A Review of Bioremediation and Natural Attenuation of MTBE

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Methyl tert-butyl ether (MTBE) has been the focus of much attention because it is used in large amounts and was reportedly relatively recalcitrant to bioremediation or natural attenuation. Beginning with a few papers a decade ago, evidence has been presented that, in fact, under suitable conditions it is amenable to bioremediation. Many species from widely disparate microbial genera are able to consume it either as a sole carbon source or as a cometabolite. Optimal conditions differ from site to site. Both aerobic and anaerobic conditions may permit MTBE degradation, with a range of electron acceptors, from oxygen through Fe(III), Mn(IV), sulfate, nitrate, and methanogenesis. MTBE metabolism in the vadose zone can be highly active. The published literature suggests that natural populations are adapting to MTBE, and reported rates of biodegradation appear to be larger in the more recent literature. Plants may serve as efficient conduits to withdraw MTBE from the wet subsurface, releasing it to the atmosphere or the vadose zone, where it may be metabolized or diffuse into the atmosphere where it is quickly photodegraded. The major remaining issues are the time required to attain specified criteria of cleanup or whether augmentation is necessary for effective remediation. © 2004 American Institute of Chemical Engineers Environ Prog, 23: 243–252, 2004

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INTRODUCTION

MTBE, more formally methyl *tert*-butyl ether, has been extensively used as an octane booster and oxygenate additive in reformulated gasoline in the United States. With MTBE being distributed nationwide, the potential for contamination of soil and groundwater is substantial. Efforts to develop strategies for cleanup have produced a large number of publications, a selection of which are discussed below [1–60]. MTBE has certain advantages over ethanol in the behavior of gasoline blends to which it is added, and it is efficiently produced from *iso*-butylene. Current production (2003) of MTBE is over 200,000 barrels per day ($\sim 8 \times 10^6$ L) in the United States and U.S. usage is about 1/3 greater [12].

Physical and organoleptic properties of MTBE make it a challenge to deal with effectively. Unlike most gasoline constituents, MTBE is highly soluble in water. At room temperature its solubility is about 50 g/L, 20 times greater than that of BTEX, the most soluble gasoline constituents (benzene, toluene, xylenes, and ethyl benzene). As discussed by Kinner [30] it also partitions strongly from air to water. The dimensionless Henry's Law constant is in the range of 0.01 to 0.04, depending on temperature [10], whereas that of benzene is about 0.2. Thus MTBE is more likely to dissolve in water and less likely to volatilize from gasoline or water to air than is the case for other gasoline constituents.

Some of the important physical and chemical properties of MTBE and BTEX compounds are shown in Tables 1 and 2. Because of its small Henry's constant, MTBE is expensive to remove from water by aeration. Removal using adsorption is expensive because the quantity that adsorbs to activated carbon is relatively small based on the small values of the octanol/water partition coefficient and sorption coefficient. Keller *et al.* [27] evaluated four physicochemical treatment technologies and reported estimated costs associated with each technology. When no air treatment is required, air stripping is the lowest-cost technology for high flow rates, whereas hollow-fiber membranes are cost effective for low flow rates. Granular activated carbon is most cost effective if air treatment costs are included. Advanced oxidation processes are the most expensive of the four options.

Although health effects of low-level MTBE contam-

Table 1. Physical and chemical properties of MTBE (from [54]).*

Physical state	Colorless liquid
Molecular formula	$C_5H_{12}O$
Molecular weight	88.15
Melting point	−109° C
Boiling point	55.2° C
Water solubility	51 g/L @ 25° C
Density	0.74 (g/mL) @ 25° C
K_{oc}	12.3; 11.0 (estimated)
log K_{ow}	1.24
Vapor pressure	245 mmHg @ 25° C
Henry's law constant	0.02 at 25° C
Odor threshold	15–40 ppb
Taste threshold	40–140 ppb
Volume in gasoline	Up to 15%
Water solubility when in gasoline	Up to 5100 ppm

Similar values are presented by Keller *et al.* [27], Jacobs *et al.* [24], and Seagren and Becker [45].

ination are uncertain and disputed (see [13]), it has a potent taste impact in water at levels of 10–30 μg/L (ppb). Thus even small spills of MTBE are detectable, and the USEPA [49] issued a health advisory recommending that drinking water levels be kept below 20–40 μg/L. This would provide a very large safety margin compared to known biological effects. Many states have implemented regulations on MTBE contamination of water and a number have banned its use in gasoline. Through the year 2000, 38 states had action levels, cleanup levels, or drinking water standards for MTBE [37].

