Anaerobic biodegradation of gasoline oxygenates in soils

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ABSTRACT: This study evaluated the biodegradation of methyl tertiary butyl ether (MTBE), ethyl tertiary butyl ether (ETBE), and tertiary butyl alcohol (TBA)—three chemicals used as gasoline additives. The biodegradation under sulfate reducing, methanogenic and denitrifying conditions was measured in static soil and water microcosms using soils of different origin and varying characteristics. The results indicate that TBA was the easiest compound to biodegrade, whereas MTBE was the most recalcitrant. It is thought that cleavage of the ether bond in MTBE and ETBE is the first and rate-limiting step in the degradation of these organics. TBA degradation was enhanced by nutrient addition in the nutrient poor soils but hindered by the presence of other easily-degraded organic compounds. Degradation of MTBE and ETBE occurred only in the soil with the lowest organic matter content and with a pH around 5.5. No degradation of MTBE and ETBE was observed in the organic-rich soils, and in the organically poor soil the addition of easily degradable organic compounds inhibited MTBE and ETBE degradation. *Water Environ. Res.*, **66,** 744 (1994).

KEYWORDS: anaerobic treatment, biodegradation, gasoline, hazardous wastes, soils.

The Clean Air Act Amendments, which were enacted in 1992, require oxygenates to be added in reformulated gasoline to reduce carbon monoxide emissions. Methyl tertiary butyl ether (MTBE), ethyl tertiary butyl ether (ETBE), and tertiary butyl alcohol (TBA)—three major gasoline octane enhancing chemicals—are likely candidates for use as gasoline oxygenates. Among these three, MTBE is the most favored (Anderson, 1988; Alnsworth, 1991). It is estimated that the production of MTBE will increase 25% per year over the next four years to meet the expected demand (Alnsworth, 1991).

These oxygenates are very water soluble (100%, 48 000, and 12 000 ppm for TBA, MTBE, and ETBE, respectively) and can account for more than 10% in gasoline (Budavari, 1989; Black, 1991). The lethal concentration of MTBE for 50% of tested mice (LC_{50}) was reported to be 1.6 mmole/L of atmosphere, and the suggested Maximum Contaminant Level-Goal (MCLG) for MTBE is 0.03 mg/L/day (Budavari, 1989; Dietrich, 1988). Oral LC_{50} of tested rates for TBA is 3.5 g/kg (Budavari, 1989). Toxicity data for ETBE is unavailable.

The ability of indigenous soil microorganisms to degrade many gasoline components is well known; however, there is little data on the biological fate of gasoline oxygenates. Past studies in our lab have demonstrated that the degradation of TBA is slow compared to methanol and phenol and varies considerably from one soil to another (Novak *et al.*, 1986; Hickman *et al.*, 1989; and Hickman and Novak, 1989). In soils where TBA was relatively recalcitrant, sulfate reducers appeared to inhibit TBA degradation (Morris, 1988). Comparable data for MTBE and ETBE is not available.

The information on biodegradation potential and degradation rates of these compounds is important for risk assessment and cleanup strategies. The purpose of this work was to study the biodegradability of these gasoline oxygenates in soils by indigenous organisms and to determine the factors that influence their biodegradation in anoxic and anaerobic subsurface environments. Specific objectives were to evaluate the biodegradation potential of MTBE, ETBE, and TBA in soils previously uncontaminated with fuels; study the effect of site variation on degradation; and determine the environmental factors that influence MTBE, ETBE, and TBA biodegradation.

Materials and Methods

Sites and sampling. Soils were collected from various depths at three sites. Site 1 was located in a wooded area at Virginia Polytechnic Institute (VPI) in Blacksburg, Va., and consisted mainly of unsaturated clay. Soil samples from two different points about 30 m away from each other were taken at this site. Soils from the first sampling point were taken in the Fall of 1988 and were designated as site 1(A). The second location, site 1(B), was taken in the Spring of 1990. Site 2 was also at VPI but positioned in a low area which received runoff from a nearby feedlot. The groundwater table was about 0.6 m below the surface. Soils at this site were sandy loam. Site 3 was in Newport News, Va. The groundwater table was about 1.5 m below the surface. Soils here were mainly silty loam. In addition to the site identification, the depth in feet where a soil sample was collected is placed after the site number. For example, site 2-1.5 represents a soil sample from 1.5 m below the surface at site 2. Soils from these sites have been extensively studied in our laboratory. Site 1 has been characterized as a "slow" site in terms of their relative degradation rates, while sites 2 and 3 have been categorized as "fast" sites (Hickman *et al.*, 1989).

Subsurface material was collected by hand auger and transferred to sterilized mason jars fitted with teflon caps. Soils were then transported to the laboratory and stored at 10°C for a maximum of one month before they were used to prepare microcosms.

Chemical and microbial characterization. The soils were characterized as to their anaerobic microbial populations, soil anions (nitrate, nitrite, sulfate, and phosphate), soil moisture content, organic content, nitrogen availability, ammonium fixation rate, and soil pH. These chemical and microbial characteristics can be used to relate the soil and groundwater variations to the biological response.

