higher. In MW-11, the concentrations of TBA were always higher than the concentrations of MTBE. In the period around 2000, the concentrations of TBA were from 7- to 15-fold higher. Monitoring wells MW-3 and MW-4 are downgradient of MW-5 and MW-6. In wells MW-3 and MW-4, the concentration of TBA was initially lower than the concentration of MTBE. By 1998, the concentration of TBA was 11-fold higher in MW-3, 40-fold higher in MW-4, and 600-fold higher in MW-1.

In 1999, a product line at the site failed an integrity test. Gasoline spilled from the line may account for the spike in concentration of MTBE in MW-3, MW-4, and MW-1 in 1999 (Figure 4). By 2000, the concentrations had

Table 2
Concentrations of Fuel Components in Ground Water at the Time the Monitoring Wells Were First Installed

Well	Date	MTBE (µg/L)	TBA (µg/L)	Benzene (µg/L)	Toluene (µg/L)	Ethylbenzene (µg/L)
In source area						
MW-2	May 1, 1991	240	<10	590	4500	430
MW-5	March 12, 1993	1500	<10	8200	14,000	1700
MW-6	March 12, 1993	140	140	490	1200	290
MW-11	September 23, 1994	2200	4600	6200	8000	940
Margin of source area						
MW-3	May 1, 1991	620	<10	230	400	310
MW-4	May 1, 1991	240	<10	360	110	300
Outside source area						
MW-1	May 1, 1991	37	<10	13	<1	3
MW-7	March 12, 1993	19	67	<1	<1	<1
MW-8	May 11, 1993	73	25	<1	<1	<1
MW-9	May 11, 1993	140	94	<1	<1	7.7
MW-10	May 11, 1993	290	72	<1	<1	<1
MW-12	May 17, 1996	3	43	0.7	0.8	2.2
MW-13	September 2, 2000	<1	<10	<1	<1	<1

returned to normal, and the concentrations of TBA in MW-3, MW-4, and MW-1 were consistently manyfold higher than the concentrations of MTBE.

In monitoring wells at the site, the relative concentration of TBA increased with distance away from MW-5. The concentration of TBA also increased over time in individual monitoring wells. This is anecdotal evidence that the TBA in the ground water was produced from biodegradation of MTBE and not from TBA that was originally present in the gasoline.

Materials and Methods

Construction of Microcosms

Sediment for the microcosm study was acquired from locations near MW-6 because it occupied an intermediate position in the plume and because the field data suggested that natural biodegradation of MTBE at that location in the plume was extensive but was not complete (Table 2 and Figures 1 and 4). The sediment was collected and stored in 1-L glass jars. To protect the anaerobic microorganisms that might be present in the samples from oxygen in the atmosphere, the headspace above the sediment was replaced with ground water from MW-6 immediately after collection. The sediment samples were shipped by air-freight and were stored at 4°C until they were used to construct microcosms.

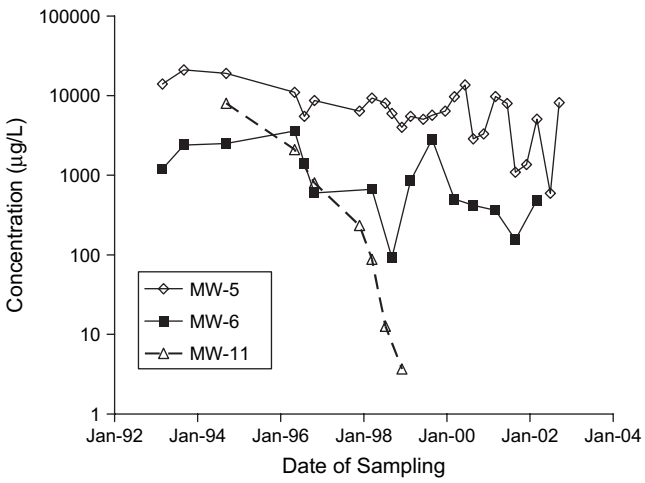

Figure 3. Persistence of toluene in monitoring wells in the source area of the MTBE plume.

All manipulations to prepare the microcosms were carried out in an anaerobic glove box. An oxygen meter indicated that the concentration of oxygen in the atmosphere of the glove box was <1 ppm by volume. Microcosms were prepared in glass serum bottles with a volume of 25 mL. Ground water from MW-6 was added to the sediment to make a slurry; then the sediment samples were stirred to blend them well. The slurry was transferred to the serum bottles with a scoop. Each microcosm received 45 g of slurry and 1.0 mL of a dosing solution containing various amendments as described subsequently. The microcosms were sealed with a Teflon®-faced gray butyl rubber septum and a crimp cap. The microcosms were stored at room temperature (20°C to 22°C) in the same glove box, under an atmosphere containing 2% to 5% v/v hydrogen.

