Methyl *tert*-Butyl Ether Biodegradation by Indigenous Aquifer Microorganisms under Natural and Artificial Oxic Conditions

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Microbial communities indigenous to a shallow groundwater system near Beaufort, SC, degraded milligram per liter concentrations of methyl *tert*-butyl ether (MTBE) under natural and artificial oxic conditions. Significant MTBE biodegradation was observed where anoxic, MTBE-contaminated groundwater discharged to a concrete-lined ditch. In the anoxic groundwater adjacent to the ditch, concentrations of MTBE were >1 mg/L. Where groundwater discharge occurs, dissolved oxygen (DO) concentrations beneath the ditch exceeded 1.0 mg/L to a depth of 1.5 m, and MTBE concentrations decreased to <1 μg/L prior to discharge. MTBE mass flux calculations indicate that 96% of MTBE mass loss occurs in the relatively small oxic zone prior to discharge. Samples of a natural microbial biofilm present in the oxic zone beneath the ditch completely degraded [U-^{14}C]MTBE to [^{14}C]CO$_2$ in laboratory liquid culture studies, with no accumulation of intermediate compounds. Upgradient of the ditch in the anoxic, MTBE- and BTEX-contaminated aquifer, addition of a soluble oxygen release compound resulted in oxic conditions and rapid MTBE biodegradation by indigenous microorganisms. In an observation well located closest to the oxygen addition area, DO concentrations increased from 0.4 to 12 mg/L in <60 days and MTBE concentrations decreased from 20 to 3 mg/L. In the same time period at a downgradient observation well, DO increased from <0.2 to 2 mg/L and MTBE concentrations decreased from 30 to <5 mg/L. These results indicate that microorganisms indigenous to the groundwater system at this site can degrade milligram per liter concentrations of MTBE under natural and artificial oxic conditions.

Introduction

Methyl *tert*-butyl ether (MTBE) is a gasoline oxygenate that results in decreased emissions of carbon monoxide (CO) and ozone (O$_3$) precursor compounds. In CO and O$_3$ nonattainment areas defined by the Clean Air Act Amendments, gasoline contains MTBE in volumes no less than 10% (*1, 2*). However, gasoline with 5% or less MTBE by volume has been used throughout the United States for octane enhancement since the phase-out of tetraethyllead in the late 1970s. Due to its widespread use and hydrophilic properties, MTBE is detected throughout the hydrosphere (*3, 4*). The potential human health risks associated with MTBE have led the U.S. Environmental Protection Agency (EPA) to issue an Advance Notice of Proposed Rulemaking under Section 6 of the Toxic Substances Control Act (TSCA) that is intended to reduce or eliminate the use of MTBE as a gasoline additive in the United States (*5*).

Even if MTBE is removed from fuel supplies in the future, the legacy of MTBE usage will continue to be a significant environmental challenge. In shallow gasoline contaminated aquifer systems, for example, the half-life of MTBE is thought to be considerably longer than for the other soluble gasoline compounds such as benzene, toluene, ethylbenzene, and xylenes (BTEX) (*6*). In addition, dissolved-phase MTBE has been observed to migrate farther laterally and vertically from leaky underground storage tanks (USTs) than BTEX (*7, 8*). Although some evidence has been reported that MTBE can be degraded under the anoxic conditions characteristic of most gasoline releases (*7, 9–14*), the characteristically low rates are not significant compared to rates of MTBE transport by groundwater flow. Likewise, although faster MTBE biodegradation rates have been reported for oxic conditions where MTBE is either the sole substrate (*15–18*) or is cometabolized (*19–21*), these rates are derived from laboratory studies using enrichment cultures, and their relevance to in situ microbial populations and conditions remains unclear.

Recent laboratory studies, however, have indicated that MTBE can be rapidly mineralized to CO$_2$ under oxic conditions by microorganisms indigenous to surfacewater bed sediments regardless of prior redox condition (*22*) or previous MTBE exposure (*23*). In all cases, MTBE degradation was observed in aerobic microcosms but not under strictly anaerobic conditions (*22*). These observations suggest that the supply of oxygen is a major constraint on the rate and extent of MTBE biodegradation under in situ conditions and that microorganisms indigenous to groundwater systems may be capable of efficient MTBE biodegradation if this oxygen constraint is relieved by a natural or artificial source of oxygen. In this paper field evidence indicates a significant decrease in MTBE concentrations associated with the onset of oxic conditions at a groundwater discharge location. Laboratory evidence indicates that MTBE removal is related to the aerobic activity of a natural microbial biofilm that has developed at the discharge location. Moreover, this paper demonstrates that artificial oxygen addition to the upgradient anoxic aquifer stimulated efficient MTBE biodegradation by indigenous microorganisms.

Methods

Study Site. In 1991, gasoline that contained MTBE was detected in the shallow water-table aquifer near a former UST area at a gasoline station near Beaufort, SC (Figure 1). Since 1993, the U.S. Geological Survey (USGS) has been monitoring the evolution of the dissolved-phase plumes of MTBE and BTEX, as well as geochemical parameters in the aquifer (*7, 24, 25*) and vadose zone (*26*). As shown in Figure 1, the MTBE plume extends from the former UST area to 230 m downgradient, where additional transport is truncated at a location of groundwater discharge to a concrete-lined ditch. This transport and lack of migration transverse to the direction of groundwater flow are due primarily to the rapid rates of groundwater flow (minimum of 33 m/year) and low rates (~0.0001 per day) of MTBE biodegradation in