Soil samples for nitrate, nitrite, and phosphate analysis were mixed with distilled water at a 1:1 volume ratio of soil to water. The resulting solution was shaken for 1 hour, and the supernatant

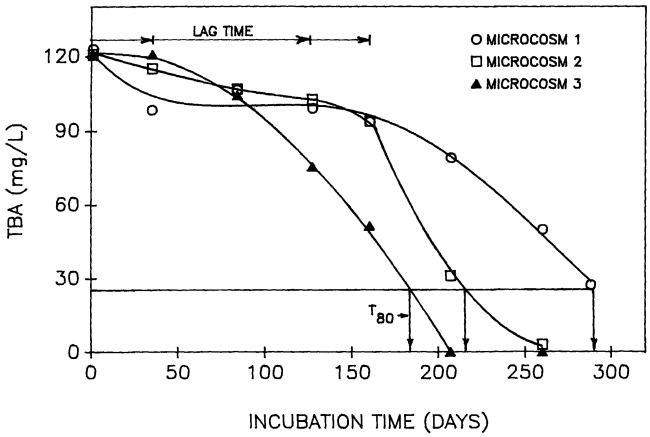

Figure 1—Degradation responses observed in three replicate microcosms with same soil and amendments.

was analyzed using a Dionex Model 2010i Ion Chromatograph with a Dionex HPIC-AS3 column. For sulfate analysis, soil samples were prepared by the same procedure except extraction was with a solution of 100 ppm phosphate as K_2HPO_4 (Tabatabi and Bremer, 1972). The soil moisture content was determined by drying soils at 104°C for 24 hours. The organic content was expressed as mg COD per kg dry soil (Nelson and Sommers, 1982). The biodegradable soil organic matter content was determined by measuring the soil organic content before and after incubation in water under aerobic conditions for 30 days after addition of inorganic nutrients (N and P). Nitrogen availability and ammonium fixation tests followed the methods described by Keeney (1982) and Blasco and Cornfield (1966), respectively. Soil pH was determined by mixing 5 g of soil with 5 mL of distilled water for 10 minutes and measuring the pH with a pH meter.

Bacterial densities were analyzed by the Most Probable Number (MPN) method (Alexander, 1982). Three anaerobic metabolic groups were enumerated: denitrifiers, lactate-using and acetate-using sulfate reducing bacteria (SRB), and acetate-using methanogens. The culture media and enumeration methods for denitrifying bacteria, SRB and methanogens followed the methods described by Tiedje (1982) and Kaspar and Tiedje (1982), respectively.

Microcosm setup. Biodegradation was evaluated in static soil/water microcosms. Aquifer material was aseptically transferred to septum-capped test tubes (13 × 10 mm) and then saturated with water which contained the oxygenate of interest. The initial concentration of organics was approximately 100 mg/L. Each microcosm contained approximately 5.0 grams (dry weight) of the appropriate soil. All microcosms were stored in the dark at 20°C. Microcosms of a particular organic compound and amendment condition were generally prepared in triplicate, but in a few instances single microcosms were used.

Two microliters of samples were periodically withdrawn from the aqueous phase by a heat-sterilized syringe and analyzed for TBA, MTBE, and ETBE by gas chromatography using a flame ionization detector and a 1.83 m × 3.18 mm (6 ft. × ⅛ in.) stainless steel column packed with 0.2% Carbowax 1 500 on 80/100 mesh Carbopack C. The carrier gas was nitrogen (24 cc/min), and the oven temperature was 120°C for TBA, 140°C for MTBE and ETBE. The injection port temperature was 150°C, and the detector temperature was 25°C.

Amendment studies. Amendment assays were conducted to examine the anaerobic degradation of these oxygenates using different metabolic pathways. Three major anaerobic microbial processes were studied including denitrification, sulfate reduction and methanogenous. Five mM of nitrate and 2 mM of sulfate were added to stimulate denitrification and sulfate reduction, respectively. Anaerobic processes were encouraged by adding reductants (0.05 g/L cysterine and 0.024 g Na_2S) in the microcosms.

The effects of environmental factors were evaluated. The factors examined in denitrification included the availability of inorganic nutrients and the presence of primary organic substrates (LaPat-Polasko *et al.*, 1988; McCarty *et al.*, 1981; Rittmann, *et al.*, 1980). The inorganic nutrient amendment consisted of 0.54 g/L KH_2PO_4, 1.23 g/L Na_2HPO_4, 0.3 g/L NH_4Cl, and 50 ml/L of mineral solution (6 g NaCl, 2.2. g $CaCl_2$, 2 g $MgCl_2 \cdot 6H_2O$ per liter of tap water). Sodium molybdate (a specific metabolic inhibitor for sulfate reduction) was added at 0.2 g/L in the microcosms to inhibit the activity of sulfate reducers.

Estimation of degradation. After the microcosms were prepared, concentrations of the added oxygenates were monitored for more than 250 days. Studies in the past have shown that TBA exhibits a long lag phase followed by a zero-order degradation pattern (Hickman *et al.*, 1989). Therefore, fast degradation rates do not necessarily indicate that compounds will degrade in a short time. Three parameters were used in the evaluation of degradation: degradation rate during the period where degradation is most active, lag time, and time for 80% of the compound to be degraded (T_{80}).

Degradation rates were calculated from the slope of portion in the concentration-time plot where active degradation occurred. The slope was then divided by the calculated dry weight of soil in each individual microcosm (Figure 1). Figure 1 also shows an example of a soil with a variable degradation response. The reported value is the average of three microcosms. The rate determined in microcosms may be lower than would occur in natural soil systems because the saturation of the soil results in a lower ratio of organisms to organic matter than would be found in soils. Error bars showing the standard deviation of the mean

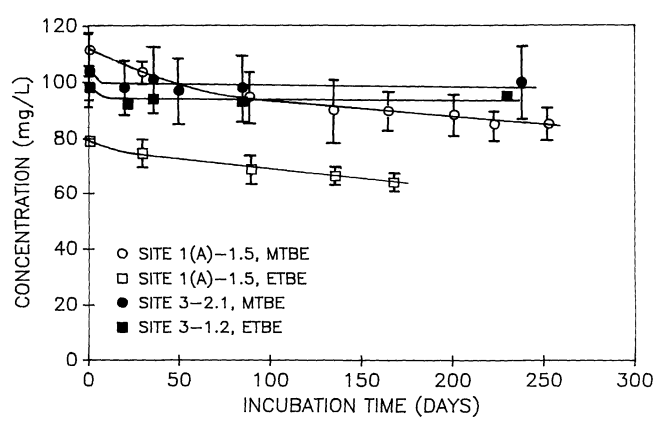

Figure 2—Fate of MTBE and ETBE in control microcosms. (Error bars are for the standard deviation of the autoclaved mean).