The drinking water levels varied but were all below 250 μg/L. As of March 2003, restrictions or outright bans were pending in 16 states. Five states proposing bans or severe restriction depend on MTBE for oxygenates and account for 45% of MTBE use nationwide [12]. Thus if implemented, these restrictions could markedly reduce new incidents of MTBE contamination of groundwater.

One liter of MTBE/ha could contaminate all the yearly rainfall on that area to a level of about 100 ppb (assuming 1 m/yr rain). Avoiding such contamination in areas where there is a lot of reformulated gasoline use has proven challenging. When MTBE constitutes 10–15% of the gasoline, containment systems have to maintain nearly perfect efficacy to avoid some contamination of groundwater. This has been a particular issue in California, which consumes a large fraction of all U.S. MTBE, although the expected magnitude of the problem is debated [26, 33].

Given the widespread nature of MTBE contamination and the intense efforts to remediate it, a large number of studies have been published that detail an extensive amount of research. Prince [40] provided an excellent critical summary of what was known about microbial degradation of MTBE. More recently, Fayolle *et al.* [14], Fiorenza *et al.* [16], and Seagren and Becker [45] provided reviews that describe some of the additional progress that has occurred. Fayolle *et al.* [14] examined the broader issues of remediating other oxygenates such as *tert*-amyl methyl ether and *t*-butyl alcohol. Fiorenza *et al.* [16] considered the distribution of contamination problems, the nature of plumes, and examples of natural attenuation. Seagren and Becker reviewed the natural attenuation of BTEX and MTBE [45]. Here we focus on a limited selection of recent papers (since 2000) to show how prospects for bioremediation of MTBE have improved.

THE CHALLENGE OF DEGRADING MTBE

Because MTBE is a highly hindered ether, the initial step of hydrolysis to the alcohols is difficult. In addition, the *tert*-butyl group is not easily metabolized by many organisms. Initial experience with MTBE contamination suggested that there were far fewer organisms able to effectively degrade it than there were for gasoline constituents such as BTEX. Thus initial efforts to remediate MTBE contamination focused on traditional remediation strategies such as pump and treat, air sparging, or chemical oxidation *in situ*. Kinner [30] offers a useful summary of some strategies implemented before 2001. For very dispersed, low-level contamination these techniques prove to be very costly per unit of contaminant removed, even though MTBE is amenable to the above-mentioned removal strategies that are usable for other gasoline constituents [2]. Odencrantz [38] described several case studies in which very extensive MTBE plumes were monitored, with little evidence for intrinsic remediation. One interesting implication is that there may be many BTEX spills that pass undetected because of intrinsic remediation, whereas the accompanying MTBE leaves a clearly detectable plume.

Table 2. Physical and chemical properties of BTEX compounds at 25° C.*

Property	Benzene	Toluene	*p*-Xylene	Ethyl benzene
Molecular weight	78.11	92.13	106.16	106.16
Solubility, g/L	1.8	0.5	0.2	0.2
log K_{ow}	2.1	2.7	3.2	3.2
Vapor pressure, mmHg	95	28	9	10
Henry's law constant, dimensionless	0.22	0.26	0.31	0.32

From references [45] and [60].

MTBE CLEANUP STRATEGIES AND COST

Current cleanup technologies for MTBE still remain much the same as those of 5 years ago. At a recent meeting [36] there were presentations on *in situ* chemical oxidation (permanganate and persulfate), ozonation, Fenton's reagent, in-well air stripping, and advanced oxidation combined with granulated activated charcoal (GAC) treatment. However, there was also consideration of bioremediation strategies including fluidized bed bioreactors, oxygen infusion, propane-stimulated cometabolism, oxygen-release compound, biostimulation of indigenous microorganisms, and use of a biobarrier, iron, and humates.

