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# Bioremediation of MTBE: a review from a practical perspective

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## Abstract

The addition of methyl *tert*-butyl ether (MTBE) to gasoline has resulted in public uncertainty regarding the continued reliance on biological processes for gasoline remediation. Despite this concern, researchers have shown that MTBE can be effectively degraded in the laboratory under aerobic conditions using pure and mixed cultures with half-lives ranging from 0.04 to 29 days. Ex-situ aerobic fixed-film and aerobic suspended growth bioreactor studies have demonstrated decreases in MTBE concentrations of 83% and 96% with hydraulic residence times of 0.3 hrs and 3 days, respectively. In microcosm and field studies, aerobic biodegradation half-lives range from 2 to 693 days. These half-lives have been shown to decrease with increasing dissolved oxygen concentrations and, in some cases, with the addition of exogenous MTBE-degraders. MTBE concentrations have also been observed to decrease under anaerobic conditions; however, these rates are not as well defined. Several detailed field case studies describing the use of ex-situ reactors, natural attenuation, and bioaugmentation are presented in this paper and demonstrate the potential for successful remediation of MTBE-contaminated aquifers. In conclusion, a substantial amount of literature is available which demonstrates that the in-situ biodegradation of MTBE is contingent on achieving aerobic conditions in the contaminated aquifer.

*Abbreviations:* BTEX compounds (benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene and *p*-xylene); DO (dissolved oxygen); MTBE (methyl *tert*-butyl ether); TBA (*tert*-butyl alcohol)

## Introduction

The addition of methyl tertiary butyl ether (MTBE) to gasoline began on a relatively small scale in the late 1970's with its use as an octane enhancer to replace tetraethyl lead. The use of MTBE as a fuel oxygenate was approved by the EPA in the 1980's. In the last decade, the production and addition of MTBE to gasoline have increased significantly as a result of the Clean Air Act Amendments of 1990 which mandated the use of reformulated gasoline (RFG, 2.0% oxygen by weight) and oxygenated fuel (Oxyfuel, 2.7% oxygen by weight) in ozone and carbon monoxide non-attainment areas, respectively. In addition, some areas have freely elected to market oxygenated fuels to improve air quality. Currently, an average of 11% MTBE by volume is added to about 30% of the gasoline sold in the United States (Andrews 1998). The estimated production of MTBE in the United States was 20 to 24 billion pounds in 1993 and its production has increased every year since then (Reisch 1994; OSTP 1997). Most of the MTBE produced in the United States during the last decade has been used for fuel oxygenation.

In general, MTBE can enter subsurface environments with the other 200+ components of gasoline during the refining, distribution, and storage of oxygenated fuels. Due to its unique physical and chemical properties relative to other gasoline components, high concentrations of MTBE have been shown to migrate in subsurface environments at speeds similar to groundwater with minimal retardation (Shaffer and Urchin 1997; Squillace et al. 1997). Consequently, the occurrence of MTBE in drinking water supply wells has increased over the past several years (Squillace et al. 1999) resulting in significant national attention focused on the use of MTBE in gasoline (USEPA Blue Ribbon Panel 1999). Table 1 shows the results of three recent MTBE occurrence surveys.

*Scope and objective*

Petroleum hydrocarbons are naturally occurring chemicals that have been exploited in a wide range of industries. Many of these hydrocarbons have been successfully bioremediated at contaminated sites. The major aromatic components of gasoline, benzene, toluene, ethylbenzene, and xylenes (BTEX), degrade relatively quickly under aerobic and anaerobic conditions. Thus, in a large majority of sites where gasoline releases have occurred, BTEX plume lengths tend to stabilize and recede at less than 260 feet from the release point (Mace 1997; Rice et al. 1995). When gasoline containing MTBE is released, BTEX plumes may still stabilize on average within 300 feet of the source but MTBE may continue to migrate. Thus, more active measures may be required to halt movement of MTBE plumes.