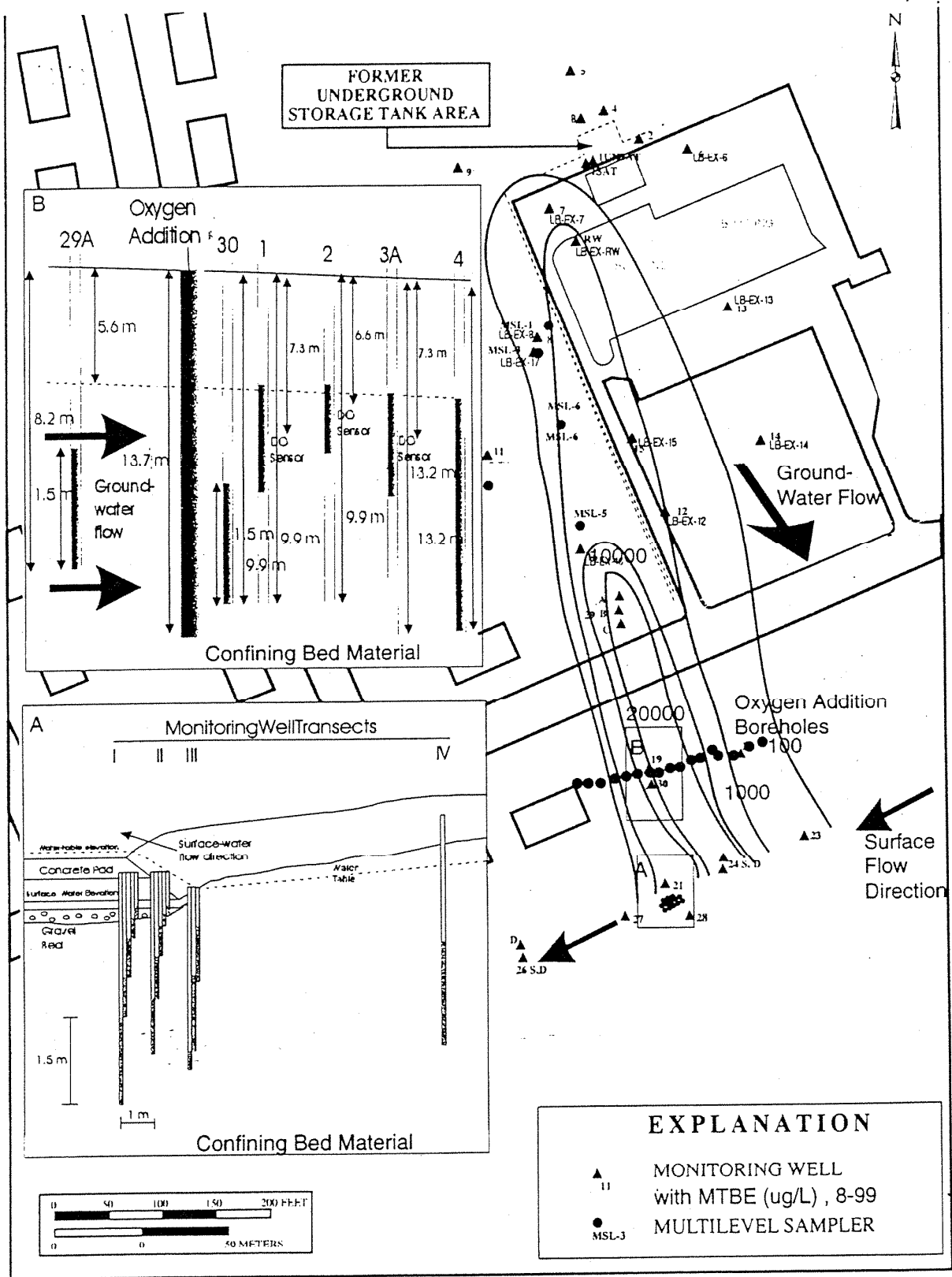* Corresponding author phone: (803) 750-6128; fax: (803) 750-6183; e-mail jlandmey@usgs.gov.

FIGURE 1. Map of the former underground storage tank area (location of present-day source area), isoconcentration lines for MTBE (g/L) in groundwater collected in August 1999. (Inset map A) Location of four transects of nested monitoring wells and structure of the surfacewater/groundwater interface at the concrete-lined ditch. Groundwater containing MTBE discharges through cracks and joints in the 10 ft 10 ft 4 in. and 10 ft 9 ft 2 in. concrete pads. The pads are underlain by a 6.6-cm-thick gravel bed, coated with a visible biofilm. (Inset map B) Location of the oxygen addition test area and monitoring wells along a groundwater flowpath.

the anoxic aquifer (24). The aquifer and vadose zone are composed of well-sorted sand grains of Pleistocene to Holocene age with <0.01% natural sedimentary organic matter and are underlain by a regional clayey confining unit at 13.7 m. Rainwater provides 33−44 cm/year of recharge to the aquifer in this semitropical location. The depth to water is 3.9 m near the former UST area in the northern part of the site and from 2.7 to 0.6 m near the concrete-lined ditch.

The structure of the concrete-lined ditch provided the opportunity to quantitatively evaluate the natural attenuation of milligram per liter concentrations of MTBE by a naturally selected microbial community where anoxic groundwater discharges to oxic surfacewater. Although designed primarily to convey surfacewater runoff, discharge measurements made upgradient and downgradient of the point of MTBE-contaminated groundwater discharge indicate that between precipitation events most of the water in the ditch consists of groundwater (7). Further evidence that the ditch is a groundwater discharge location is that groundwater level elevations made in wells drilled through the concrete are consistently higher than the water level in the ditch. This discharge of groundwater occurs primarily through cracks and joints in the 8.8-cm-thick concrete. This concrete pad significantly reduces volatilization losses of MTBE prior to groundwater discharge. The concrete pad also restricts the transport of foreign organic matter from the surface water to the groundwater beneath the ditch. Underlying the concrete is a 6.6-cm-thick bed of gravel coated by a dense microbial biofilm. This biofilm extends through gaps and cracks in the concrete pad and is visible on the surface of the ditch near cracks.