Wilson *et al.* [50] presented information on the cost associated with the remediation of sites contaminated with MTBE and other petroleum compounds. The average cost of 311 sites was $200,827; however, the average cost was $414,273 for the 32 sites that were affecting drinking water wells or supplies. The average values of monitored natural attenuation costs were $74,000 in Tennessee and $113,574 in Texas. In both states, the cost of monitored natural attenuation was less than each of the other alternative technologies that had been used at other field sites. Bioremediation rates are important in monitored natural attenuation.

BIOREACTORS ARE EFFECTIVE *EX SITU*

Kharoune *et al.* [29] developed a bioreactor with an inoculum from contaminated soil. A biomass of 1 g dry wt/L accumulated and was able to consume 99% of an input of 80 mg/L MTBE with a hydraulic retention time of 24 h. Similar results were obtained by Wilson *et al.* [51], with a bioreactor depleting 150 mg/L MTBE with a 4-day residence time. The biomass was about 1 g/L, indicating somewhat less active culture than that of Kharoune *et al.* The starter culture for this reactor was obtained from several sources and no particular organisms were identified.

A cometabolic reactor was developed by Dupasquier *et al.* [11] using a pentane-oxidizing *Pseudomonas aeruginosa.* It serves as a biofilter for MTBE vapors, but not for MTBE in water. The specific activity was relatively low and pentane was required as the cosubstrate. Fortin and Deshusses described a biotrickle filter to treat MTBE vapors and established a stable microbial consortium that mineralized 100 mg/L MTBE within a day in a liquid culture [17]. The estimated specific activity was in the range of 240 mg MTBE per gram dry wt culture per day, suggesting a somewhat more active culture than that of Kharoune *et al.* [29]. Many other compounds could be metabolized by the consortium and evidently one or more of these supported growth of the MTBE degrading organisms, because no single strain able to degrade MTBE could be isolated from the consortium.

ISOLATED ORGANISMS CAN DEGRADE MTBE

Mo *et al.* [34] identified three pure cultures of single species able to degrade MTBE, albeit at very modest rates. Genera represented were *Rhodococcus*, *Arthrobacter*, and *Methylobacterium.* Other microbial species including bacteria and fungi were described shortly thereafter. Numerous consortia have also been reported. The earliest report of an effective bacterial consortium was published in 1994 by Salanitro *et al.* and a presumably different consortium was identified 2 years later by Cowan and Park. Prince [40] provided a thoroughly documented review of examples for consortia and pure cultures described to that time. (See his paper for citations of those earlier studies.)

Bruns *et al.* [9] reported on a compost biofilter from which a strain PM1 was isolated [19]. Bruns *et al.* found that PM1 was the dominant organism when measured by molecular (PCR)-based methods, although the most abundant microbial isolates from the cultures were more rapidly growing yellow and white colonies of organisms unable to metabolize MTBE. The strain PM1, which can grow on MTBE as a sole carbon source, produced pinpoint cream colonies under standard plate culture conditions. Molecular characterization by ribosomal RNA intergenic transcribed spacer (ITS) indicated that PM1 is in the general β-proteobacteria subgroup including *Leptothrix* and *Rubrivivax.* Hanson *et al.* [19] showed that it converted MTBE to CO_2 and cell biomass with a yield of 0.18 mg cells/mg MTBE. The organism was active with MTBE concentration as high as 500 mg/L and fully degraded low levels of MTBE (20 mg/L).

Hatzinger *et al.* [20] observed biodegradation of MTBE by a pure culture of a strain identified as *Hydrogenophaga flava* ENV 735. This organism grew well with hydrogen as an energy source. Growth was slow on pure MTBE and was stimulated by addition of yeast extract. Cells grown on higher levels of yeast extract had constitutive MTBE degrading ability, but degradation of *t*-butyl alcohol was inducible. In addition, the metabolism of *t*-butyl alcohol responded differently to addition of oxidase inhibitors, indicating that there are two independent oxidase systems for the limiting steps of MTBE and *t*-butyl alcohol degradation. The organism was applied to MTBE biotreatment as described by Steffan *et al.* [47]. A variant organism with reduced adhesion has been tested for its ability to migrate within aquifer sediments, and the effect of surfactant on mobility of the wild-type has been tested [48]. The variant organism is apparently a subpopulation of the typical culture. Many surfactants proved toxic but Tween 20 enhanced mobility in sand columns.