This paper will assess the variables that influence the biodegradation of MTBE in the natural environment with a focus on those potentially responsible for optimizing biodegradation processes in both in-situ and ex-situ applications. Several case studies will be presented to assess the state of the knowledge on MTBE biological treatment in the field and to identify future directions in this area of research.

## MTBE biodegradation

While much is known about the biodegradation of many gasoline components under both aerobic and anaerobic conditions, the biodegradation of MTBE is not fully understood. In general, alkyl ethers are relatively stable and unreactive compounds due to the difficulty associated with the cleavage of the ether bond (Mo et al. 1997; White 1996) and the resistance to microbial attack of the tertiary or quaternary carbon structures (Squillace et al. 1997). Many studies have reported that MTBE is recalcitrant under both aerobic and anaerobic conditions (Fujiwara et al. 1984; Jensen and Arvin 1990; Yeh 1992; Suflita and Mormile 1993; Yeh and Novak 1995). However, recent studies have reported the ability of several bacterial and fungal cultures from various environmental sources to degrade MTBE under anaerobic or aerobic conditions either as the sole source of carbon and energy (Salanitro et al. 1994; Eweis et al. 1997; Mo et al. 1997; Park and Cowan 1997; Fortin and Deshusses 1999a,b; Hanson et al. 1999) or cometabolically (Hardison et al. 1997; Steffan et al. 1997; Garnier et al. 1999).

*Anaerobic biodegradation*

Yeh and Novak (1994) evaluated the biodegradation of MTBE in soils from three different sites under various anaerobic and anoxic conditions. These researchers reported that the biodegradation of MTBE was observed under methanogenic conditions in a soil with a low organic carbon content. The co-existence of easily degraded organic compounds was shown to inhibit MTBE degradation. While there have been few successful laboratory or field experiments showing MTBE biodegradation under anaerobic conditions, there is some evidence that MTBE is reduced under methanogenic conditions in the field (Hurt et al. 1999; Wilson 1999).

*Aerobic degradation*

Numerous laboratory and field studies have reported the biodegradation of MTBE under aerobic conditions (Table 2). In a number of these studies, it was suggested that a monooxygenase enzyme is responsible for the biotransformation of MTBE to *tert*-butyl alcohol (TBA) (Hardison et al. 1997; Steffan et al. 1997). In general, monooxygenase enzymes catalyze reactions in which only one of the two oxygen atoms of molecular oxygen is incorporated into the organic substrate. Results from several studies indicate that the monooxygenase responsible for MTBE biodegradation may be a cytochrome P450 enzyme (Hardison et al. 1997; Steffan et al. 1997).

Most of the cultures reported to degrade MTBE have been shown to require oxygen concentrations greater than 2 mg/L. Park and Cowan (1997) reported that a mixed culture enriched from a petroleum refinery activated sludge was able to degrade MTBE

*Table 1.* Summary of recent MTBE occurrence data

| Conc. ($\mu$/L) | Maine[1] | California[2] | USGS[3] | |
|---|---|---|---|---|
| | Private Wells (n = 946) | Public Wells (n = 793) | (n = 5,418) | (n = 376) |
| Non detect | 85.0% (<0.2 $\mu$g/L) | 85.1% (<0.2 $\mu$g/L) | 98.7% (<3 $\mu$g/L) | 82.2% (<0.2 $\mu$g/L) |
| <5 | 12.0% | 13.6% | 0.5% | 16.2% |
| 5–35 | 1.9% | 1.3% | 0.3% | 1.0% |
| >35 | 1.1% | 0.0% | 0.2% | 0.5% |

Adapted from 1) Maine DHS 1999; 2) California DHS 1999; 3) Moran et al. 1999.