Monitoring Wells. The site has been heavily instrumented with monitoring wells to delineate the MTBE and BTEX plumes and to document the direction of groundwater flow (7) (Figure 1). To characterize the potential for MTBE biodegradation by indigenous microorganisms near the ditch, 13 new wells were installed adjacent to and in a relatively small area of the concrete-lined ditch perpendicular to the predominant direction of groundwater flow (labeled transect I and II [in ditch] to III [ditch bank], Figure 1, inset map A). Transects I−III consist of wells made of 2.2-cm-diameter PVC pipe, with 2.2-cm-diameter vertically segregated and variably screened intervals. All wells were installed using direct-push technology and referenced to sea level. The most upgradient monitoring point (IV) was a previously installed 2.2-cm-diameter well (well 21). The potential for dilution of MTBE-contaminated groundwater by relatively cleaner water was minimized by locating the in-ditch wells near the downstream edge of the discharging MTBE plume, thereby ensuring that any parallel flow beneath the ditch from upstream areas would contribute elevated concentrations of MTBE to the monitoring wells (see Figure 1). Water-level measurements and groundwater samples were collected from these wells in August 1999 and January 2000. The analytical method used for MTBE has been described elsewhere (7).

To observe the effect of artificial oxic conditions on MTBE biodegradation by indigenous microorganisms in an anoxic portion of the aquifer upgradient from the ditch, two previously installed wells (wells 29A and 30) and four new wells (1, 2, 3A, and 4) were installed along a groundwater flowpath through the oxygen release area (Figure 1, inset map B). These wells consist of 4.4-cm-diameter PVC pipe and 4.4-cm-diameter PVC screen. Specifically, well 29A is screened from 6.7 to 8.2 m, well 30 is screened from 8.25 to 9.9 m, wells 1 and 2 are both screened from 6.6 to 9.9 m, well 3A is screened from 9.9 to 13.2 m, and well 4 is screened from 9.9 to 13.2 m. The average depth to the water table as measured in January 2000 was 5.61 m below land surface. Concentrations of MTBE and geochemical parameters were

analyzed in these wells in August 1999 before oxygen was added in September and after in October 1999, December 1999, January 2000, and September 2000.

MTBE Mass Flux. The monitoring wells installed in and adjacent to the ditch were used to estimate changes in MTBE mass flux attributable to biodegradation by indigenous microorganisms from the anoxic aquifer to the concrete-lined ditch using a modification of the Theis polygon method originally developed for surfacewater applications (27). In that method, the mass flux of a dissolved substance in a surfacewater body can be quantified by dividing a transect located at right angles to flow direction into a series of polygons of known area. For groundwater systems, these polygons can be envisioned as being centered around the screened interval midpoint of a series of wells in a transect, with the total area being bound on top by the water-table surface, below by the bottom of the screened interval, and on either lateral side by the same distance that exists between the well of interest and its closest neighbor (0.3 m in most cases for this study).

The mass flux of MTBE from an upgradient to a downgradient well transect can be related to groundwater discharge through the cross-sectional area of each transect by

$$F = \sum (CAq) \tag{1}$$

where F = total mass of MTBE (M/t), C = MTBE concentration in each polygon (M/L^3), A = area of each polygon (L^2), and q = specific discharge of groundwater through each polygon [(L^3/t)/L^2].

As shown in Figure 1, groundwater flowlines to the surfacewater body are normal to the ditch where groundwater discharges, so the resultant cross-sectional areas used to determine MTBE mass flux are oriented normal to groundwater flow and parallel to the ditch. As stated previously, mass flux changes in MTBE concentrations could not be attributable to dilution, because parallel flow beneath the ditch from upstream areas would contribute higher, rather than lower, concentrations of MTBE to the monitoring wells.

MTBE Biodegradation Study. The potential for microorganisms indigenous to the groundwater discharge area to degrade MTBE was investigated in liquid culture microcosms using uniformly labeled [U-^{14}C]MTBE (10.1 mCi/mmol; 10% MTBE in ethanol) obtained from New England Nuclear Research Products, Du Pont (Boston, MA). The radiochemical purity of the [U-^{14}C]MTBE was determined by the manufacturer and independently confirmed in our laboratory using liquid scintillation counting and radiometric detection gas chromatography (GC/GRD) to be >97%.

Microcosm Studies. [U-^{14}C]MTBE mineralization was examined in aerobic liquid cultures containing biofilm material collected from the cracks in the concrete-lined ditch. As described above, a dense microbial biofilm coats the gravel that underlies the oxic portion of the concrete-lined ditch. Biofilm material was scraped from the concrete bed material and suspended in a small volume of surfacewater under an air atmosphere. Liquid culture microcosms were created by transferring 0.1 mL of the biofilm suspension to 25 mL serum tubes containing 9.9 mL of sterile, minimal medium and an air atmosphere. The minimal medium consisted of 98% phosphate buffer (8.3 mM, pH 7.2), 1% Wolfes mineral solution, 1% Wolfes vitamin solution, and 1.9 μCi of [U-^{14}C]-MTBE. Initial dissolved MTBE concentrations in equilibrium with the headspace were estimated to be 18.4 μM (1.6 mg/L), representative of the MTBE concentration reaching the ditch. Five replicate experimental treatments were prepared. Duplicate killed control microcosms were prepared in the same manner and autoclaved twice for 1 h at 15 psi and 121 °C. Duplicate cell-free controls were prepared as described without the addition of the biofilm inoculum. Headspace

concentrations of CH_4, $^{14}CH_4$, CO_2, and $^{14}CO_2$ were monitored by analyzing 0.5 mL of headspace sample using GC/GRD combined with thermal conductivity detection. Headspace concentrations of MTBE and [U-^{14}C]MTBE were monitored using GC/GRD combined with flame ionization detection. The headspace sample volumes were replaced with oxygen (99.9% Scotty II, Scott Specialty Gases, Plumsteadville, PA). The GC/GRD output was calibrated by liquid scintillation counting using H^{14}CO$_3$ or [U-^{14}C]MTBE. The results of the [U-^{14}C]MTBE mineralization studies were corrected for the loss of constituents due to headspace sample collection.