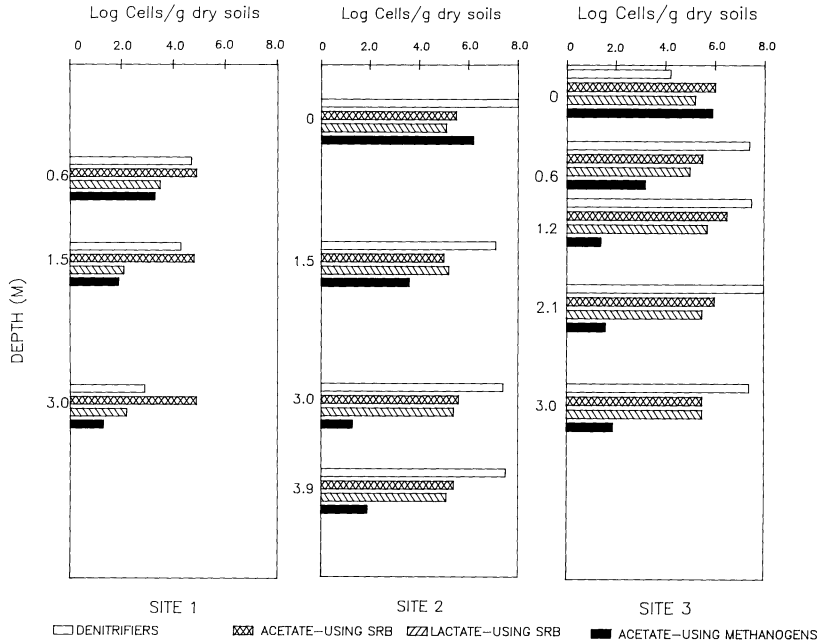

Figure 3—Distribution of anaerobic microorganisms in subsurface soils. (Error bars are for the standard deviation of the autoclaved mean).

are shown for several of the degradation studies to provide information regarding variability of the data.

Autoclaved controls. Controls were used to monitor and quantify the adsorption volatilization losses in the system. These contained soils which were autoclaved once a day for five consecutive days at 120°C and 15 psi pressure for 30 minutes. The soils were then transferred to the microcosms and autoclaved two more times. Figure 2 shows that following adsorption, MTBE and ETBE losses during sampling and incubation were less than 2% over 100 days of incubation.

Results and discussion

Soil characterization. Soil characteristics for the sites are listed in Table 1. Except for the surface soils, the soils at three sites were moderately acidic with a pH between 5.0 and 6.0. The degradable organic matter, nitrogen availability and ammonium fixation rates were reflective of the background at each site. In the soils from sites 1 and 2, where the organic flux came from surface infiltration, organic matter, nitrogen and sulfate contents were high in the upper layers and decreased with depth. Site 2 surface soil, which received runoff from a feedlot, was very high

Table 1—Chemical and physical characteristics of soils.

Soil	Moisture %	Soil pH	Organic, mg/g as COD		$NH_4^+ - N$, mg/kg		Major anion, mg/L[b]		
			Total	Degradable	Available	Fixation %	NO_3^-	PO_4^{-3}	SO_4^{-2}
1-0.6	26	5.0	10 800	3 700	14.0	53.8	0.2	0.07	34.0
1-1.5	29	4.8	3 300	<100	8.4	7.7	0.3	0.03	19.0
1-3.0	27	5.1	3 100	400	3.0	15.4	<0.1	0.40	5.0
1-4.2	30	5.0	1 600	<100	3.0	69.2	1.0	0.03	5.0
2-0	23	6.8	66 500	14 800	51.4	0	15.0	0.14	40.0
2-1.5	31	5.0	4 200	<100	26.6	0	7.0	0.14	24.0
2-3.0	30	5.1	1 700	200	8.7	0	8.7	0.14	20.0
2-3.9	26	5.2	1 100	<100	7.0	0	7.0	0.14	22.0
3-0	6	7.2	9 000	2 200	38.7	30.8	0.6	0.26	99.4
3-0.6	8	6.8	10 400	2 900	8.1	15.4	4.1	0.29	65.4
3-1.2	17	5.2	2 900	<100	22.6	0	0.5	0.12	7.8
3-2.1	22	5.7	6 500	3 200	19.5	0	0.2	0.21	20.0
3-3.0	41	6.2	155 000	11 000	72.9	0	<0.1	0.05	230.0

[a] Average value of site 1(A) and Site 1(B).
[b] in 1:1 soil extract.

in organic matter. At Site 3, organic matter entered from both the surface and groundwater, so the concentration of organic in soils from the unsaturated zone decreased with depth and then increased in the saturated zone.

The densities of anaerobic groups at the sites are given in Figure 3. Substantial numbers of anaerobic microorganisms were found at these sites. The acetate-using methanogens decreased with depth, but the other organisms showed little change. Soils from the sites 2 and 3 had microbial populations two orders of magnitude higher than site 1 the low organic content soil. Denitrifiers and sulfate reducers were the dominant groups, while methanogens were much fewer in the subsurface.

The chemical and biological characteristics indicate that soils from site 1 were relatively oligtrophic compared to the others. Sites 2 and 3 soils were the most eutrophic with respect to organic and nutrient contents.

Degradation of TBA, MTBE, and ETBE without amendment. In this series of studies, the microcosms were incubated without addition of any external amendment to stimulate the indigenous subsurface microorganisms. Because oxygen and nitrate were excluded from the microcosms and sulfate and carbon dioxide were abundant, the likely metabolic pathways involved in the biodegradation in the unamended microcosms would be fermentation, sulfate reduction and methanogenesis. These degradation studies were used not only to obtain background information on biodegradation under natural conditions but also as a set of controls to be used to evaluate the effectiveness of the alternations imposed on the microcosms in the amendment studies.