*Table 2.* Summary of laboratory and microcosm MTBE biodegradation studies

| Culture | Initial cell density [cells $ml^{-1}$] | Initial MTBE concentration [mg $L^{-1}$] | MTBE half life[1] [d] (lag Time [d]) | Reference |
|---|---|---|---|---|
| Mixed culture from sludge (BC-1)[2] | ~3 × $10^8$ | 120 | 0.08 | Salanitro et al. 1994 |
| Groundwater microcosm | NA | 5–80 | 2(14)–69(21) | Salanitro & Wisnjewski 1996 |
| Groundwater field study | NA | ~1 | 693(20) (similar for benzene) | Borden et al. 1997 |
| Pure fungal culture (cometabolic on *n*-butane in gas phase) | 2.5 × $10^6$ | 1 $\mu$mole in gas phase | 1.4 | Hardison et al. 1997 |
| Pure culture from sludge and gingko | 5–40 × $10^5$ (CFU) | 200 | 29 | Mo et al. 1997 |
| Pure culture (cometabolic on propane)[2] | ~5.7 × $10^8$ | 20 | 0.04 | Steffan et al. 1997 |
| Groundwater field study | NA | NA | 578 | Schirmer et al. 1998 |
| Stream-bed sediments microcosm | NA | 0.15 (0.4 for TBA) | <56 (<15 for TBA) | Bradley et al. 1999 |
| Pure culture (cometabolic on pentane)[2] | ~2.9 × $10^6$ | 336 | 5 (1) | Gamier et al. 1999 |
| Pure culture (PM-1) | 2 × $10^6$ | 5–50 | 0.8–1.7 | Hanson et al. 1999 |

Note: 1) Assumes first-order decay; 2) Assumes 0.002 mm cell diameter: 55% of the cell is protein and 70% of the cell is water. NA = information not collected.

under aerobic conditions with the transient accumulation of TBA. A decrease in MTBE degradation rates was observed at dissolved oxygen concentrations below 2 mg/L. It was concluded that MTBE-degrading microbial populations could be more sensitive to low oxygen tensions than other hydrocarbon-degrading populations.

In another laboratory study, the effect of oxygen supply on MTBE degradation was evaluated (Yang et al. 1998). Results from this study revealed that a continuous supply of oxygen compared to a one-time oxygen addition greatly enhanced MTBE degradation rates.

Ex-situ studies have also demonstrated the dependence of MTBE degradation on adequate concentrations of molecular oxygen. For example, results from studies in suspended growth bioreactors have indicated that MTBE-degrading cultures are sensitive to suboxic conditions (Sun et al. 1996; Tang & Sun 1997). In addition, studies using two pilot-scale biotrickling filters treating MTBE-contaminated air streams revealed that MTBE biodegradation rates are a strong function of dissolved oxygen concentrations (Fortin & Deshusses 1999a,b).

Finally, several in-situ field studies have demonstrated the importance of oxygen in stimulating MTBE degradation (Koenigsberg et al. 1999; Mackay et al. 1999; Salanitro et al. 1999). In an MTBE-impacted aquifer at Vandenberg Air Force Base, Mackay et al. (1999) found that increasing dissolved oxygen (DO) concentrations from less than 1 mg/L to 10–15 mg/L caused MTBE concentrations to rapidly decrease from

200–300 $\mu$g/L to less than 5 $\mu$g/L. Similarly, other researchers have observed that biosparging an MTBE plume at a site caused a decrease in MTBE concentrations from up to 10 mg/L to non-detectable levels in a period of three months (Javanmardian & Glasser 1997). Laboratory microcosm studies using soil from both sites confirmed that oxygen stimulated MTBE biodegradation by indigenous microorganisms (Wilson et al. 1999; Yang et al. 1998).

*Growth-dependent degradation.* Growth rates less than 0.25 $d^{-1}$ and cellular yields of approximately 0.2 g cells/g MTBE have been reported for MTBE-metabolizing mixed and pure cultures (Salanitro et al. 1995; Fortin & Deshusses 1999a,b; Hanson et al. 1999). These cell yields are much lower than those for cultures grown on simple sugars (0.4–0.8 g cells/g sugar) or gasoline constituents such as pentane (0.9 g cells/g pentane) (Fortin & Deshusses 1999a; Garnier et al. 1999). It has been suggested that MTBE behaves as a metabolic or electron transport inhibitor or as an uncoupler of ATP formation (Salanitro et al. 1995). In addition, there is some indication that low growth rates on MTBE might be due to the formation or accumulation of metabolic intermediates which potentially inhibit cellular growth (Salanitro 1998).