A methylotrophic bacterium, *Mycobacterium austroafricanum* IFP 2012, efficiently degraded MTBE with a high yield of biomass of 0.44 g per g MTBE consumed [18]. The specific rate of degradation of MTBE by resting cells previously grown on *t*-butyl alcohol was about 1.4 g MTBE per g dry wt per day, but the doubling time of cells grown with *t*-butyl alcohol was rather long, about a day. With MTBE as growth substrate there was no exponential phase of growth; *t*-butyl alcohol accumulated during the initial stages of MTBE use and then was in turn consumed.

Kern *et al.* [28] used molecular microbial community analysis techniques to study MTBE degraders from a contaminated aquifer in Montana. An active consortium was isolated and from it two MTBE degraders, *Rhodococcus koreensis* and *Pseudomonas* Ant9, were obtained. However, both required propan-2-ol as a cosubstrate and both strains were lost from the

consortium on repeated subculturing. Growth of the consortium was stimulated by spent culture medium, indicating that growth factors might be produced and excreted by some members.

Thus, there are multiple species naturally present and able to use MTBE, but not necessarily under simple conditions. Rather different conditions permit or enhance MTBE degradation.

SOME MTBE DEGRADERS ARE ACTIVE UNDER "NATURAL" CONDITIONS

Most studies in the laboratory or field indicate a primarily aerobic degradation process for MTBE, but there are reports implying degradation under methanogenic or other anaerobic conditions [6, 15, 23, 45, 52]. The study of Finneran and Lovley [15] showed that humic substances were important as mediators for the facilitated degradation of MTBE using reduction of Fe(III) as the oxidant. Hurt *et al.* [23] inferred the anaerobic degradation of MTBE from the presence of *t*-butyl alcohol and disappearance of MTBE from the middle of a plume having a high concentration of BTEX. Wilson *et al.* [52] showed that MTBE was removed only after BTEX, in laboratory microcosms constructed from material obtained in a contaminated plume. Bradley *et al.* [5–7] examined several electron acceptors, under anoxic conditions, as discussed below.

The paucity of examples of complete *in situ* MTBE degradation in earlier years and the much more general finding of effective degradation more recently may reflect the intensity of research effort, or the enhancement of microbial populations when MTBE became more widespread in the environment. Bradley *et al.* [5] first found MTBE degradation in streambed sediments near an MTBE contaminated site. Later work by the same group [8] indicated that the potential to degrade MTBE is widespread. They identified activity at 11 widely separated sites with 15–66% mineralization of MTBE occurring in 7 weeks in microcosms. A history of MTBE exposure did not seem to be necessary for the activity to be present. Sandy sediments were more active than those with a high clay and silt content, but organic matter content was only weakly related to activity. Because the microcosms were incubated static but aerobic, diffusion of oxygen to active microbes could have been dependent on porosity of the sediment, and diffusion in turn might have limited the activity.

Bradley *et al.* [6] also showed that MTBE degradation could occur in microcosms with streambed organisms under denitrifying conditions. One sediment was from MTBE contaminated waters and another from an uncontaminated site. Both were able to mineralize added MTBE when nitrate was available. Under methanogenic conditions (no added nitrate) the undesirable intermediate *t*-butyl alcohol was produced, whereas relatively little MTBE was transformed during the period of the incubation, and there was no mineralization of the MTBE.

Bradley *et al.* [9] documented the dependency of MTBE degradation rate and extent on the redox state of the system for three sandy sediments from different locations, only one of which was known to have been exposed to MTBE. When there was an electron acceptor to avoid methanogenesis, mineralization occurred, rather than production of *t*-butyl alcohol. Sulfate, Fe(III), and Mn(IV) were less effective than nitrate or oxygen. Results varied from one site to another for particular electron acceptors.