TBA. TBA biodegradation data for the three sites are summarized in Table 2. Typical degradation patterns are shown in Figure 4. In the absence of amendments, TBA degraded very slowly in the site 1 soils. In general, TBA degradation at site 1 followed an approximately zero order degradation pattern with no obvious lag phase. The average degradation rate in site 1(A) soils was less than 0.05 mg/L/day/g dry soil, and the projected T_{80} was 2 years or more. Soils from upper layers had slightly higher degradation rates. Similar results were obtained from site

Table 2—TBA degradation in unamended microcosms.

Soil	Degradation rate, mg/L/day/g dry soil	Approximate lag time, day	T_{80}, day
1(A)-0.6	0.04	[a]	360
1(A)-1.5	0.02	[a]	>600
1(A)-3.0	<0.01	[a]	>600
1(B)-0.6	0.06	[a]	300
1(B)-1.3	0.03	[a]	>600
1(B)-3.0	0.05	[a]	>600
1(B)-4.2	0.04	[a]	>600
2-0	0	—	—
2-1.5	0.13	<30	110
2-3.0	0.11	110	200
2-3.9	0.12	40	160
3-0	0.06	short, <30	200
3-0.6	0.06	30	300
3-1.2	0.08	short, <30	200
3-2.1	0.15	short, <30	150
3-3.0	0.08	[a]	400

[a] No clearly defined lag phase.

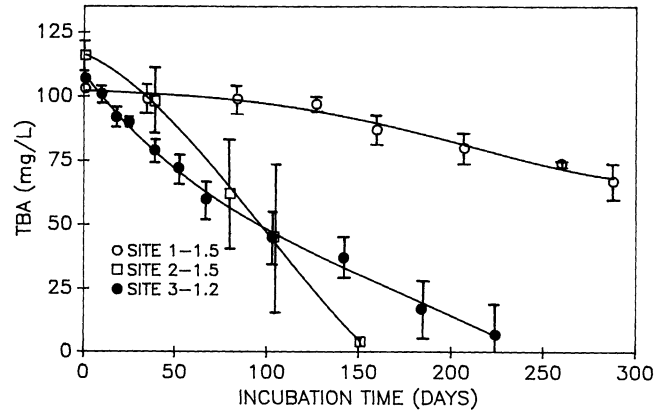

Figure 4—Typical TBA degradation patterns in unamended microcosms.

1(B) soils, indicating little variation in TBA degradation for separate soil samples from the same area.

Except for the site 2 surface soil (site 2-0) in which a high concentration of methane was detected in the microcosms and no TBA degradation occurred, TBA degradation rates in site 2 soils were two or more times higher than in the site 1 soils and exhibited short lag phases. TBA degradation in the site 3 soils was faster than in site 1 soils but slower than site 2 soils, except for the site 2.1 soil which had a degradation rate similar to those in site 2.

The results from unamended microcosms indicate that the unique chemical and microbial characteristics of different soils could influence the degradation of TBA. Sites 2 and 3 soils contained relatively abundant organic matter and nutrients, as a result, microbial groups were more diverse and active; TBA degraded rapidly at these sites. Site 1 soils, on the other hand, were low in organic matter and nutrients, thus the microbial populations and activity were low, resulting in slow rates of TBA degradation.

A comparison of the variability in degradation rates can be made from the standard deviations shown in Figure 4. These variations are typical of the variability found throughout the study in triplicate microcosms. When degradation was slow, little difference was seen in replicate microcosms. When degradation was rapid, considerable variation was observed. This is because in three replicate microcosms, degradation in one might differ significantly from the other two. Such differences may also be seen in the data presented in Figure 1. Considerable variability in microbial densities in soils is known to exist due to microchanneling so such variations are to be expected.

MTBE and ETBE. No significant MTBE degradation was observed at any depth at site 1 during the 250-day study period. Approximately 10% of the MTBE was lost during the first 2 months of incubation, probably due to adsorption, and the concentrations in the microcosms remained steady after that.

In contrast to MTBE, ETBE was biodegraded in the site 1(A)1.5 and site 1(A)-3.0 soils. After about a 120-day lag, the TBA concentration increased (confirmed by GC/MS) as shown in Figure 5. This suggests that ETBE is being metabolized to TBA. The moles of TBA accumulated per moles of ETBE degraded ranged from 0.4 to 0.8. This variation in TBA accumulation, also evidenced by a large standard deviation, may be

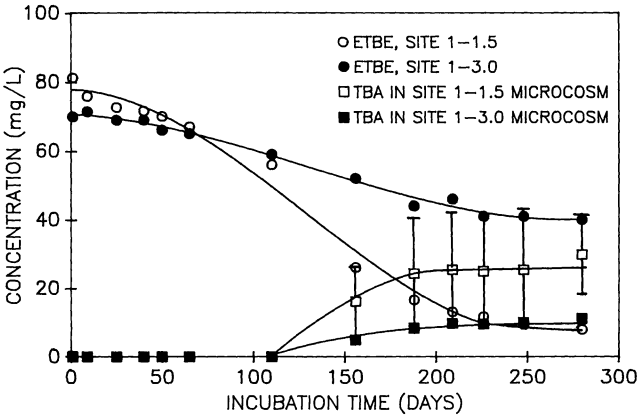

Figure 5—Degradation of ETBE in unamended site 1(A) soils, showing TBA as an intermediate. (Error bars are provided for TBA produced in site 1-1.5 microcosms).

a function of the relative degradation rates of ETBE and TBA. The accumulation of TBA suggests that breakage of the ether bond in ETBE is the first step in ETBE degradation. MTBE, if it were degraded, would be expected to undergo a similar reaction but at a much slower rate or with a very long lag phase. It is possible that MTBE degradation would have occurred if the degradation process was monitored beyond the study period of 250 days.