*Co-occurring contaminants.* MTBE is most often found at sites that are impacted by complex mixtures of gasoline hydrocarbons, some of which have been shown to stimulate the cometabolic biodegradation of MTBE (Hyman & O'Reilly 1999). Hardison et al. (1997) and Hyman et al. (1998) isolated an *n*-alkane grown filamentous fungus (*Graphium* sp.) that was shown to cometabolically degrade MTBE to TBF (*tert*-butyl formate), and then TBA. There is some indication that monooxygenase enzymes might be responsible for the cometabolism of MTBE by cultures grown on *n*-alkanes (Hardison et al. 1997). Steffan et al. (1997) reported that several propane-grown pure cultures were able to cometabolically biodegrade MTBE under aerobic conditions. Stoichiometric amounts of TBA were produced but were eventually degraded at a rate 50–75% lower than that of MTBE. Garnier et al. (1999) isolated a pure culture (*Pseudomonas aeruginosa*) capable of cometabolically degrading MTBE in the presence of pentane.

Alternatively, MTBE biodegradation may be inhibited in the presence of more easily biodegradable compounds (Mo et al. 1997). This inhibition can occur when MTBE-degrading cultures preferentially utilize easily degradable hydrocarbons instead of MTBE. It will also occur when this preferential hydrocarbon use results in the depletion of electron acceptors and nutrients necessary for MTBE biodegradation in mixed contaminant groundwater plumes.

*Environmental factors.* Environmental conditions in the subsurface such as temperature, pH, nutrient availability, water content, and availability of electron acceptors can greatly affect the rate of biodegradation. Generally, soils and groundwater contaminated with organic compounds have sufficient quantities of trace nutrients, and thus, limiting elements for growth are typically nitrogen and phosphorus. The required carbon/nitrogen/phosphorus ratio varies depending on the nature of the contaminant but is commonly in the range of (100–300): 10: (0.05–1) (Alexander 1994). Near-neutral aquifer pH values are usually optimum for the biodegradation of organic compounds. Groundwater temperature is often one of the most important factors controlling microbial activity and rates of organic matter decomposition. Generally, enzymatic activity and microbial metabolism rates double for every 10 °C increase in temperature until close to inhibitory temperatures which are usually around 40 °C to 50 °C for most microorganisms (Gerhardt 1994).

*Literature conclusions*

Even though the reports discussed above vary to some extent, several conclusions can be made regarding the biodegradation potential of MTBE in the subsurface by microbial populations:

1. MTBE biodegradation can be expected to occur aerobically either with MTBE as the sole carbon and energy source or cometabolically with alkanes (Table 2);
2. The cellular yield of microorganisms utilizing MTBE as the sole organic carbon source can be expected to be very low;
3. The presence of more easily biodegradable organic compounds in the subsurface can either inhibit MTBE biodegradation or possibly promote cometabolic MTBE biodegradation; and
4. While MTBE has been observed to be degraded anaerobically, the most rapid biodegradation rates seem to occur under aerobic conditions.

The dependence of MTBE biodegradation on dissolved oxygen concentrations has field implications and indicates that the effectiveness of engineered bioremediation systems in subsurface environments may depend upon the ability to transport and sus-

tain adequate oxygen concentrations throughout the MTBE biodegradation zone. This will be discussed below with an emphasis on strategies for optimizing conditions to promote MTBE biodegradation processes.

## Implementation of bioremediation for MTBE

Successful remediation can be defined by several objectives: 1) prevention of drinking water well contamination; 2) prevention of off-site plume migration; 3) stabilization of contaminant plume; 4) demonstration of active on-going biological processes that effect contaminant destruction; or 5) achievement of cleanup goals. Implementation of a bioremediation field strategy will depend on the selected objective; however, in nearly all cases, the objective includes some type of plume control.