In other work, Landmeyer *et al.* [31] observed that enhancement of MTBE degradation in a gasoline plume depended on introduction of oxygen, either by rainfall recharge or by deliberate addition of an oxygen-releasing compound. When gasoline is released with MTBE, degradation of hydrocarbons rapidly consumes available oxygen, resulting in MTBE remaining present even as the hydrocarbons disappear. If the MTBE contaminated water comes into the presence of either nitrate or oxygen and in absence of BTEX, MTBE may be consumed.

Kane *et al.* [25] examined aquifer materials in the vicinity of four leaking underground storage tanks in California. The microcosms that they studied were initially oxygen limited. For two of the sites, aerobic incubation resulted in effective degradation of MTBE, whereas for two others it did not. Thus, appropriate organisms or conditions may not be present at all sites. Where there was activity, organisms similar to a known MTBE degrader, strain PM1, were found to be present.

Schirmer and Barker [43] described a long-term study at the Borden aquifer in Ontario, Canada, where a known amount of MTBE was introduced, along with gasoline constituents, to produce a plume below the water table. Chloride was introduced as a conservative tracer. After about 6 years, only 3% of the MTBE mass remained, corresponding to a half-life of about 1 year. The BTEX of gasoline was more rapidly degraded, showing near total disappearance in the first 16 months of the study. Recently Schirmer *et al.* [44] reported laboratory evidence with microcosms, to show that MTBE degradation occurs in aquifer materials from that site. Three microcosms showed MTBE disappearance, but only after a lag of 189–300 days. Several other microcosms, prepared at the same time with a variety of amendments, failed to show MTBE degradation. Thus the occurrence of MTBE degradation seemed to be sporadically present at the site and factors determining expression of that activity remain to be identified.

A study from Europe reports that only one aquifer, out of four MTBE-contaminated aquifers tested, was positive for intrinsic MTBE degradation capability [35]. However, all four could degrade benzene. The MTBE degrader was not identified.

Zoeckler *et al.* [57] searched for intrinsic bioremediation of MTBE in aquifer sediments in the vicinity of a gasoline-contaminated aquifer in Virginia. A sample from upgradient of the contamination showed toluene degradation but no MTBE loss. A sample from downgradient showed MTBE loss but at a lesser rate than that of a sample from the source area. The more active source area sample degraded MTBE with an apparent first-order rate of about 0.06/day, decreasing the input concentration from 4.8 to 1.5 mg/L in about 15 days. Aquifer material was diluted to maintain aerobic microcosms with 5 g material added to 10 mL water. Thus initial rates were in the range of 1 mg/kg material/day.

The original source area sediments contained variable levels of MTBE from 1 to >50 mg/L. *In situ* at the source area, the MTBE degradation may have been oxygen limited because there was a significant amount of petroleum hydrocarbon present.

DEGRADATION IS DOCUMENTED *IN SITU*

Bioremediation and natural attenuation have been investigated at a number of field sites [1, 32, 39, 45, 52, 60]. Significant differences have been found from site to site with respect to the rate of biodegradation. Values range from the rate being so small that biodegradation is not measurable to clear evidence of biodegradation. Peargin [39] found 15 sites with no observed bioremediation and eight sites with a significantly larger remediation rate. Robb and Moyer [41] also found significant differences at the two sites described in their work. These differences appear to be attributable to site characteristics that affect rates of mass transfer. Disappearance of MTBE appears to be attributable to volatilization and biodegradation so the natural attenuation process may require several years because of mass transfer limitations. Oxygen transfer rates are generally greater in unsaturated soil where gas-phase diffusion contributes to mass transfer. The slowest mass transfer rates are in the saturated zone where there is little or no convective flow.

First-order rate constants as large as 0.4 per day have been observed under aerobic conditions [19]. Under anaerobic conditions values are as large as 0.01 per day [52]. These values correspond to half times of 1.7 and 69 days, respectively. Because rates at most field sites are less than these values, remediation times may often exceed one year where natural attenuation is used and biodegradation is one of the processes that contributes to disappearance of MTBE.