Unlike TBA degradation in the site 1 soils, where the degradation rate was slower in the deep soil, the highest ETBE degradation rate was found at site 1(A)-1.5 and site 1(B)-4.2. It was observed that the microcosms containing these two soils had higher final pH values than the others, suggesting that the pH might influence ETBE degradation. It was also observed that the final pH in microcosms with MTBE and ETBE was lower than those with TBA. Both MTBE and ETBE are reported to be unstable in acidic solution (Budavari, 1989). Tests conducted by Yeh (1992) showed, however, that the rate of chemical hydrolysis is not affected by moderate pH adjustment, suggesting that microbial catalysis is required for degradation of MTBE and ETBE. Cleavage of the ether bond as the first step in the degradation of methoxylated compounds can occur for lignin-derived monoaromatic compounds under methanogenic conditions (Colberg, 1988; Heaty and Young, 1979; Kaiser and Hanselman, 1982), and studies have shown that compounds with methoxyl groups (or ether bonds) are more resistant to biodegradation. The cleavage of the ether bond (demethoxylation) must occur before degradation proceeds further (Bache and Pfennig, 1981; Boyd *et al.*, 1983; Taylor, 1983). These studies suggest that in this soil, cleavage of the MTBE and ETBE ether bonds proceeds slowly, if at all. MTBE is more resistant to degradation than ETBE.

No degradation of MTBE and ETBE was observed over 250 days of incubation in the site 2 and 3 soils. These sites were microbially active and a variety of organic compounds including methanol, phenol, and TBA were found to be degraded rapidly at these sites (Hickman *et al.*, 1989; Hickman and Novak, 1989). It might be that at microbially active and organically rich sites the population of microorganisms capable of synthesizing the enzymes to catalyze the breakage of ether bonds in MTBE and ETBE were not present or were repressed or inhibited.

The degradation results from unamended microcosms indicate that TBA can be biodegraded naturally in the subsurface. MTBE, on the other hand, was resistant to biodegradation. ETBE was biodegraded, but this reaction was limited to soils which had the lowest microbial population and contained the lowest levels of organic matter.

Degradation under denitrifying conditions. A series of experiments was designed to evaluate whether degradation of TBA, MTBE, and ETBE would occur under denitrifying conditions and, if so, to determine the rates of degradation. The effects of subsurface environmental factors such as nutrient availability and the existence of a cosubstrate were also studied in these experiments. To monitor nitrate and nitrite, a larger microcosm system containing 15 g of soil was used in some of the experiments. The soil to water ratio was kept the same as in the smaller microcosm system.

TBA. TBA degradation at site 1 was enhanced by providing nitrate as an electron acceptor (Figure 6). The most significant improvement was the reduction in the lag phase and T_{80} as seen in Figure 7. Nutrient enrichment further increased the degradation rate of TBA from 0.05 to 0.2 mg/L/day/g dry soil, and the T_{80} was shortened to 140 days. TBA degradation rates at all depths at site 1 were similar under denitrifying conditions after addition of nitrate and nutrients. This suggests that one of the primary factors controlling the TBA degradation rate at site 1 is the nutrient availability in this soil. Denitrifiers are reported to be ubiquitous in soils (Tiedje, 1988). The microbial enumeration data showed that denitrifiers existed in high population at

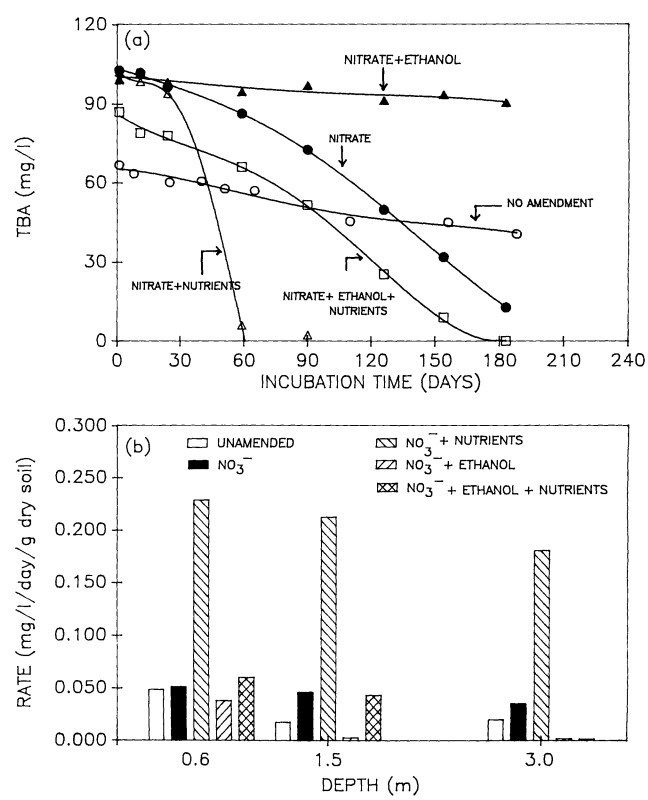

Figure 6—TBA degradation at site 1 under denitrifying conditions: (a) degradation pattern in site 1(A)-1.5 soil, (b) depth profile of degradation rates.