Control of contaminant plume migration can be accomplished by either achieving hydraulic controls, or by stimulating a contaminant degradation rate equal to or greater than the contaminant migration rate. Plumes can be captured hydraulically using pump-and-treat (NRC 1994) where the extracted groundwater is treated using an ex-situ treatment process (e.g., air stripping, granular activated carbon). Control of contaminant plumes by degradation can be accomplished using either biological or physiochemical (e.g., in-situ oxidation) methods. For easily biodegradable organic chemicals such as BTEX compounds, biodegradation zones occur naturally and require minimal enhancement. In such a case, the bioremediation strategy is referred to as natural attenuation and typically requires continued monitoring to verify plume control (Kampbell et al. 1995; USEPA 1999). However, for less biodegradable substrates such as MTBE, biological degradation zones must often be created in the subsurface using bioenhancement or bioaugmentation techniques to enhance MTBE removal rates. Case studies for each of these bioremediation approaches are discussed in detail later in this paper.

### *Ex-situ treatment*

Treatment of MTBE-contaminated groundwater has been successfully performed using both suspended growth bioreactors and fixed film bioreactors.

*Suspended growth bioreactors.* Suspended growth bioreactors typically utilize concentrated suspensions of active cells to biodegrade high concentrations of organic contaminants. The main advantage of using suspended cultures in stirred bioreactors is the inherent contact of microorganisms with aqueous phase organic contaminants and dissolved oxygen and nutrients. The defining kinetic parameters governing the efficiency of suspended growth bioreactors are the Mean Cell Residence Time (MCRT) and the Hydraulic Retention Time (HRT). One way of maintaining a high cell concentration in bioreactors is by decoupling the HRT of the wastewater stream from the MCRT. This is achieved by recycling the cells from the effluent stream back to the contact tank (Shuler & Kargi 1992). Cell recycling has been shown to be an important step in ex-situ MTBE bioremediation applications (Pitre & Steffan 1999) since it conserves the biomass of the slow-growing MTBE population and ensures high cell concentrations of MTBE-degraders in the MTBE/microorganism contact zone. Great care must be taken when designing a suspended growth bioreactor for MTBE bioremediation. Because MTBE-degrading organisms are typically sensitive to sudden changes in environmental conditions, the installation and use of an equalization tank prior to the contact tank is recommended to dampen variations in influent concentrations and to prevent shock loadings of MTBE (Pitre & Steffan 1999).

*Fixed-film bioreactors.* Unlike suspended growth bioreactors, fixed film systems, such as biotrickling filters and fluidized bed bioreactors, contain a physical surface inside the bioreactor that allows microorganisms to attach themselves as a biofilm. Biofilm reactors have the advantage of retaining slow-growing cultures, such as MTBE-degraders, that could potentially be washed-out of suspended growth reactors due to low growth rates. Biofilm reactors have been successfully used to treat MTBE-contaminated water and air streams (Eweis 1997; Tang & Sun 1997; Fortin & Deshusses 1999a,b). For example, in a laboratory scale biotrickling filter, MTBE-grown cultures were shown to fully convert 97% of influent MTBE to $CO_2$ following an acclimation period (Fortin & Deshusses 1999a,b).

*Ex-situ conclusions.* The most comprehensive study on the biological treatment of MTBE in a complex waste stream compared the use of suspended growth reactors to fixed-film reactors for the treatment of MTBE-contaminated water (Tang & Sun 1997). This study showed that suspended growth reactors can be used for MTBE removal but that fixed-film reactors

were more efficient in retaining slow growing MTBE-degrading populations (Tang & Sun 1997). Thus, it is generally agreed that fixed-film systems will be more practical than suspended growth reactors for MTBE treatment in the field.