Laboratory Analytical Procedures

The concentrations of MTBE and TBA were determined by headspace gas chromatography/mass spectrometry (GC/MS) using a modification of EPA Method 524.2 (U.S. EPA 1998). Samples were collected for analysis with an automated static headspace sampler. Analytes were determined by GC/MS using a Finnigan Ion Trap Detector. The lowest calibration standard was 1.0 µg/L for MTBE and 10 µg/L for TBA; the method detection limit was 0.28 µg/L for MTBE and 2.4 µg/L for TBA. The lowest

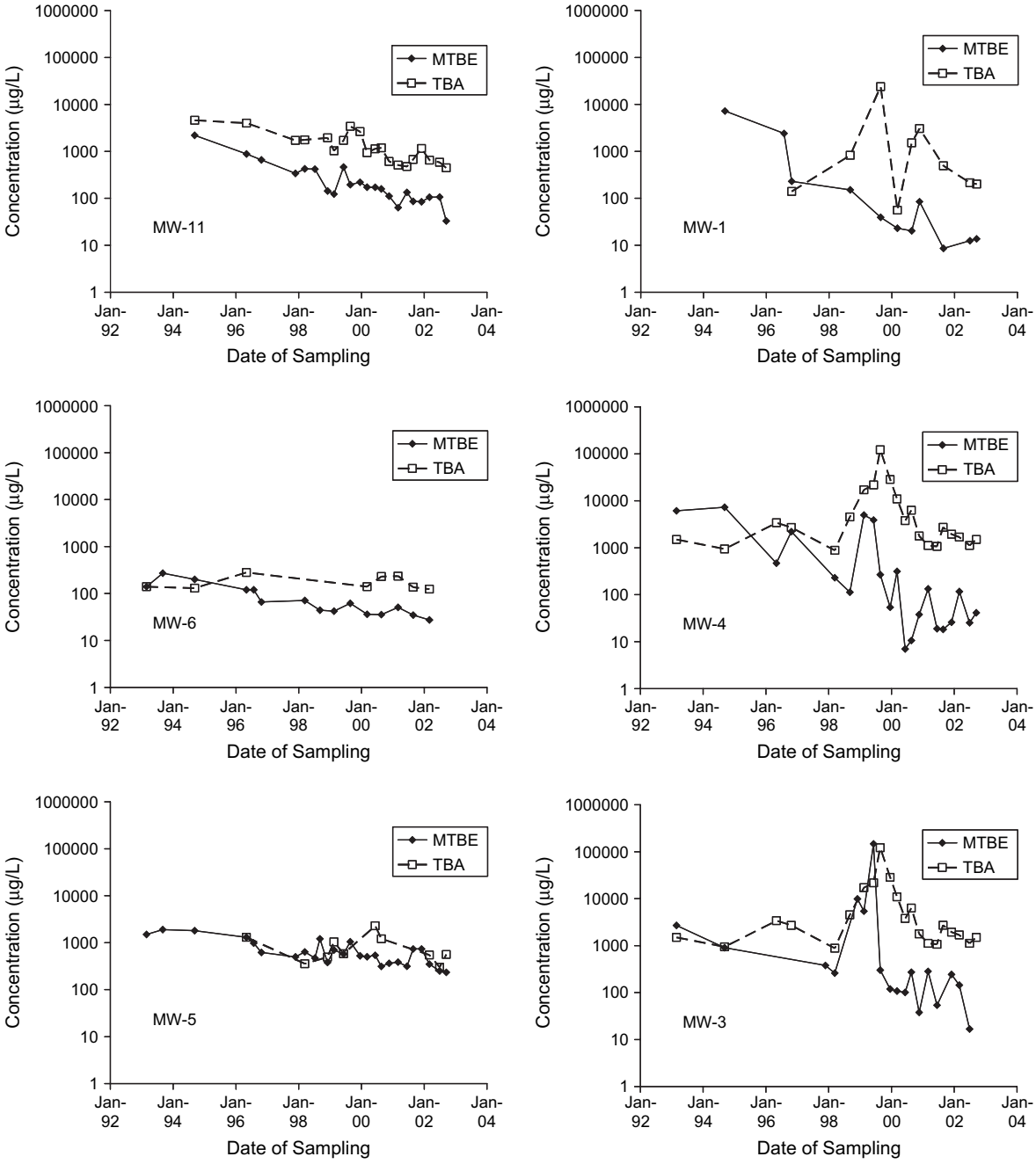

Figure 4. Changes in the concentration of MTBE and TBA in monitoring wells over time. Wells MW-1, MW-4, and MW-3 are downgradient of wells MW-11, MW-6, and MW-5.

calibration standard for the alkylbenzenes (BTEX and trimethylbenzenes) was 1.0 µg/L.

The concentration of methane was determined by headspace analysis and gas chromatography using a flame ionization detector (Kampbell and Vandegrift 1998). The lowest calibration standard for methane was 10 ppm (v/v) and the method detection limit was 0.3 ppm (v/v). The concentration of hydrogen was determined by headspace analysis and gas chromatography using a reducing gas detector. The lowest calibration standard for hydrogen was 0.5 ppm (v/v) and the method detection limit was 0.22 ppm (v/v).

Concentrations of sulfate and nitrate plus nitrite nitrogen were determined by capillary electrophoresis. The method detection limit for sulfate was 0.17 mg/L, and the method detection limit for nitrate plus nitrite nitrogen was 0.3 mg/L. Acetate was determined by high-performance liquid chromatography. The limit of quantitation was 0.1 mg/L and the method detection limit was 0.04 mg/L.

Prior to sampling, while the microcosms were still sealed, the contents of each microcosm were mixed with a vortex mixer and then the sediment was allowed to settle. The septum was removed, and ~1 mL of the standing water was taken and diluted in 14 mL of distilled water containing 1% trisodium phosphate as a preservative. The diluted samples contained ~15 mL of diluted water and 6.0 mL of headspace. The diluted samples were sealed with a septum and crimp cap and then shaken to bring the water and headspace to equilibrium.