Artificial Oxic Conditions. Oxygen in the form of a metal peroxide slurry (magnesium peroxide [MgO$_2$] and water) was added to an anoxic part of the aquifer upgradient of the ditch. The slurry slowly releases dissolved oxygen upon hydrolysis with groundwater, according to

$$MgO_2 + H_2O \rightleftharpoons {}^1\!/_2O_2 + Mg(OH)_2 \qquad (2)$$
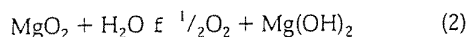
The MgO$_2$ slurry was a proprietary mixture consisting of 25–35% MgO$_2$ by weight (Regenesis Bioremediation Products). The release of dissolved oxygen (DO) into the adjacent aquifer material is governed by chemical diffusion, and transport to downgradient areas is governed by advection. Alternative methods to add oxygen to aquifer systems exist: addition of pure H$_2$O$_2$ (see ref 28 for review), addition of H$_2$O$_2$ with UV for generation of hydroxyl radicals (·OH) (29), addition of pure air (100% oxygen) (28, 30, 31), sparging with ambient air (21% oxygen) (28), and release of ambient air via semipermeable tubing placed in the aquifer (32, 33). However, these methods were not selected for use in this study because of the significant potential for MTBE mass to be lost due to volatilization. The MgO$_2$ material has been used by other workers to decrease BTEX levels in groundwater at gasoline spill sites (34–37), and reportedly MTBE at another site (38), although that evidence is equivocal because MTBE concentrations were already decreasing prior to MgO$_2$ addition.

In the oxygen addition area, a slurry consisting of 30 kg of MgO$_2$ mixed with 30 L of uncontaminated water was injected into each of 18 boreholes on 1.7-m centers located in a transect across the MTBE plume (Figure 1 and inset map B) through the drill rods of a direct-push rig using a high-pressure grout pump. The slurry was injected in each borehole from the bottom of the contaminated aquifer at 13.7 m to 0.6 m above the average water-table surface (to account for seasonal fluctuations in water-table elevation). The remainder of the borehole was filled to land surface with bentonite pellets. The total volume of uncontaminated water added to the aquifer as part of the slurry was <10% of the volumetric flux of groundwater through the area amended with oxygen, so injection will not significantly affect the concentrations of MTBE in the aquifer. This line of oxygen addition boreholes was designed to ensure that anoxic, MTBE-contaminated groundwater from upgradient areas passed through a permeable barrier of oxygenated aquifer material, thereby creating a long-term source of oxic conditions in the anoxic aquifer.

DO concentrations were measured in wells in the oxygen addition area before and after oxygen addition. DO concentrations were measured in August 1999 at the wellhead in all six wells in the test area using a colorimetric method (CHEMets) and in wells 1 and 2 with in-well sensor technology that measured DO concentrations at 15-min intervals. The in-well DO sensors (Hydrolab Minisonde) were installed in May 1999 in wells 1 and 2 at 7.3 and 6.6 m bls, respectively. A third DO sensor was installed in November 1999 in downgradient well 3A, at 7.3 m bls. After oxygen addition in September 1999, field measurements of DO concentrations using the CHEMets occurred in October and December 1999 and January and September 2000, and these field values were used to calibrate the DO sensors that had been in continuous operation since May 1999.

Results and Discussion

MTBE Fate under Natural Oxic Conditions. The concrete-lined ditch is a site of localized groundwater discharge as indicated by groundwater level measurements made during periods of low and normal rainfall. For example, Figure 2A depicts equipotential lines drawn for water-level measurements made during a period of low rainfall (August 1999). These equipotential lines indicate that the surfacewater body receives groundwater from both a horizontal component of flow from upgradient areas adjacent to the ditch (A) and an upward, vertical component of flow from beneath the ditch. During a period of higher rainfall and correspondingly high surfacewater flow rates in January 2000, groundwater continued to discharge to the ditch, along both horizontal (A) and upward, vertical (B) flowpaths (Figure 2B). However, the magnitude of the upward, vertical head gradient remained unchanged. The position of the equipotential lines with respect to the water-table surface also indicated little influence of downward, vertical flow from local infiltration of precipitation near the ditch and, hence, lack of significant dilution of MTBE prior to discharge.

The distribution of DO concentrations measured using low-flow sampling of the monitoring wells installed in and adjacent to the ditch indicated the presence of an oxygen-rich zone directly beneath the ditch. As depicted in Figure 3A, the extent of this oxic zone reaches 1.5 m below the bottom of the concrete-lined ditch and at progressively shallower depths nearer the ditch bank. The presence of DO below the ditch and the lack of DO at deeper depths suggest that a combination of oxygen diffusion and advective mixing of surfacewater and groundwater may be responsible for the oxic conditions in the relatively permeable gravel bed beneath the concrete pad (Figure 1, inset map A). Such hydrologic connection between surfacewater and groundwater systems is well-known (39) and widespread (40).

Groundwater sampling indicated that MTBE concentrations rapidly decrease as anoxic groundwater comes into contact with oxygenated water beneath the concrete-lined ditch. For example, both sampling events showed a similar pattern of decreasing MTBE concentrations in the direction of groundwater flow to the ditch (Figure 3A,B), although overall concentrations of MTBE were higher during the dry summer of 1999 and lower in January 2000 following more extensive dilution of groundwater solutes across the entire study area by rainfall events. A representative groundwater flow line from transect IV to II in August 1999 depicts MTBE decreasing from 3140 to 1090 μg/L before the oxic zone and from 1090 to 92 μg/L in the oxic zone (Figure 3A). The shallowest screened wells in cross sections I and II were characterized by either no detection or up to 2 orders of magnitude lower MTBE concentrations than were measured in cross section IV located ~6.5 m upgradient. These shallowest screened wells also contained >1.0 mg/L DO, whereas the deeper wells in transect III had no measurable DO and MTBE concentrations ranging from 294 to 1090 μg/L.