*In-situ processes*

*Natural attenuation.* Natural attenuation involves physical, chemical, or biological processes that, under favorable conditions, occur naturally to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil or groundwater (55 CFR 46 March 8, 1990). Natural attenuation processes, particularly biological degradation, are currently best documented at petroleum fuel spill sites for BTEX compounds (Kampbell et al. 1995; USEPA 1999). Natural attenuation of MTBE-contaminated sites has not been well documented. Some MTBE plumes, such as in Port Hueneme, California and East Patchogue, New York have extended to beyond 4000 ft suggesting limited or no natural attenuation. Researchers at Lawrence Livermore National Laboratory have suggested that natural attenuation of MTBE plumes occurs primarily from dispersion and not from biological activity (Happel et al. 1998). While the LLNL study evaluated a substantial number of sites, it neither evaluated a large amount of time series data nor did it evaluate MTBE concentration decreases once the leading edge of the MTBE plumes migrated past the BTEX plumes. Conversely, several case studies have demonstrated a decrease in MTBE concentrations due to the combined effect of dispersion and biodegradation (Barker et al. 1998; Schirmer & Barker 1998; Tyner et al. 1998; Cho & Wilson 1999; Hurt et al. 1999). While seemingly contradictory, the available literature suggests that naturally occurring biodegradation of MTBE is a function of site specific variables which may include the co-occurrence of MTBE and BTEX in contaminant plumes and/or a sufficient MTBE residence time in the aquifer.

MTBE is typically degraded to TBA, which in most cases has been shown to further degrade to harmless intermediates (Salanitro et al. 1994; Steffan et al. 1997). The reports in the literature on the rate of TBA degradation relative to that of MTBE are inconsistent; TBA has been shown to degrade faster than MTBE (Bradley et al. 1999) or slower than MTBE (Steffan et al. 1999). As a result, it is difficult to determine from the available laboratory data whether TBA oxidation is the rate-limiting step for MTBE biodegradation in the field. Certainly, more information on the natural attenuation of MTBE is needed prior to acceptance of this technique by the regulatory community as a control strategy for MTBE remediation.

*Bioenhancement.* Bioenhancement is a remediation strategy that relies on naturally occurring microorganisms to degrade organic compounds with the support of introduced electron acceptors, nutrients, and/or co-substrates. A common approach to bioenhancement involves pumping groundwater to the surface, dissolving the necessary nutrients, electron acceptors, and/or co-substrates in the groundwater, and pumping it back into the contaminated aquifer. Alternatively, enhancements can be directly sparged or injected into the subsurface with air or with oxygen release compounds. The most common form of bioenhancement is the addition of oxygen to the subsurface to promote aerobic biodegradation. Bioenhancement by oxygen addition has been performed at MTBE-contaminated sites using air sparging (Javanmardian & Glasser 1997; Johnson 1998), oxygen sparging (Mackay et al. 1999; Salanitro et al. 1999), oxygen release compounds (Barcelona et al. 1999; Koenigsberg et al. 1999), or hydrogen peroxide (USEPA 1998; Yeh & Novak 1995). Finally, bioenhancement may include the addition of a cometabolite to effect the fortuitous biodegradation of MTBE during the energy-producing degradation of the cometabolite. Successful cometabolites for MTBE degradation include *iso*-propanol, propane, *n*-butane, and diethyl ether (Hardison 1997; Steffan et al. 1997).

*Bioaugmentation.* Enhancing the activity of microorganisms that are naturally growing on organic contaminants in the subsurface is less expensive and less difficult than relying on introduced organisms to survive, compete, and degrade target contaminants. However, there are several cases in which the addition of microorganisms is needed for the degradation of some organic compounds. A primary motivation to bioaugment would be the presence of a small indigenous microbial population commonly defined as less than $10^5$ cells/g soil, or a population that has not developed mechanisms to degrade the target contaminant (Forsyth et al. 1995). Under such conditions, the addition of a slurry of microorganisms and necessary nutrients can then serve to "jump start" biological processes by increasing the size of microbial populations by several orders of magnitude. A second motivation for bioaugmentation is the need to rapidly biodegrade the target contaminant with little or no

start-up period. By adding a large population of known degraders to the subsurface, remediation managers are assured that microorganisms with the ability to metabolize the contaminant are initially present in the subsurface and that degradation should occur (Forsyth et al. 1995). Finally, bioaugmentation has been used in cases where multiple contaminants create toxic concentrations for indigenous organisms while exogenous organisms are capable of surviving and degrading the mixed contaminant stream.