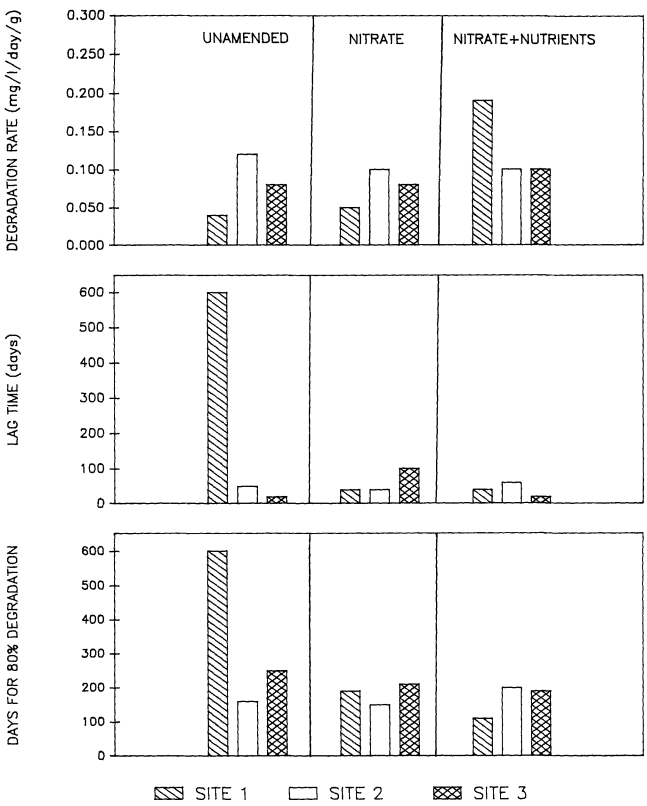

Figure 7—TBA degradation at three sites under denitrifying conditions. Each value is the average of all depths at a site.

all depths (Figure 3). Site 1 was categorized as an oligotrophic site in which nutrients were confined to the upper layers of soils, and TBA degradation was slower in deeper soils. With proper nutrient amendment TBA, degradation occurred at nearly the same rate at all depths.

The addition of 100 mg/L ethanol as a co-substrate retarded TBA degradation under denitrifying conditions in the site 1 soils (Figure 6). Many organic compounds, either naturally occurring or man-made, co-exist in soil systems. Studies report some success in the degradation of recalcitrant compounds by adding a primary substrate to provide energy for microbial growth (secondary substrate utilization) (McCarty *et al.*, 1981; Rittmann *et al.*, 1980). However, in the case of TBA degradation under denitrifying conditions, degradation was retarded in the presence of ethanol. Ethanol was completely degraded within two weeks. However, TBA degradation did not occur following degradation of ethanol unless additional nutrients were provided.

TBA degradation in organic-rich soils (site 2 and 3) was not influenced by nutrient addition. In fact, the addition of nitrate plus nutrients resulted in higher degradation rates in the site 1 soils than in the organic-rich site 2 and 3 soils (Figure 7). Although the organic-rich soils have a higher nutrient content than for the site 1 soils, they also contained higher degradable organics and these appear to decrease the degradation rate of the TBA, possibly because of competition for nutrients.

MTBE and ETBE. ETBE degradation under denitrifying conditions was observed only in the site 1(A)-1.5 and 1(B)-4.2 Soils (Figure 8). MTBE degradation under denitrification was not observed at any depth at site 1. No TBA accumulation was observed in the microcosms undergoing active ETBE degradation. This suggests that either ETBE cleavage of the ether bond in ETBE is much slower than TBA degradation or that a different ETBE degradation mechanism occurs under denitrification conditions.

Degradation of ETBE under denitrification was very sensitive to the presence of other organic compounds (Figure 8). While TBA degradation could be stimulated by nutrient addition in the system after ethanol was degraded, ETBE degradation did not occur even after all the ethanol was degraded. This same phenomenon might also account for the lack of degradation of MTBE and ETBE in the organically rich site 2 and 3 soils.

Other highly water-soluble compounds such as methanol or ethanol may be blended together with TBA, MTBE, and ETBE in reformulated gasoline. Because of their high solubility, these compounds will exist with the oxygenates in groundwater contaminated by these fuels. The degradation data clearly show that MTBE and ETBE were very resistant to biodegradation in organic-rich soils. Although ETBE can be anaerobically degraded in oligotrophic soils, when other easily-degraded organic compounds were present, the degradation was inhibited. Therefore, the biodegradation of MTBE and ETBE can be expected to be difficult in almost all fuel contaminated systems. Single substrate degradation studies should be interpreted cautiously because results may differ significantly when mixtures of organics are likely to occur. The addition of nitrate to promote the activity of denitrifiers and enhance the degradation of organic pollutants is a

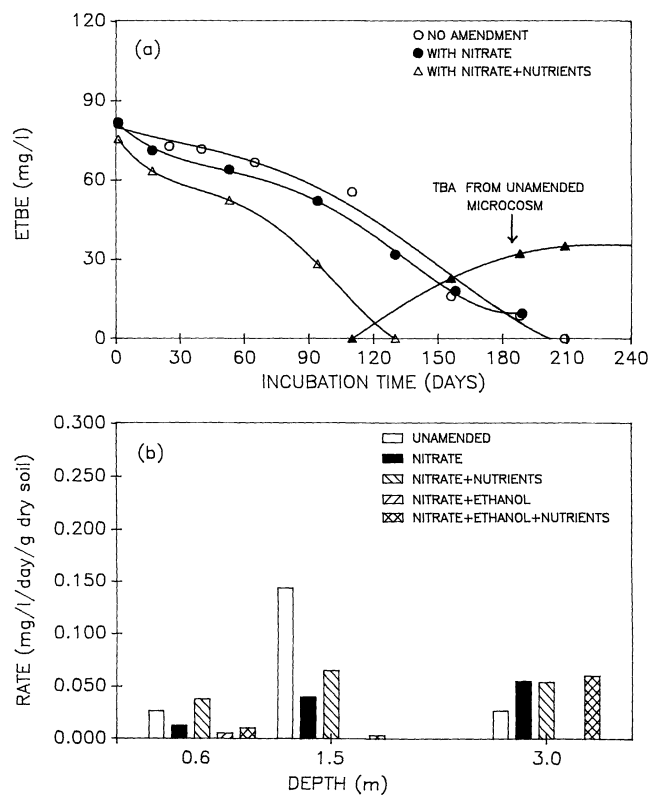

Figure 8—ETBE degradation at site 1(A) under denitrifying conditions: (a) degradation patterns (b) depth profile of degradation rates.