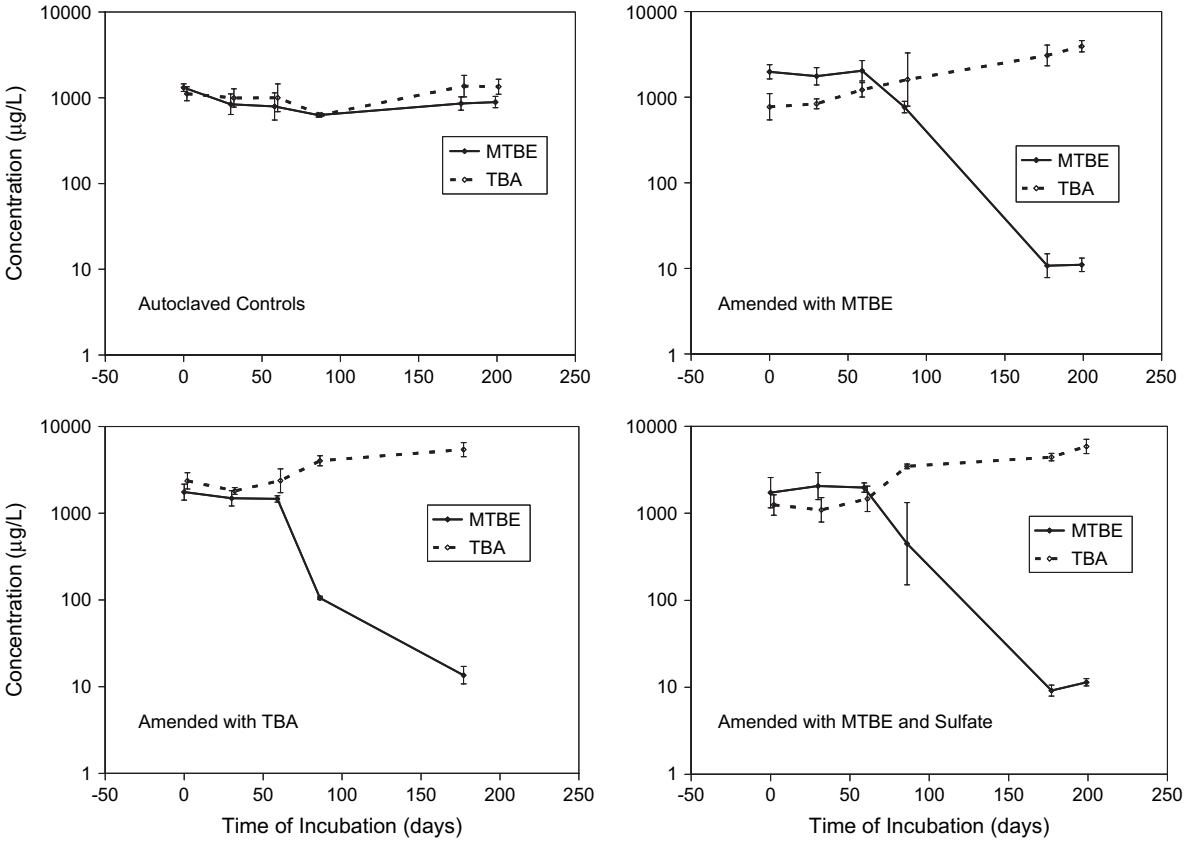

Figure 5. Removal of MTBE and production of TBA in sediment microcosms. Symbols are the geometric mean of analyses in three replicate microcosms. Error bars are the 95% confidence interval on the means.

Depending on the amount of standing water that was sampled from each microcosm, the pore water in the microcosms was diluted in a range between 15:1 and 30:1 before analysis. As a result, the effective detection limit for MTBE in the microcosms was near 10 µg/L, and the effective detection limit for TBA was near 72 µg/L. The effective method detection limits for sulfate, nitrate, and acetate in diluted samples were near 5, 30, and 1.2 mg/L, respectively.

To determine the concentration or quantity of hydrogen and methane in the microcosms, the concentration of hydrogen or methane was determined in the headspace of the aforementioned diluted samples. The concentration of hydrogen and methane in the water in the diluted samples was estimated using Henry's law. The total amount of hydrogen or methane in the diluted samples was assumed to be the amount in the 1.0 mL of water originally taken from the microcosm. The total amount of hydrogen or methane in the microcosm was calculated from the measured concentration in the water in the microcosm, using the Henry's law constant, and the amount of water and headspace originally in the microcosm. The effective method detection limits for methane and hydrogen in the water originally sampled from the microcosms was 1.2 µg/L for methane and 0.11 µg/L for hydrogen.

The concentration of biologically available iron in the sediments used to construct the microcosms was determined using the Biologically Available Iron Assay (New Horizons Diagnostics Corporation, Columbia, MD).

Results and Discussion

MTBE Biodegradation in Microcosms

It was not known beforehand if the sediment as collected contained appreciable concentrations of MTBE or its degradation product TBA. The measured concentrations of MTBE and TBA are the sum of the concentrations added and the concentrations of MTBE and TBA that were present in the sediment as collected. The control microcosms were constructed with autoclaved sediment and amended with MTBE and TBA to a final concentration in the microcosm of 1.3 ± 0.14 mg/L of MTBE and 1.1 ± 0.19 mg/L of TBA (means and 95% confidence intervals on the means). In the second treatment, living microcosms were amended with MTBE to produce a final concentration of 2.1 ± 0.31 mg/L of MTBE and 1.0 ± 0.008 mg/L of TBA. In the third treatment, living microcosms were amended with TBA to produce a final concentration of 1.8 ± 0.35 mg/L of MTBE and 2.4 ± 0.48 mg/L of TBA. The fourth treatment was intended to determine if available sulfate had an effect on anaerobic MTBE biodegradation. The fourth treatment was amended with MTBE to produce a final concentration of 1.8 ± 0.58 mg/L of MTBE and 1.3 ± 0.32 mg/L of TBA and 120 mg/L of sulfate.