The selection of a microorganism can be done using one of the multitude of vendors selling slurries or freeze-dried powders. Slurries involve microorganisms that are currently metabolizing carbon and do not need any start-up period prior to contaminant degradation. Alternatively, freeze-dried powders require the introduction of water, nutrients, and a readily assimilable carbon source to initiate cell activity prior to subsurface injection. Mixed microbial slurries, as opposed to pure cultures, have been found to be the most effective due to their expected wide range of degradation mechanisms (Forsyth et al. 1995). Finally, the use of naturally occurring microorganisms instead of genetically engineered microorganisms (GEMs) is generally more acceptable from a regulatory standpoint (Betts 1999). Once selected, the microorganisms, nutrients, and/or cosubstrates can be injected into the subsurface. The location and density of injection wells, the rate of subsurface microbial recharge, and the need for supplemental nutrients and electron acceptors are site-specific.

The addition of an exogenous microbial population to the subsurface requires overcoming several obstacles. First, if the indigenous population is abundant and surviving under current contaminated conditions, it may out-compete the exogenous population. This challenge has been successfully addressed at some sites by creating a temporary subsurface zone that favors a trait of the exogenous organisms. For example, Dybas et al. (1995) created a zone with low trace mineral concentrations that favored microorganisms with efficient trace metal scavenging systems.

Second, bioaugmentation may result in clogging or the creation of a low permeability zone within the aquifer (MacDonald et al. 1999). This can result from the addition of high concentrations of microorganisms and nutrients, or from the production of extra-polymer substances (EPS) by microorganisms when introduced to new environments with limited substrates and nutrients. Aquifer clogging could cause a portion of the contaminated plume to by-pass low permeability areas thus negating the effect of bioaugmentation. This limitation has been overcome at some sites by pulsing nutrients or electron acceptors into the subsurface (NRC 1993). For MTBE, clogging is not expected to be a limiting factor due to the low observed growth rates and cell yields of MTBE-degraders.

Third, care should be taken to ensure that the exogenous microorganisms are capable of degrading the target contaminant and all secondary by-products. Incomplete mineralization can result in the formation of secondary contaminant plumes that may prove to be more toxic and/or more mobile than the initial plume. This limitation can be mitigated by ensuring that a mechanism exists for complete contaminant mineralization prior to injection and by installing monitoring systems to verify that mineralization is actually occurring in the subsurface.

*Bioaugmentation for MTBE.* For MTBE, bioaugmentation is being implemented at several field sites. Pure and mixed cultures have been derived with the ability to effectively degrade MTBE (Hanson et al. 1999; Salanitro et al. 1999). Slurries of these cultures have been recently injected into the subsurface at two MTBE-contaminated sites. In both cases, the remedial systems were designed such that the plume migrated through a microbial barrier. After 1 year of operation at one of the sites, neither the migration of the injected microorganisms nor the need for multiple microbial injections were observed (Salantiro et al. 1999). In addition, other researchers have isolated a microorganism grown on propane, ENV425, which has been shown to successfully and rapidly degrade MTBE in the presence of a cometabolic substrate, propane (Steffan et al. 1997). These researchers are currently in regulatory negotiations to inject a combination of a slurry of ENV425 and dissolved propane at a gasoline-contaminated site in California.