Because the oxic conditions lead to the aerobic biodegradation of MTBE as groundwater passes through the oxygenated ditch-bed sediments, the mass loss of MTBE due to this biodegradation can be estimated. The MTBE mass loss (reported here as the flux of MTBE) through transects I–IV was determined using eq 1 for both August 1999 and January 2000 sampling events. In August 1999, the MTBE mass flux for the most upgradient, anoxic area closer to the source area of groundwater contamination was 1.4 g of MTBE/day (Table 1). In contrast, the MTBE mass flux to the concrete-lined ditch was only 0.053 g of MTBE/day. Hence, between transects IV and I (a distance of <6.5 m) there was
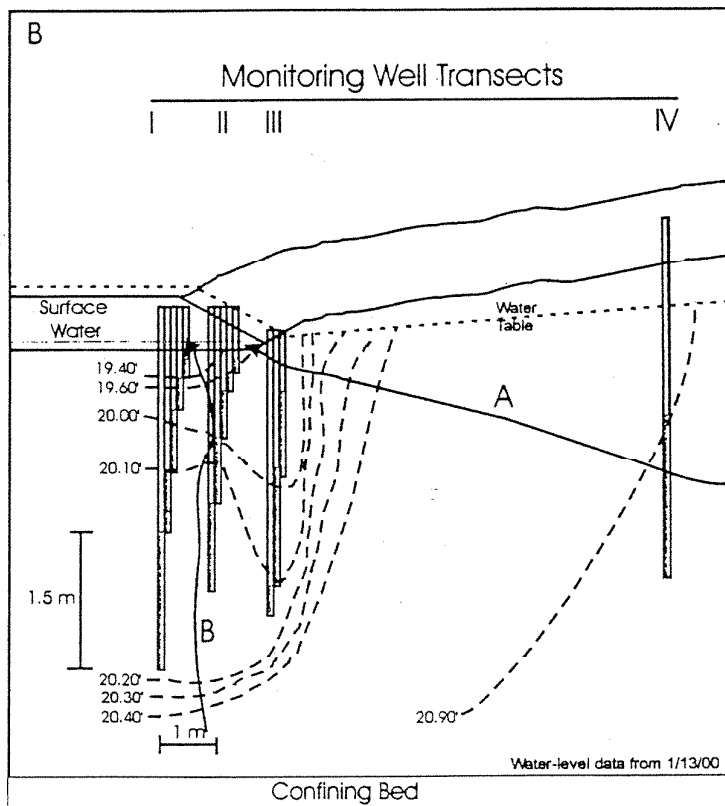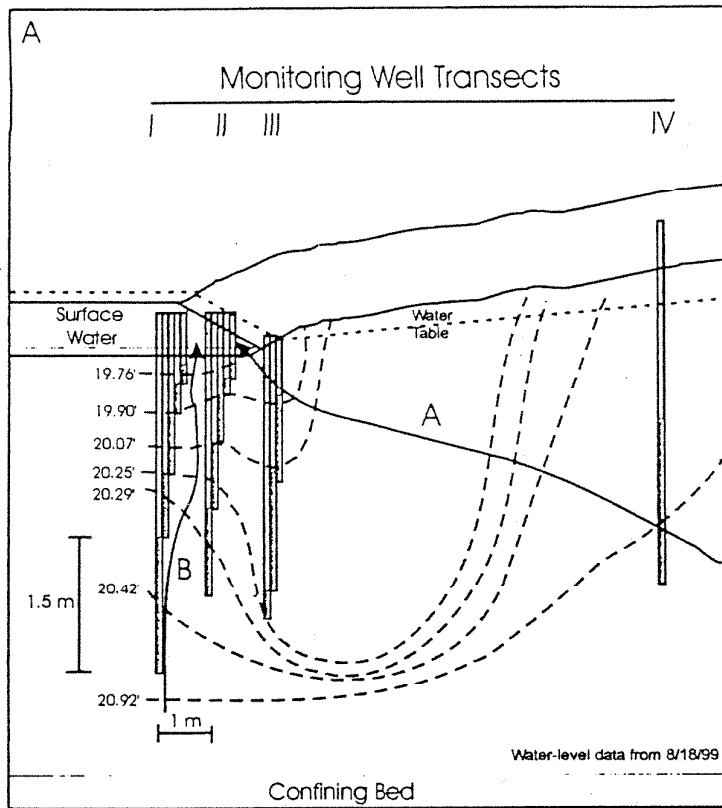
FIGURE 2. Cross-section view of four well transects, showing vertically segregated screened intervals, groundwater equipotential lines for August 18, 1999 (A), and January 13, 2000 (B). Representative horizontal (A) and upward, vertical (B) components of groundwater flow are depicted.
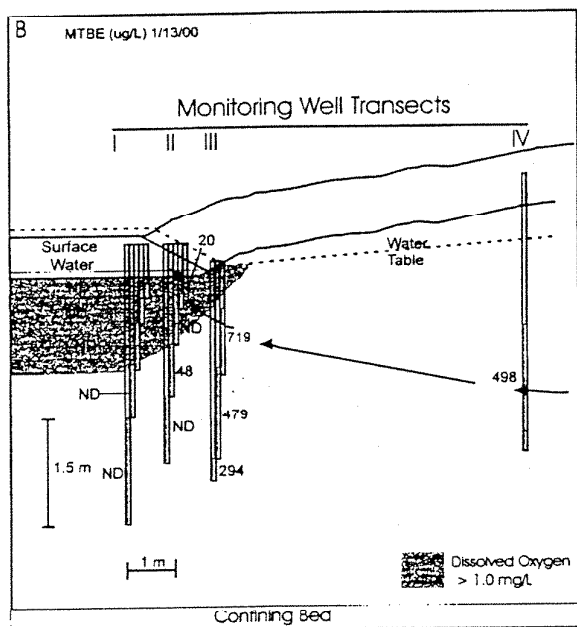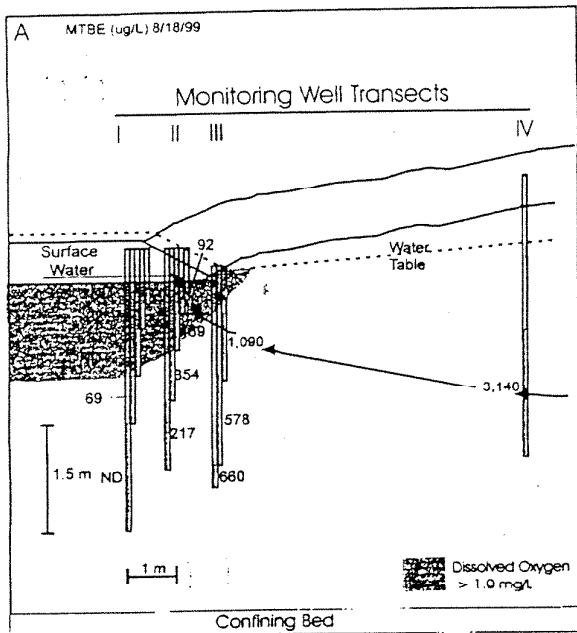
Panel A labels: A, Monitoring Well Transects, I, II, III, IV, Surface Water, Water Table, 19.76', 19.90', 20.07', 20.25', 20.29', 20.42', 20.92', B, 1.5 m, 1 m, Water-level data from 8/18/99, Confining Bed