Triplicate microcosms from each treatment were sampled and analyzed after 0, 30, 58, 86, 177, and 199 d of incubation. Figure 5 presents results for MTBE and TBA. There was no significant consumption of MTBE or

production of TBA in the autoclaved controls during 199 d of incubation. There was no significant consumption of MTBE or production of TBA in the living microcosms after 30 or 58 d of incubation. After 86 d of incubation, all of the living microcosms showed statistically significant evidence for removal of MTBE. After 177 d of incubation, the concentration of MTBE in all of the living microcosms was below the effective method detection limit. The means and confidence intervals presented in Figure 5, for living microcosms that were sampled after 177 and 199 d of incubation, are calculated using the individual detection limits for the replicate microcosms. They are presented to show an estimate of the upper boundary on the concentrations remaining in the microcosms as allowed by the sensitivity of the chemical analyses and the variation in the experimental manipulations.

In the treatment amended with sulfate (120 mg/L), the sulfate was consumed within the first 58 d of incubation, while degradation of MTBE began at some time after 58 d of incubation. In the time interval when MTBE was removed in the treatment amended with sulfate, sulfate was not detectable in the microcosms (effective method detection limit 3 mg/L).

A first-order rate constant for removal of MTBE from the pore water of the microcosms was calculated for the time period from 0 through 177 d of incubation. The lag as seen in Figure 5 was included. The rate was calculated as the slope of a linear regression of the natural logarithm of concentration on time of incubation. For the microcosms amended with MTBE, the rate constant and 95% confidence interval on the rate was 11 $\pm$ 2.7 per year. For microcosms amended with MTBE and sulfate, the rate constant for MTBE biodegradation was 12 $\pm$ 2.9 per year; biodegradation of MTBE in this treatment began after the sulfate was consumed. For microcosms amended with TBA, the rate constant for MTBE biodegradation was 11 $\pm$ 2.3 per year. The rate of attenuation in the autoclaved controls was not statistically significant at 95% confidence (0.66 $\pm$ 0.96 per year).

Wilson et al. (2000) demonstrated rapid and extensive MTBE degradation in methanogenic ground water at a spill of JP-4 jet fuel near Elizabeth City, North Carolina. The rate of MTBE natural biodegradation that was extracted from the field data was 2.7 per year, with an upper bound of 5.0 per year and lower bound of 2.2 per year. The rate of MTBE biodegradation in laboratory microcosms was 3.5 $\pm$ 0.14 per year. These rates are roughly equivalent to the rates in the microcosms constructed with material from Parsippany, New Jersey.

Production of TBA in the Microcosms

The amount of TBA produced in the microcosms exceeded the amount that would be expected from the metabolism of MTBE that was present in the pore water. Approximately twice as much TBA was produced as was expected.

The partitioning of MTBE from water to aquifer solids was estimated from the "spike recovery" of MTBE in the microcosm treatments where MTBE was added to the sediment. The spike recoveries were determined after 30 d of incubation when the microcosms had come to sorptive equilibrium, but before there was any evidence of biodegradation. The average concentration of MTBE in the microcosms that received only TBA was 1487 µg/L. The concentration of MTBE in the dose solution added to the microcosms that only received MTBE would have added an additional 990 µg/L. The expected total concentration of MTBE was 2477 µg/L, but the average measured concentration of MTBE was only 1754 µg/L. Of the 990 µg/L added, only 266 µg/L was recovered. The concentration of MTBE in the dose solution added to the microcosms that received MTBE and sulfate would have added an additional 1017 µg/L. The expected total concentration of MTBE was 2504 µg/L, but the average measured concentration of MTBE was only 2057 µg/L. Of the 1017 µg/L added, only 570 µg/L was recovered. The "spike recovery" of MTBE from the pore water of the microcosms was 27% and 56%. This suggested that approximately half of the MTBE in the microcosms was sorbed to the sediment.

The TBA may have been produced from MTBE that was originally sorbed to the sediments used to construct the microcosms. The microcosms that were only amended with MTBE had significant concentrations of ethylbenzene plus xylenes (1.1 to 1.8 mg/L) and total trimethylbenzenes (3.7 to 5.4 mg/L). It is likely that the sediment used to construct the microcosms contained residual non–aqueous phase gasoline and that the MTBE was partitioned to the gasoline.

Physiological Processes That May Consume MTBE or Produce Methane

Wells at the site were sampled in November 1999, June 2000, and July 2001. In ground water from MW-12, at the outside margin of the plume, the concentrations of methane were low, concentrations of nitrate met or exceeded 0.8 mg/L, and concentrations of sulfate exceeded 20 mg/L (Table 3). Inside the plume, methane accumulated and sulfate was depleted. Uniformly throughout the plume, the concentration of nitrate was <0.1 mg/L. In 1999, the concentration of iron II in MW-5 was 18 mg/L. Dissolved oxygen was not detected. Natural biodegradation in the plume must occur under anaerobic conditions and specifically under iron-reducing, sulfate-reducing, or methanogenic conditions.