AUGMENTATION SPEEDS ACTIVITY WITHIN AQUIFERS

Laboratory studies of water and sediments from Vandenberg Air Force Base, California were used to try to identify suitable geochemical conditions for aerobic MTBE degradation [53]. Stimulation of cometabolism and stimulation of indigenous organisms were considered, along with the possibility of introducing an active strain. However, indigenous activity was associated with the preexisting presence of strain PM1-like organisms [22]. Supplementation with oxygen was sufficient to increase MTBE degradation, even though the organism was generally not detectable before that time by molecular techniques. Increased oxygenation of an anaerobic MTBE plume resulted in MTBE degradation at the same site [53]. Once an effective treatment zone was established, increased MTBE was effectively degraded at input levels up to 2 mg/L.

Recent work from Scow's group [22] documented the behavior of strain PM1, within an MTBE plume at Vandenberg Air Force Base. At that location the numbers of PM1 type organisms were estimated, by quantitative PCR, to range from 10^3 to 10^4 per mL under oxygen stimulated conditions, although the organism was nearly undetectable without oxygen stimulation. *In situ* it was probably oxygen limited. When a little MTBE (10 μg/L) was added in oxygen-stimulated microcosms, cell numbers rose to 10^5 per mL. In field studies, numbers of organisms rose in proportion to added MTBE.

Salanitro *et al.* [42] described the development of a biobarrier at the U.S. Navy Environmental Test Site, Port Hueneme, CA. Earlier they had used a mixed culture of MTBE degrading organisms, whereas in their more recent work they used an isolated organism identified as a *Rhodococcus*. Oxygenation was ensured by intermittent sparging with air or pure oxygen. Within 9 months, levels of MTBE declined from about 1 mg/L to a few μg/L in the biobarrier zone. The identity of the active MTBE degraders was not confirmed but was assumed to be the same as the introduced strain.

Propane biostimulation was tested at Port Hueneme, CA. Using deuterated MTBE and groundwater tracers to monitor the process, it was found insufficient to meet the state's treatability criteria [49].

A cometabolic system has been field-tested [3] using cyclohexane as carbon source with added oxygen. The MTBE concentration declined 80% over the time of treatment. Stimulation of MTBE degradation by supplying oxygen, nutrients, and strain PM1 as an MTBE degrader was successful [46]. Levels of MTBE were reduced as much as 85% within a year at a location in Montana that showed no prior evidence for the presence of strain PM1. However, naturally occurring MTBE degraders were also present.

MTBE DEGRADATION CAN OCCUR IN THE VADOSE ZONE

In this laboratory, studies of MTBE have been conducted over a number of years, with sandy soil obtained near the Kansas River adjacent to the closed Riley County landfill. Zhang [54] and Zhang *et al.* [55, 56] examined MTBE losses by groundwater movement and volatilization through the vadose zone for six channels, 10 × 65 × 110 cm, packed with the sandy soil. In five channels with growing alfalfa plants, losses of MTBE to the atmosphere were significantly greater than those for an unplanted channel. Disappearance of MTBE was also slightly greater for four channels that had been inoculated with strains of *Rhodococcus* (two channels) or *Arthrobacter* (two channels) obtained from C. Kulpa; however, there is not sufficient replication to show that these differences are statistically significant. These were two of the three strains reported by Mo *et al.* [34]. (The other strain derived in their study was a *Methylobacterium*.) The duration of this first experiment [54, 56] was a few months and there was no clear evidence for a large fraction of the MTBE not accounted for through water outflow or surface volatilization. Obtaining reliable mass balances from surface gas flux rates is difficult. Zhang *et al.* [55] monitored MTBE flux through plants in this experiment before adaptation to MTBE occurred and found a considerable fraction of the total volatilization loss was by the plants. A portion of MTBE was transpired from the leaves but a larger fraction diffused from roots within the vadose zone and from the relatively small diameter alfalfa stems. For this experiment MTBE was introduced with the water for 83 days and washed out for 96 days.