Panel B labels: B, Monitoring Well Transects, I, II, III, IV, Surface Water, Water Table, A, 19.40', 19.60', 20.00', 20.10', 20.20', 20.30', 20.40', 20.90', B, 1.5 m, 1 m, Water-level data from 1/13/00, Confining Bed

FIGURE 3. Cross-section view of four well transects and MTBE concentrations (g/L) for groundwater samples collected August 18, 1999 (A), and January 13, 2000 (B). The approximate location of the oxygenated zone beneath the ditch, defined as DO concentrations >1 mg/L for groundwater sampled on June 1, 2000, is represented by the gray fill. ND means MTBE was not detected at concentrations >1 g/L. Representative horizontal groundwater flowline is depicted.

a 96% loss of MTBE. Moreover, because no MTBE was detected beneath the ditch in January 2000, there was essentially 100% mass loss of MTBE in the oxic/anoxic interface before discharge to the ditch. These MTBE mass losses are attributable to biodegradation and not dilution or dispersion, because flow from upstream areas would contribute higher concentrations of MTBE to the monitoring well network, rather than lower concentrations (Figure 1). Overall, the MTBE mass loss at the oxygenated groundwater discharge location, which occurs over a distance of <1 m, is

TABLE 1. Mass Flux of MTBE between Four Well Transects Located in and near the Concrete-Lined Ditch where Anoxic, MTBE-Contaminated Groundwater Discharges, near Beaufort, SC[a]

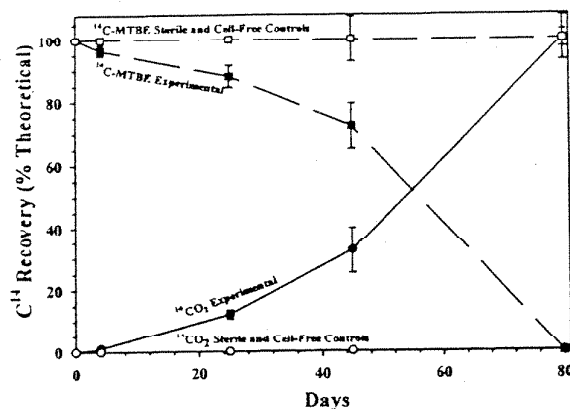|	MTBE mass flux, g/day	
well transect	Aug 18, 1999	Jan 13, 2000
IV	1 387 000	220 000
III	241 900	27 820
II	386 100	18 750
I	53 600	0

[a] Data were collected Aug 18, 1999, and Jan 13, 2000.

FIGURE 4. Aerobic mineralization of [U-^{14}C]MTBE to $^{14}CO_2$ in liquid cultures containing biofilm material from beneath the concrete-lined ditch. Data are means ± SD for five replicate experimental, duplicate sterile control and duplicate cell-free control microcosms.

much higher than the MTBE mass loss over the tens of meters in the MTBE plume shown in Figure 1 (7).

Laboratory evidence confirmed that much of this MTBE mass loss can be attributed to natural microbial activity in the oxygenated groundwater discharge area. The natural microbial biofilm associated with the surface of the concrete-lined ditch and the underlying gravel bed demonstrated complete mineralization of [U-^{14}C]MTBE (Figure 4). After 80 days, no [U-^{14}C]MTBE was detected in experimental liquid cultures and the final recovery of [U-^{14}C]MTBE radiolabel as $^{14}CO_2$ was 99 ± 3%. No $^{14}CH_4$ was detected in any treatment in this study, and no intermediate compounds (i.e., tert-butyl alcohol) were detected. [U-^{14}C]MTBE mineralization was attributable to biological activity, because the recovery of $^{14}CO_2$ in autoclaved and cell-free control microcosms was e 2% and the recovery of [U-^{14}C]MTBE was 100% (Figure 4).