The iron-reducing culture described by Finneran and Lovley (2001) degraded both MTBE and TBA. In contrast, there was a stoichiometric accumulation of TBA from biodegradation of MTBE at the Parsippany, New Jersey, gasoline spill site. The concentration of biologically available iron in the sediment used to construct the microcosms varied from 1600 to 4000 mg/kg. The water content of the microcosms was near 12.5 mL, and the dry weight of sediment was near 32 g. Six moles of biologically available iron III is needed to metabolize 1 mol of MTBE to TBA, and 30 mol are needed to completely degrade MTBE to carbon dioxide and water (Schmidt et al. 2004). The biologically available iron in the microcosms could support the biodegradation of ~1000 mg/L of MTBE to TBA and 200 mg/L of MTBE to carbon dioxide and water. The actual consumption of MTBE was 2 mg/L from the pore water, equivalent to 4 mg/L when corrected for MTBE that partitioned to residual gasoline.

Table 3
Distribution of TBA, Sulfate, and Methane in a Plume of MTBE in Ground Water
(See Figure 1 for the location of the wells.)

Well	Date Sampled	MTBE (µg/L)	TBA (µg/L)	Sulfate (mg/L)	Nitrate (mg/L)	Methane (mg/L)
MW-5 (near source)	November 3, 1999	792	598	<1	<2	1.5
	June 20, 2000	420	526	<1	<0.5	3.9
	July 31, 2001	367	436	<1	<0.5	5.1
MW-6 (sediment for microcosms)	November 3, 1999	43.2	185	<1	<2	3.5
	June 20, 2000	51.2	223	<1	<0.5	6.8
	July 31, 2001	37.7	166	<1	<0.5	1.2
MW-11 (further from source)	November 3, 1999	155	2350	12.7	<2	6.6
	June 20, 2000	146	1000	<1	<0.5	6.4
	July 31, 2001	134	933	<1	<0.5	6.4
MW-12 (cross-gradient from plume)	November 3, 1999	3	<10	114	2.8	0.019
	June 20, 2000	1	<10	28.2	0.8	0.106
	July 31, 2001	1	<10	26.4	0.8	0.153

Iron reduction is a plausible process for metabolism of MTBE at the Parsippany site.

Mormile et al. (1994) noted that acetogenic bacteria can metabolize phenyl methyl ethers under anaerobic conditions. These organisms can derive energy by combining molecular hydrogen and carbonate with a methyl ether to produce the corresponding alcohol and acetate. It is theoretically possible for acetogenic bacteria to degrade MTBE to TBA and acetate. Mormile et al. (1994) examined two representative cultures of acetogenic bacteria. Although the pure cultures could degrade a series of methyl esters of alkanoic acids, they could not degrade MTBE.

Despite the negative results of Mormile et al. (1994), it is possible the organisms that degrade MTBE in sediment from the Parsippany site, New Jersey, are acetogenic bacteria. In the mixed bacterial community in the aquifer and in the microcosms, the acetate produced from degradation of MTBE would be further metabolized to methane and carbon dioxide. It is also theoretically possible that the organisms that degrade MTBE at the Parsippany site, New Jersey, act by a direct hydrogenation of the ether bond in MTBE to TBA and methane. In this process, MTBE would serve as an alternate electron acceptor. In either case, degradation of MTBE would require molecular hydrogen as a source of reducing power and carbonate as either a cosubstrate or a growth substrate.

Source of Molecular Hydrogen for MTBE Degradation

Molecular hydrogen can be produced during the anaerobic biodegradation of alkylbenzenes to methane and carbon dioxide (Wiedemeier et al 1999, 205). In the initial reaction, the benzene ring is first reduced and then opened to produce long-chained fatty acids (Young 1984). Subsequent anaerobic biodegradation of the fatty acids to acetate produces molecular hydrogen as a product. On a theoretical basis, 3 mol of molecular hydrogen would be produced for each mole of benzene that was degraded to acetate.

The glove box used to incubate the microcosms had a headspace of 2% to 5% v/v hydrogen. If sufficient hydrogen diffused through the septum of the microcosms, there is a possibility that the hydrogen concentration in the microcosms was not representative of conditions in the aquifer, and the production of TBA from MTBE in the microcosms was an artifact of incubation in a glove box.

A set of container controls was incubated along with the microcosms containing sediment. In this experimental treatment, the serum bottles contained sterile water spiked with MTBE and TBA, but no sediment. Any hydrogen present in the water in the container controls must have diffused into the microcosm through the septum. The total amount of hydrogen in the container controls was taken as a reasonable estimate of the amount of hydrogen supplied to the living microcosms from hydrogen in the glove box. After 582 d of incubation, microcosms were sampled to determine the concentration and quantity of hydrogen and methane. The concentration of TBA in water was determined as described previously. The production of TBA was determined by subtracting the initial concentration from the concentration after 582 d of incubation.

Table 4 compares the concentrations of hydrogen and methane in water in the microcosm and also compares the total amount of hydrogen and methane in the microcosms to the production of TBA in the microcosms. Water in equilibrium with 2% (v/v) hydrogen should have a hydrogen concentration of 16,000 nM. The achieved concentration in the container controls was ~13% of the expected equilibrium concentration in the headspace of the glove box. If hydrogen is required for metabolism of MTBE to TBA, at least 1 mol of molecular hydrogen would be required to produce 1 mol of TBA. There was two to four times more TBA production in the living microcosms than there was hydrogen supplied in the container controls. Hydrogen diffusing through the septum was a component of the hydrogen available for MTBE metabolism, but it was not the major component.