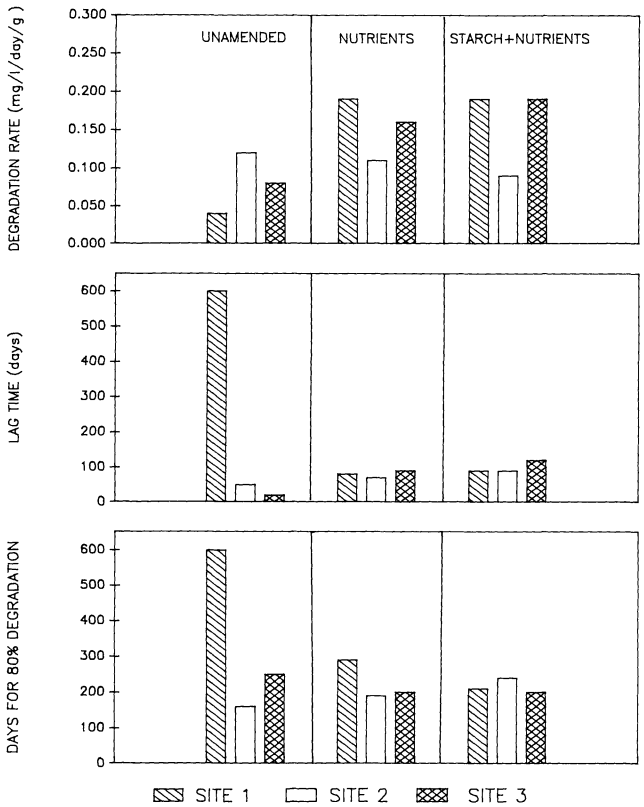

Figure 9—TBA degradation with nutrient and nutrient + starch amendments. Each value represents the average of all depths at a site.

potential alternative for the subsurface system in which the supply of oxygen is limited. Many organic compounds have been reported to be degraded under denitrifying conditions (Tiedje, 1988). However, for the gasoline oxygenates, this study indicates that enhancing the activity of denitrifiers did not promote degradation of MTBE and ETBE.

Degradation under anaerobic conditions. In this phase of the study, nutrient availability and the effect of a primary substrate were evaluated.

TBA. Figure 9 shows typical results for the enhancement of TBA degradation at the three sites by nutrient addition. Similar to the responses observed under denitrifying conditions, the most significant enhancement by nutrient addition occurred for the site 1 soils. The results also show that by supplying nutrients, TBA degradation would occur at nearly the same rates at all sites, indicating the controlling factor for TBA degradation again is nutrient availability.

The degradation of some organic compounds under methanogenic conditions may involve interspecies hydrogen transfer. Addition of a hydrogen donor such as acetate to promote the activity of methanogenic bacteria has reported to be successful in some cases (Gibson and Suflita, 1986). The results in the first study without amendments indicated that the degradation of TBA in unamended microcosms with site 1 soils was very slow. Ethanol or starch were added to the microcosms to provide a potential source of hydrogen. Ethanol addition resulted in inhibition of TBA degradation under anaerobic conditions (data not shown here). Repeated addition of ethanol (200 mg/L every

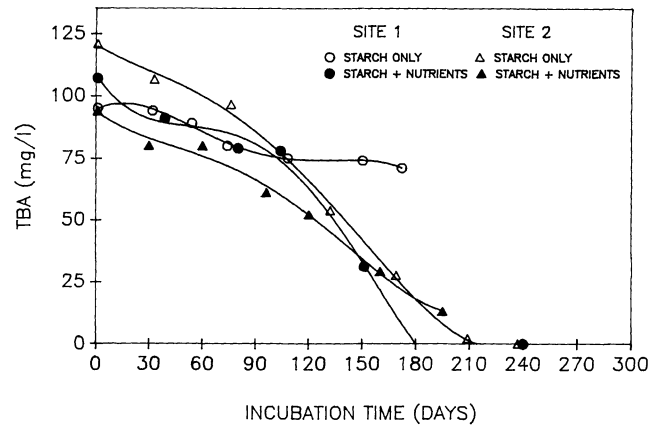

Figure 10—Effect of starch on TBA degradation.

2 weeks) in site 1 soils inhibited TBA degradation over the entire 200 day incubation period. It was observed that starch (200 mg/L, one dose) exhibited some adverse effects on TBA degradation in soils at site 1 but not at site 2 (Figure 10). When nutrients were added, starch had no influence on TBA degradation at site 1.

MTBE and ETBE. The repeated addition of ethanol under anaerobic conditions inhibited ETBE degradation (data not shown here). ETBE was degraded in the unamended microcosms

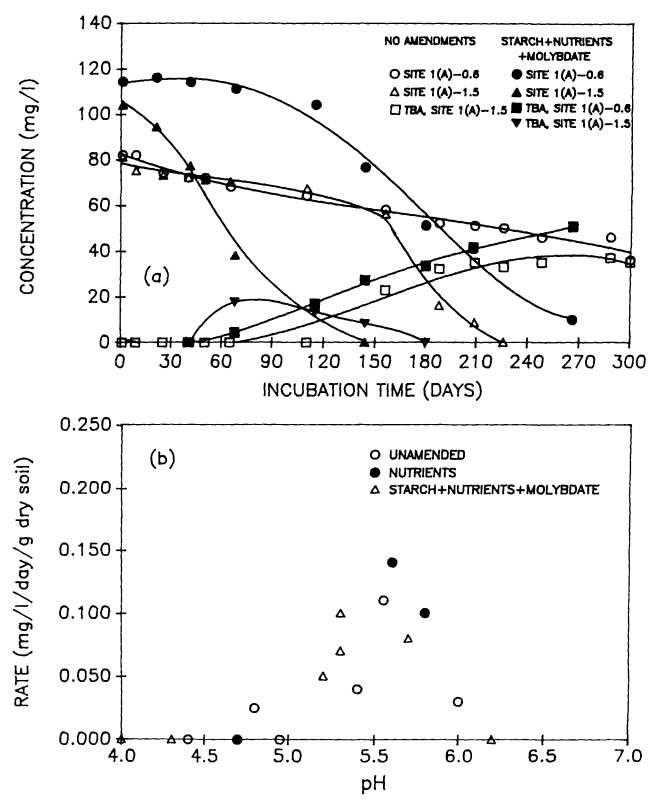

Figure 11—Degradation of ETBE at site 1 under methanogenic conditions: (a) degradation patterns (b) effect of pH (each point represents the average of triplicate microcosms).