Microcosms were prepared from vadose zone soil

from all six channels during the washout period and were incubated with MTBE. After 5 months there was definite evidence for MTBE disappearance in some microcosms. Repeated feeding of the same microcosms, which are kept just moist, has resulted in activities up to 10 mg per kg soil per day. These have remained active with just MTBE feeding and occasional aeration, by opening the bottle for a few minutes, for more than 3 years. This shows that there is no rapid buildup of substances that might be inhibitory to MTBE degradation. When soil samples were incubated under saturated conditions there was no evidence for MTBE disappearance. One microcosm prepared fully saturated became highly methanogenic but showed no evidence of MTBE disappearance.

More recently, the possibility of microbial adaptation in the vadose zone has been explored. Two channels were exposed to MTBE in the inflowing groundwater, with a water table maintained about 20 cm from the bottom of the channels. In this study only the latter half of each channel had plants in place. Initial MTBE levels in the input water were 100 μL MTBE/L water (~74 mg/L). Significant fluxes of MTBE from the soil surface were not observed except in the planted area, after 8 months' exposure. The concentration of MTBE in the input water was increased to 250 μL/L and surface gas fluxes were observed after 2 months, but only small amounts, and only in the planted area. After 8 months they were negligible. Finally the input concentration was increased to 1 mL/L (~740 mg/L). Once again there was a period of adaptation when MTBE flux was readily measurable followed by return to a no flux condition. After 3 months the rate of MTBE transport at the surface was only about 10% of the rate predicted from water usage. By 8 months there was negligible surface flux.

The experimental setup is such that a fully saturated zone is present (~20 cm) with a relatively short retention time for that portion of water that flows through without vertical movement (1–2 days). Sampling of both inlet and outflow water gives no indication that there is degradation of MTBE in the groundwater during that short residence time. The available oxygen in the inlet water would be sufficient for only a minute fraction of the total MTBE to be fully mineralized (~50 μmol of 8.4 mmol/L at the highest tested concentration).

Soil samples taken from the channels at various periods during this prolonged treatment regimen show the ability to consume MTBE aerobically. After 9 months of MTBE exposure at 100 μL/L, samples were taken at three depths in both planted and unplanted regions. Activities were in the range of 1 mg kg^{-1} day^{-1} MTBE consumption (Table 3). This activity is sufficient to degrade the calculated vertical flux of MTBE within the vadose zone.

Rates in soil taken from 7.5 to 15 cm below the surface are 6–8.5 mg kg^{-1} day^{-1} after 18 months of channel treatment at the highest MTBE level (1 mL/L). Activities at the 15–22 cm depth are somewhat lower, about 5 mg kg^{-1} day^{-1}. This *ex situ* estimate is about right to account for all of the MTBE that is in the water that is currently consumed, whereby >250 mL water passes vertically through <50 kg soil/day. A portion of the water enters the plants, carrying some MTBE with it, but this can account for only a small fraction of the loss. There is no detectable surface loss by volatilization.

Table 3. MTBE degradation rates with vadose zone soil samples.

Location*	Planted** (mg kg^{-1} day^{-1})	Unplanted (mg kg^{-1} day^{-1})
Channel 1		
0–7.5 cm	0.6	0.8
7.5–15 cm	2.5	0.7
15–22 cm	1.7	1.0
Channel 2		
0–7.5 cm	0.9	0.5
7.5–15 cm	2.1	0.7
15–22 cm	1.0	1.0

Samples were taken with a cork borer (~1 cm) at four positions along planted or unplanted portion of the channel and pooled for analysis. Indicated depths are approximate, assuming that a complete core is obtained at each depth. Total sample mass varied from 18 to 32 g, and soil moisture fraction varied from 0.07 to 0.19. For the topmost samples (18–23 g), moisture was augmented by adding 0.5 mL water. Each pooled sample was incubated in a 125-mL serum bottle with a serum stopper.

**Rates were determined with known amounts of MTBE (0.57 mg) added to each bottle. Values shown are per kilogram of dry soil.