The hydrogen concentrations in the living microcosms were more variable than the concentrations in the control with no sediment. This would be expected if measured concentrations reflect a pool of hydrogen that turned over as hydrogen is produced and consumed. In some of the

Table 4
Comparison of the Production of TBA in the Microcosms to the Total Quantity of Hydrogen and Methane Contained in the Microcosms and to the Concentration of Hydrogen and Methane Dissolved in Water in the Microcosms. Microcosms Were Sampled after 582 d of Incubation. Reported Are Means ± 95% Confidence Intervals. If the Interval Included 0, the Raw Data Are Reported. Concentrations of Hydrogen Expected Are Calculated from the Removal of Alkylbenzenes

Treatment	TBA Production (μM/microcosm)	Hydrogen Measured		Hydrogen Expected (μM/microcosm)	Methane Measured	
		μM/microcosm	nM		μM/microcosm	mg/L
Autoclaved control no sediment	Not produced	0.17 ± 0.043	2100 ± 550	None	<0.004	<0.001
BTEX added to sediment	0.50 ± 0.047	0.29	13,800	2.9	16.1 ± 4.4	12.0 ± 1.16
		0.107	4000			
		0.098	2600			
MTBE added to sediment	0.63 ± 0.064	0.65	16,000	1.9	20.4 ± 3.4	13.7 ± 0.38
		0.038	1200			
		0.013	490			
TBA added to sediment	0.83 ± 0.11	0.086	1490	1.15	22.4 ± 12	11.7 ± 2.3
		0.072	1250			
		0.011	380			

living microcosms, the concentration of hydrogen was two to three times higher than in the container controls. In other living microcosms, the hydrogen concentration was less than one-tenth the concentration in the container controls.

At the beginning of the microcosm experiment, the pore water in the microcosms had substantial concentrations of alkylbenzenes. In the treatment where BTEX was added to the sediment, the total concentration of BTEX and trimethylbenzenes was 8400 μg/L. In the treatments where only MTBE was added or TBA was added, the concentrations of total alkylbenzenes were 5100 and 3000 μg/L, respectively. The majority of the alkylbenzenes in the microcosms were originally present in the sediment used to construct the microcosms. Approximately 46% of the total alkylbenzenes was 1,2,4-trimethylbenzene, 14% was 1,2,3-trimethylbenzene, 14% was *m*-xylene, 12% was ethylbenzene, 10% was 1,3,5-trimethylbenzene, and less than 1% was benzene, toluene, or *o*-xylene. After 177 d of incubation, all the alkylbenzenes were present at concentrations below the detection limit (Figure 6). The alkylbenzenes started to degrade before MTBE started to degrade.

The potential for production of molecular hydrogen through anaerobic biodegradation of the alkylbenzenes was estimated by multiplying the molar concentration of total alkylbenzenes in the pore water by 3. The results are presented in Table 4. The measured concentrations of molecular hydrogen were usually at least an order of magnitude lower than the concentrations that would be expected from the metabolism of the alkylbenzenes in the pore water of the microcosms. Anaerobic biodegradation of the alkylbenzenes could account for the concentrations of hydrogen measured in the living microcosms.

The microcosms that contained aquifer sediment contained appreciable concentrations of methane (Table 4); concentrations ranged from 11.7 to 13.7 mg/L. If 1 mol of methane was produced from each mole of MTBE that was transformed to TBA, the TBA produced in the microcosm would account for ~5% of the methane present in the microcosms. In the plume, the highest concentration of TBA (2.3 mg/L) was associated with the highest concentration of methane (6.6 mg/L, see Table 3). Only 0.51 mg/L of methane would be expected from production of 2.3 mg/L of TBA. This is 8% of the methane actually present in the ground water at field scale. Methane may have been produced during anaerobic metabolism of MTBE to TBA, but the amount of methane produced by metabolism of MTBE was inconsequential compared to methane produced by other processes, such as the anaerobic biodegradation of BTEX compounds.

Possible Physiological Mechanisms for MTBE Degradation

The postulated processes of MTBE carboxylation (degradation by acetogenic bacteria) and direct MTBE hydrogenation (MTBE as an alternate electron acceptor), as well

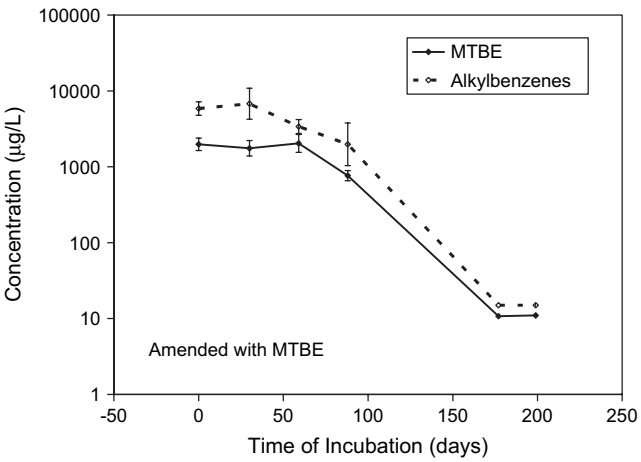

Figure 6. Concomitant removal of alkylbenzenes and MTBE in microcosms.

as chemolithotrophic methanogenesis, all consume hydrogen and lead ultimately to the production of methane. Table 5 compares the Gibbs free energy for MTBE carboxylation and for direct MTBE hydrogenation to the free energy for chemolithotrophic methanogenesis under physiological conditions in the microcosms and in the aquifer. The measured concentrations of hydrogen in the microcosms varied from 380 to 16,000 nM (see Table 4). No data are available for the hydrogen concentration in ground water at the Parsippany site, New Jersey, at the time the sediment samples were collected. The fact that the ground water was actively methanogenic suggests that the hydrogen concentration is in the range of 5 to 20 nM (Chapelle et al. 1995). In August 2003, the concentrations of dissolved hydrogen in MW-4, MW-5, MW-6, and MW-8 were 4.6, 2.6, 5.4, and 1.9 nM, respectively.