MTBE Fate under Artificial Oxic Conditions. Prior to oxygen addition, the deeper wells located closer to the oxygen addition boreholes contained the highest concentrations of MTBE and the lowest concentrations of DO. For example, well 29A upgradient of the oxygen addition boreholes had 37 mg/L MTBE, and well 30 directly adjacent to the borehole location had 17 mg/L MTBE in January 1998, almost 2 years prior to oxygen addition, with 19.6 mg/L MTBE measured in August 1999 before oxygen addition. These results indicate that the input of MTBE into the oxygen addition area was high and constant prior to oxygen addition (well 30, Figure 5A). Concentrations of benzene and toluene in these wells prior to oxygen addition were also >1 mg/L (Figure 1). As such, DO levels remained near 0 mg/L during this period and were confirmed using the in-well DO sensors to be continually low for the 3 months prior to oxygen addition (data not shown). At well 1 located 1 m downgradient from
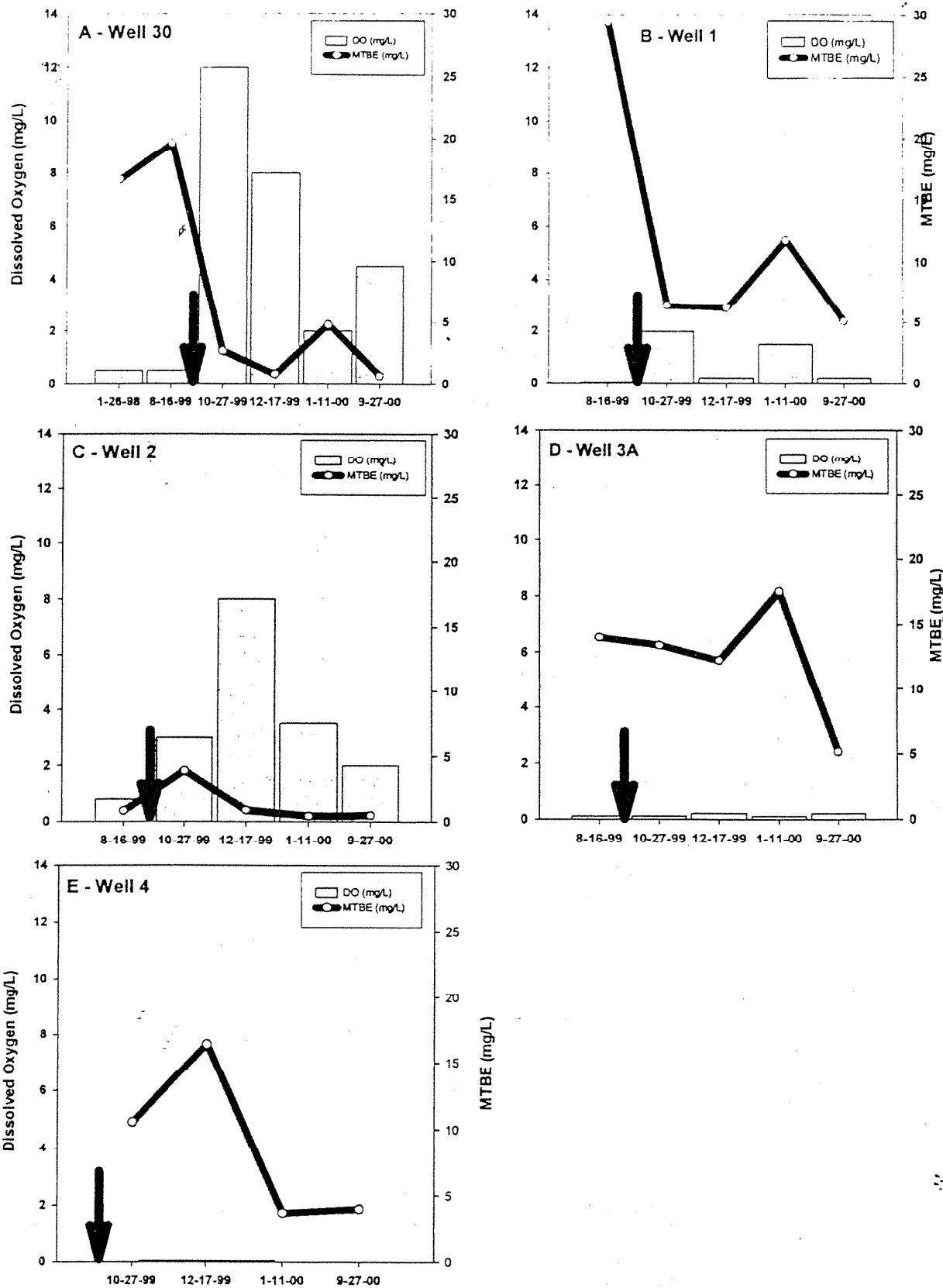
FIGURE 5. MTBE and DO concentrations in wells 30 (A), 1 (B), 2 (C), 3A (D), and 4 (E) in the direction of groundwater flow collected in January 1998 (for well 30 only); August, October, and December 1999; and January and September 2000. Refer to Figure 1, inset map B, for well locations. Arrow represents oxygen addition on September 9, 1999.

the oxygen addition area and also representative of the water chemistry at the deeper portion of the shallow aquifer. 29.8 mg/L MTBE and 0.1 mg/L DO were measured in August 1999 (Figure 5B). At the shallower conditions represented by well 2 located farther downgradient than wells 30 and 1, much lower concentrations of MTBE (<0.8 mg/L) and higher concentrations of DO (0.8 mg/L) were observed (Figure 5C), reflecting the facts that the screened interval of well 2 is above the main plume and that well 2 also receives DO from localized precipitation infiltration (7). Even farther downgradient but at the same depth interval as well 1, well 3A had MTBE concentrations near 17.5 mg/L and anaerobic conditions prior to oxygen addition (Figure 5D).