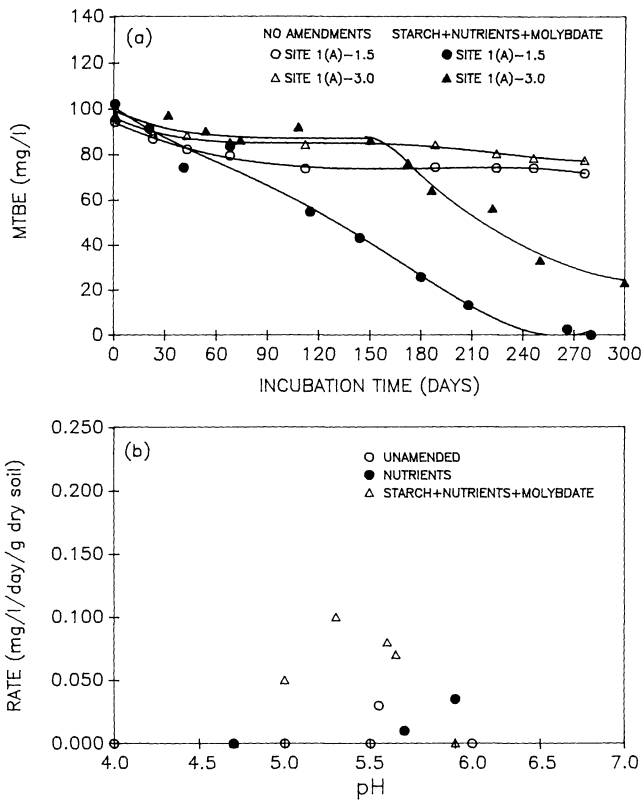

Figure 12—Degradation of MTBE at site 1 under methanogenic conditions: (a) degradation patterns, (b) effect of pH (each point represents the average of triplicate microcosms).

for site 1 soils (Figure 5), but when ethanol was added, no degradation was observed.

The addition of nutrients and starch/nutrients/molybdate enhanced ETBE degradation at site 1 (Figure 11a). However, the stimulation resulting from nutrient addition did not occur in every soil, implying that nutrients were important but not the only factor limiting ETBE degradation. Previous results in the unamended microcosms indicated that ETBE degradation may be inhibited by low soil pH. A plot of ETBE degradation rates as a function of pH reveals the important influence of soil pH (Figure 11b). ETBE degradation was observed at microcosms with a pH between 5.0 and 6.0, and the optimum pH for ETBE degradation was around pH 5.5. Site 1 soils are rich in clays which contribute to soil acidity. Although the nutrient solution added to the microcosms contained a phosphate buffer and had a pH of 7, the clay-rich soil was able to decrease the pH to near its original value and retarded degradation when that pH was less than 5.0.

Degradation of MTBE at site 1 under anaerobic conditions was the only site and condition where MTBE degradation was observed (Figure 12a). However, the degradation rates were still much slower than those for TBA and ETBE under similar conditions. In addition to nutrients, pH also appears to influence MTBE degradation in the site 1 soils (Figure 12b).

No degradation of MTBE and ETBE was observed at site 2 or site 3, indicating that MTBE and ETBE can be recalcitrant in organic-rich soils. The recalcitrant characteristics of MTBE and ETBE in organically rich environments will have profound impacts on bioremediation efforts for MTBE and ETBE cleanups. As previously stated, it is likely that other organic compounds will be present with these oxygenates in a groundwater system contaminated by fuels. Thus the degradation of MTBE and ETBE can be expected to be difficult even though microbial degradation of both MTBE and ETBE can occur under methanogenic conditions in organic-poor soils.

Conclusions

The biodegradation of TBA, MTBE, and ETBE in soil microcosms under various anoxic or anaerobic conditions was evaluated in soils from three different sites. These rates, while probably not quantitatively equal to those expected in soil because of the high dilution in microcosms, provide relative values which can be used to evaluate the factors which influence degradation of gasoline oxygenates. The influence of nutrient availability, the presence of easily-degraded organics and soil pH on the biodegradation under unamended, denitrifying and methanogenic conditions was investigated.

Based on the data, several conclusions can be drawn. In soil microcosms without external amendments, TBA biodegraded most readily, whereas MTBE was the most recalcitrant. TBA degradation occurred at every site and depth but the degradation responses varied widely. Degradation of ETBE occurred only in soil from site 1 which had the lowest natural organic matter content. MTBE was not degraded in any of the unamended soil microcosms over 250 days of incubation time. With nutrient addition, TBA degradation under denitrifying conditions could be increased to the same level soils from all sites. A similar phenomenon was observed for TBA degradation under methanogenic conditions. Addition of nitrate and nutrients did not significantly increase ETBE degradation under denitrifying conditions. Under denitrifying conditions, the presence of ethanol hindered TBA degradation. The co-existence of other easily degraded organic compounds had a detrimental effect on ETBE degradation. No ETBE degradation was observed in organic-rich soils. When ethanol was added to the organic-poor soils, where ETBE was degraded, degradation did not occur. Under methanogenic conditions, degradation of MTBE and ETBE occurred only in oligotrophic soils with low organic matter and at a pH between 5.0 and 6.0. The co-existence of ethanol and other easily-degraded organics inhibited MTBE and ETBE degradation.

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