McLoughlin et al. (2001) measured hydrogen concentrations in 94 monitoring wells at 18 gasoline spill sites. In 29 wells where the concentration of contaminants (MTBE plus TBA plus BTEX) was >500 μg/L, the concentration of hydrogen ranged from 1 to 10 nM. The maximum concentration of hydrogen in any well at the 17 sites ranged from 1.0 to 51 nM. The other assumptions for the calculations of Gibbs free energy are listed in Table 5.

The calculations suggest that in the microcosms, the free energy for chemolithotrophic methanogenesis, MTBE carboxylation, and MTBE hydrogenation is roughly equivalent. The free energy is lower than would be expected for processes using conventional soluble electron acceptors such as oxygen, nitrate, and sulfate. The calculations also suggest that under conditions to be expected in the aquifer, the free energy for MTBE carboxylation and MTBE hydrogenation is greater than the free energy for chemolithotrophic methanogenesis, and that MTBE-degrading organisms may have a competitive advantage over chemolithotrophic methanogens.

The physiological mechanism of anaerobic MTBE biodegradation at the Parsippany site, New Jersey, is still unclear. Studies with enrichment cultures are underway and the details will be reported elsewhere. Enrichment with sulfate and nitrate failed to produce MTBE-degrading cultures. Enrichment with hydrogen produced cultures that degraded MTBE to TBA and produced methane. The methane could have been produced directly by an organism that hydrogenates MTBE or resulted from further metabolism of acetate produced by an MTBE-carboxylating organism. At present, the enrichments are mixed cultures, and it is impossible to distinguish the two processes.

Anaerobic Biodegradation of MTBE at Field Scale

To evaluate the biodegradation of MTBE in the ground water, MTBE in water samples was analyzed for changes in the ratio of the stable carbon isotopes ($\delta^{13}C$). The stable carbon isotope data were reported earlier in Kolhatkar et al. (2002). As MTBE is biologically degraded, those MTBE molecules containing ^{12}C at the methyl group are degraded more rapidly than molecules containing ^{13}C at the methyl group. The ratio of ^{13}C to ^{12}C in the residual MTBE increases as biodegradation proceeds (Hunkeler et al. 2001; Gray et al. 2002; Kolhatkar et al. 2002). As described in Equation 1, the conventional notation for the ratio of ^{13}C to ^{12}C in a sample ($\delta^{13}C$) reports the ratio in terms of its deviation from the ratio in the international standard (V-PDB). Because the values of $\delta^{13}C$ are much less than 1, they are usually reported in units of parts per thousand (‰).

$$\delta^{13}C = \left[\frac{(^{13}C/^{12}C)_{sample} - (^{13}C/^{12}C)_{V\text{-}PDB}}{(^{13}C/^{12}C)_{V\text{-}PDB}}\right] \times 1000 \quad (1)$$

The fractionation of stable carbon isotopes during biodegradation of MTBE follows a Rayleigh distillation model (Hunkeler et al. 2001). As a consequence, the slope of a plot of $\delta^{13}C$ against the natural logarithm of the fraction of MTBE remaining after biodegradation approaches a straight line.

Table 5
Effect of Concentrations of Dissolved Molecular Hydrogen on the Gibbs Free Energy Available for Physiological Processes in the Laboratory Microcosms and in Ground Water at the Parsippany Site (Free energy is calculated per mole of MTBE consumed or mole of methane produced)

	Conditions in Microcosms[1]		Conditions in Aquifer[1]	
	Methane 10 mg/L		Methane 5 mg/L	
Biochemical Process	**Hydrogen (nM)**	**Energy (kJ/mol)**	**Hydrogen (nM)**	**Energy (kJ/mol)**
(Chemolithotrophic methanogenesis)	300	−62.8	5	−25.3
$4H_2$ + bicarbonate + $H^+ \rightarrow$ methane + water	3000	−84.9	10	−31.9
	30000	−107	20	−38.6
(MTBE carboxylation) MTBE + H_2 +	300	−51.9	5	−42.1
carbonate → TBA + acetate + water	3000	−57.4	10	−43.7
	30000	−62.9	20	−45.4
(MTBE hydrogenation) MTBE +	300	−86.2	5	−78.0
$H_2 \rightarrow$ TBA + methane	3000	−91.7	10	−79.7
	30000	−97.2	20	−81.4

[1]Alkalinity 200 mg/L, pH 7, acetate 0.3 mg/L, temperature 15°C.

This relationship is described in Equation 2, where F is the fraction of MTBE remaining after biodegradation. The slope of the line is the isotopic enrichment factor (ε) in Equation 2.

$$\delta^{13}C_{\text{MTBE in ground water}} = \varepsilon \ln F + \delta^{13}C_{\text{MTBE in gasoline}} \quad (2)$$

Equation 2 can be used to calculate the fraction of MTBE remaining after biodegradation from the $\delta^{13}C$ of MTBE in the ground water, the $\delta^{13}C$ of MTBE in the gasoline originally released, and the isotopic enrichment factor (ε) for biodegradation.