The above experiment does confirm that the vadose zone can serve as a highly effective sink for MTBE, when adapted organisms are present. The identity of the active organisms has not yet been determined in this experiment. The concentrations of MTBE being metabolized in this vadose zone system considerably exceed concentrations studied by other groups with organisms in liquid culture with soils from aquifers or sediments. Routine feeding of the microcosms uses about 2 mg MTBE for 4–5 mL water in the microcosm, giving concentrations in the range of 400–500 ppm in the aqueous phase. The inlet water of the channels being studied contains 740 mg/L MTBE. Its concentration declines through the vadose zone and the vertical soil substrate profile presumably is indicative of oxygen diffusion to the sites of reaction. Thus we have established a very effective passive bioreactor and demonstrated that vadose zone soil could serve as a large sink for MTBE. The work of Landmeyer *et al.* [31] indicates that a similar situation exists in natural settings, such as streambeds, where anaerobic water with MTBE is seeping into the aerobic stream from groundwater.

The extent to which the vadose zone functions in MTBE removal in the natural environment has not been examined. In locations with fluctuating water tables and intermittent recharge surrounding shallow contam-

Table 4. Summary of MTBE degradation rates.

First order rate constant, days^{-1}	Specific degradation rate, mg MTBE/g cells day^{-1}	Degradation rate, mg MTBE/kg soil day^{-1}	Reference
	79		[29]
	37		[51]
	240		[17]
	1400		[18]
0.06		1	[57]
0.1			[4]
0.01			[52]
0.005			[46]
0.3			[22]
0.3			[25]
0.4			[19]
0.001–0.1	130–3000		[60]

ination, as from gasoline spills, it could be a significant sink.

Table 4 provides a summary of MTBE biodegradation rates. In some cases, rates have been estimated from the data that are presented in the original work. These results show that biodegradation occurs at reasonable rates under aerobic conditions when microbial populations that can grow on MTBE are present. By providing nutrients, oxygen, and organisms that are adapted to grow on MTBE, remediation can occur at a reasonable rate.

PHYTOREMEDIATION IS A USEFUL OPTION

The above-mentioned studies and the work reported by Zhang [54] showed that plants such as alfalfa could grow in the presence of significant concentrations of MTBE (>100 ppm) and serve as a means to withdraw MTBE-contaminated water. This can be useful as a means to control plume migration in climates where potential evapotranspiration exceeds precipitation. More recently, Hong *et al.* [21] successfully applied hybrid poplars as a means to control a plume of MTBE at a site where potential evapotranspiration (~150 cm/yr) does not greatly exceed precipitation (~100 cm/yr). In this instance a relatively tight clay layer covered the contaminated sandy aquifer, so that little precipitation could recharge the aquifer as the plants extracted the water. A paved parking lot could serve the same purpose at a site such as a gasoline station. By use of ^{14}C-labeled MTBE, Hong *et al.* [21] documented that little MTBE metabolism occurs within the tree; most input MTBE departs to the atmosphere unchanged.

Because of the significant amount of water that plants remove from the soil and groundwater, the location of the water table may be lowered allowing aerobic biodegradation to occur in additional parts of the site. Vegetation has the potential to enhance the rate of disappearance of MTBE at contaminated sites through evapotranspiration and biodegradation. The MTBE that passes to the atmosphere is readily transformed through gas-phase reactions that occur when light is present. The half-life of MTBE in the atmosphere may be as short as 3 days because of hydroxyl radials (OH^-) that participate in chemical transformations that occur [58, 59].

Research on phytoremediation of MTBE has recently been reviewed [58]. Investigations with hybrid poplars and alfalfa have shown that MTBE passes through both poplars and alfalfa to the atmosphere as the process of evapotranspiration brings MTBE contaminated water into the plant.

CONCLUSIONS

There are now sufficient examples of MTBE bioremediation to ensure that it is feasible under many conditions. Several pilot tests and limited field applications show that biodegradation is often significant at sites where monitored natural attenuation is the remediation process. In the past decade, the number of species that degrade MTBE, and the reported rates of biodegradation, have increased significantly. Until further work is done it will not be possible to determine the extent to which bioaugmentation may be beneficial at field sites. Both aerobic and anaerobic strategies may be applicable; however, aerobic biodegradation rates are larger. Vegetation is generally beneficial at sites where monitored natural attenuation is used.

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