Following the delivery of oxygen in early September 1999, however, MTBE concentrations changed significantly in the oxygen-impacted wells. In the wells closest to the oxygen addition area (wells 30 and 1), for example, in well 30, DO concentrations increased from 0.4 to 12 mg/L and MTBE concentrations decreased from 19.6 to 2.66 mg/L, and in well 1 DO increased from 0.2 to 2 mg/L and MTBE concentrations decreased from 29.8 to 6.42 mg/L, within 60 days (Figure 5A,B). The relatively smaller increase in DO in well 1 compared to that in well 30 is due to the facts that well 1 is located farther downgradient of and screened shallower than well 30, and well 1 had almost 10 mg/L higher initial MTBE concentrations than well 30. This decrease in MTBE concentration in wells 30 and 1 equates to decreases of up to 87 and 79%, respectively. Simulations of MTBE fate using the numerical code BIOSCREEN (41) indicated that the decreases in MTBE concentrations observed in the field could not be explained solely by groundwater advection (simulation data not shown). This removal of MTBE is rapid considering concentrations in well 30 had been consistently >17 mg/L for almost 2 years of monitoring prior to oxygen addition (Figure 5A). In a bioaugmentation study (31), similar rapid MTBE removal was reported 4 months after oxygen delivery to an MTBE-only contaminated aquifer. Interestingly, they saw a less dramatic but similar response to their test plot that was not seeded but had only oxygen added, suggesting oxygen was the limiting factor (31). Moreover, in contrast to previous reports that MTBE degradation lags behind the preferential degradation of BTEX compounds in aquifers contaminated by oxygenate-containing gasoline (37), at this study site, MTBE degradation occurred simultaneously with similar orders of magnitude decreases in benzene and toluene concentrations (data not shown). In the farther downgradient and shallower well 2, the initially low MTBE concentrations did not change following oxygen addition (Figure 5C). Farther downgradient and deeper in the aquifer, wells 3A and 4 continued to have high MTBE concentrations and low DO, suggesting no oxygen transport to this area (Figure 5D,E). Moreover, sampling of well 30 in January 2000 indicated essentially no change in other potential oxygen sinks, even though this well had the largest increase in DO concentration: dissolved ferrous iron [Fe(II)] concentrations went from 1.74 to 1.17 mg/L, and hydrogen sulfide concentrations went from 0.363 to 0.483 mg/L. In well 1 farther downgradient, Fe(II) remained unchanged (from 1.02 to 1.08 mg/L), while hydrogen sulfide decreased from 3.82 to 1.37 mg/L. Because sediment-bound concentrations of these species were not quantified, it is likely that at least some oxygen was consumed by these sinks following oxygen addition.

Even a year after oxygen addition, MTBE concentrations in well 30 have continued to be <0.5 mg/L (Figure 5A), and DO concentrations have stabilized around 3.5 mg/L. In well 1, MTBE concentrations are no more than 5 mg/L a year after injection (Figure 5B). In shallow well 2 downgradient from wells 30 and 1, MTBE concentrations have remained relatively low and unchanged, and the increase in oxygen

observed was due to advection from the oxygen addition area and by input from rainfall during the wetter winter months (Figure 5C). In the most downgradient well 3A, whereas DO levels have not increased even a year after oxygen addition, MTBE concentrations that had been >10 mg/L decreased to 5 mg/L in September 2000 (Figure 5D). This concentration is similar to the MTBE concentration in the upgradient area that initially was characterized by the most complete MTBE biodegradation, and this lower value measured in well 3A represents the effluent concentration of MTBE after biodegradation being transported to downgradient areas. A similar concentration of MTBE is seen in the most downgradient well 4 (Figure 5E).

These field observations of relatively rapid aerobic MTBE biodegradation following oxygen addition suggest that the indigenous bacteria have become acclimated not only to milligram per liter concentrations of MTBE in the almost 10-year-old gasoline plume but also to the periodic delivery of oxygen by recharge events (7). At the site, rainfall events can deliver up to near saturation levels of DO (8 mg/L) into the contaminated aquifer (7). Monitoring of DO at 15-min intervals with the real-time DO sensors system described earlier indicated that this rainfall delivery of DO near 8 mg/L returns to background, anoxic levels within 24 h (unpublished data, J. E. Landmeyer). This DO loss cannot be explained solely by groundwater advection, because the conservative parameters of specific conductance and groundwater temperature also being measured returned to background values less rapidly. However, even though rainfall recharge at the study site is significant (33−44 cm/year), it is not a consistent source of DO, and consequently MTBE concentrations remained high prior to the oxygen addition experiment. However, the ability to degrade gasoline-related contaminants under these periodic oxic conditions presumably has been occurring since the release of the contamination and most likely contributed to the rapid MTBE losses observed under artificial oxic conditions.

The observed biodegradation of MTBE in a shallow groundwater system at locations of natural and artificial oxic conditions is important in light of the large amount of evidence that exists suggesting that MTBE is relatively recalcitrant in many groundwater systems (1−4, 6, 7, 9−13) and that MTBE would therefore preferentially accumulate. Those studies indicated that the success of bioremediation strategies such as natural attenuation would likely be low in plumes containing MTBE (42) and that remediation could only occur if contaminated aquifers were inoculated with specific microorganisms derived from enrichment cultures shown to degrade MTBE (31). However, the results of this study demonstrate that significant natural attenuation of MTBE can occur if the oxygen limitations naturally associated with gasoline releases can be removed, either under natural conditions where discharging anoxic groundwater comes into contact with oxygen or under artificial conditions where oxygen can be added to aquifers containing milligram per liter concentrations of MTBE. This latter solution may be an effective strategy for intercepting characteristically long MTBE plumes, particularly at those sites not characterized by groundwater discharge to land surface.

Acknowledgments

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