Kolhatkar et al. (2002) previously reported the enrichment factor (ε) for anaerobic biodegradation of MTBE in the microcosm study discussed in this paper (-9.16 ± 5.0 at 95% confidence). More recently, Kuder et al. (2004) reported a more precise value of ε calculated from enrichment cultures that were made from the microcosms (-12.4 ± 1.5 at 95 % confidence). The experimental value of Kuder et al. (2004) is in good agreement with the theoretical value of –12.2 that would be expected from the cleavage of a C–O bond in a molecule with five carbon atoms (Kuder et al. 2004).

Schmidt et al. (2004) report that the normal isotopic ratio of carbon in MTBE in gasoline ranges from $-31.7‰$ to $-28.1‰$ (the highest ratio of ^{13}C to ^{12}C). O'Sullivan et al. (2003) surveyed the isotopic composition of 27 samples of gasoline from around the world. The range of $\delta^{13}C$ was from $-32.0‰$ to $-27.4‰$. Equation 2 was used to estimate the fraction of MTBE remaining in selected monitoring wells at the Parsippany site from the $\delta^{13}C$ of MTBE in the ground water. A conservative value of $-27.4‰$ was used for the $\delta^{13}C$ of the original MTBE in gasoline and a value of –12.2 was used for ε. The estimates are a conservative upper boundary on the fraction remaining. The true values for the fraction remaining can be lower but not higher than the estimates. These estimates of the extent of biodegradation of MTBE are presented in Table 6.

Table 6
Biodegradation of MTBE in Monitoring Wells Predicted from the $\delta^{13}C$ of Residual MTBE in the Ground Water. The $\delta^{13}C$ Data Are from Kolhatkar et al. (2002)

Monitoring Well	Date of Sampling	$\delta^{13}C$ in MTBE ($‰$)	Fraction Remaining (F)
MW-5	2000	−25.33	0.84
MW-5	2002	−26.04	0.89
MW-6	2000	−7.59	0.20
MW-6	2001	−11.25	0.27
MW-6	2002	−8.95	0.22
MW-7	2001	5.52	0.07
MW-7	2002	3.52	0.08
MW-10	2001	1.83	0.09
MW-10	2002	3.37	0.08
MW-11	2000	−17.37	0.44
MW-11	2001	−13.49	0.32
MW-11	2002	−13.81	0.33

Biodegradation of MTBE in water from well MW-5 was limited (Table 6). At most, only 16% of the MTBE had degraded. However, ~70% to 80% of the original concentration of MTBE had been degraded in water from well MW-6, where the core samples were taken for the microcosm study. More than 90% of the MTBE had degraded in water from wells MW-7 and MW-10. Approximately 60% of the MTBE had degraded in water from well MW-11.

Conclusions

Conventional wisdom holds that the rates of anaerobic processes in ground water are slow. In three experimental treatments in this microcosm study, the rate of MTBE transformation to TBA varied from 11 ± 2.3 per year to 12 ± 2.9 per year at 95% confidence. The microcosm study documented that the aquifer harbored organisms that were capable of rapidly degrading MTBE under anaerobic conditions. Considering the usual residence time of ground water at UST spill sites, these rates are effectively instantaneous.

To date, most field studies of MTBE have been conducted on large plumes that show little indication from their behavior that natural biodegradation is an important mechanism. Amerson and Johnson (2002) injected deuterated MTBE into a large MTBE plume at Port Hueneme, California. At the time of their experiment, the plume was more than 1000 m long and the leading edge of the plume continued to advance into the aquifer. A mass balance on the injected deuterated MTBE showed no evidence of natural MTBE biodegradation in the aquifer. Landmeyer et al. (1998) described a plume of MTBE near Beaufort, South Carolina; the plume traveled over 200 m before it discharged to surface water. The rate of natural biodegradation in the plume was less than 0.04 per year (Landmeyer et al. 2001).

In contrast, this case study at Parsippany, New Jersey, was on a small MTBE plume ~40 m in diameter. Ground water flow at the site should be rapid, on the scale of 10 to 44 m/year. The spill is more than a decade old. The plume of ground water contamination could be hundreds of meters long. Despite the opportunity for transport of MTBE, there is little evidence of transport of MTBE to the west, to the north, or to the east of the perennial source area of MTBE in ground water. Rapid biotransformation of MTBE to TBA would confine MTBE in ground water to the region where the plume is being continually generated by dissolution of fuel components from residual gasoline in the aquifer.

In the plume at field scale and in the microcosm study, MTBE was degraded to TBA. There was no evidence for natural biodegradation of TBA in the laboratory microcosm study, and TBA accumulated in the downgradient portion of the plume at field scale.

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and objective, and they offered useful suggestions to improve the manuscript. The manuscript was extensively revised in response to their comments.

Authors' Note: Our discussion pertains to conditions at the site through 2002. In subsequent years, the concentration of MTBE has increased in monitoring well MW-3 to as much as 46,000 µg/L and in MW-4 to as much as 19,500 µg/L, with little evidence of transformation of MTBE to TBA. Our evaluation and conclusions concerning the processes operating at the site are not binding on the New Jersey Department of Environmental Protection.

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Editor's Note: The use of brand names in peer-reviewed papers is for identification purposes only and does not constitute endorsement by the authors, their employers, or the National Ground Water Association.

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