One potential challenge related to the in-situ biodegradation of MTBE is the issue of bioavailability. If MTBE-blended gasoline is released or diffuses into low permeability regions of the subsurface, biodegradation will likely be limited due to mass transfer limitations of electron acceptors, nutrients, or even microorganisms into such low permeability regions. MTBE has a relatively high solubility in water (approximately 48,000 mg/L) and is therefore thought to be more bioavailable to indigenous microorganisms than other gasoline components. However, this high solubility will most likely create larger concentration gradients which could enhance MTBE diffusion into

*Table 3.* Influent groundwater quality for bioreactors at Sparks, Nevada and San Diego, California

| Influent parameter | Nevada Mean $\mu g\ L^{-1}$ | California Mean $\mu g\ L^{-1}$ |
|---|---|---|
| MTBE | 330 | 9,570 |
| Benzene | 100 | 5,770 |
| Ethylbenzene | 7.4 | 140 |
| *m, p*-Xylene | 15 | 570 |
| *o*-Xylene | 5.9 | 290 |
| Toluene | 4.4 | 650 |
| TPH-extractable[1] | 650 | 550 |
| TPH-Purgable[1] | 410 | 16,300 |
| COD | 51,100 | 88,300 |

[1] Total Petroleum Hydrocarbons.

low permeability regions (Mackay et al. 1999) and ultimately limit the rate of biodegradation of MTBE.

## Case studies

### *Ex-situ fixed-film aerobic degradation*

Biological reactor treatment is being used to remove MTBE and other gasoline components from water at two fuel transfer stations, one in Sparks, Nevada and the other in San Diego, California (Stringfellow 1998; Stringfellow et al. 2000). The bioreactors are up-flow fluidized bed reactors with a granular activated carbon (GAC) packing medium. The California reactor is a pilot-scale unit with a volume capacity of approximately 300 gallons and is being used to treat approximately 4,000 gallons per day of MTBE-contaminated groundwater. In March of 1999, the California reactor was installed to treat MTBE. It was started with 365 lbs of coconut carbon and was operated for a pilot study. In Sparks, Nevada, there are two full-scale 3,000 gallon-reactors operating in parallel. Combined, these two Nevada reactors are being used to treat approximately 400,000 gallons per day of groundwater with a mean MTBE concentration of 330 $\mu$g/L and a mean TPH (extractable and purgable) concentration of 1,060 $\mu$g/L. These full-scale fixed-film reactors were originally installed in October 1995. Each Nevada reactor was loaded with 6,000 lbs of coconut carbon. Average influent characteristics for the two sites are presented in Table 3.

The operation of the bioreactors at the two sites exhibited three phases in the treatment of MTBE: sorption phase, growth phase, and operations phase (Stringfellow et al. 2000). The sorption and growth phases are collectively referred to as the start-up phase. The sorption phase is the period during initial operation when the GAC had significant sorption capacity and a significant microbial population had not accumulated in the reactor. With MTBE, the sorption phase was brief and complete breakthrough of MTBE was observed in the Nevada reactor after approximately 30 days. In the California reactor, the system exhibited breakthrough in less than 20 days (Stringfellow et al. 2000). Sorption of MTBE was shown to be an insignificant removal mechanism in these reactors during the operations phase (Stringfellow 1998).

*Table 4.* Statistical summary of effluent water quality and removal efficiency data for the operations phase of the Sparks Solvent Fuel Site (1996–1997)

| Statistic | MTBE Removal % | MTBE Effluent conc. $\mu g\ L^{-1}$ |
|---|---|---|
| Average | 83 | 41 |
| Standard deviation | 9.1 | 24 |
| Maximum value | 98 | 99 |
| Minimum value | 59 | 10 |

The sorption phase was followed by the growth phase, the period during which an active biomass accumulated in the reactor. Microorganisms capable of degrading BTEX compounds grew rapidly and a BTEX removing population was quickly established in the reactors. The establishment of an MTBE-degrading population took much longer. There was no measurable MTBE removal by the Nevada reactor for approximately 100 days after initial start-up. After this time, the reactor began to degrade MTBE with increases in removal efficiency over the next 300 days. Apparent steady-state operation was achieved after 400 days. A summary of effluent quality and MTBE removal efficiency for the Nevada reactor from late 1996 to early 1998 is presented in Table 4. In the California reactor, the start-up phase was very brief and full treatment was achieved in approximately 25 days.

The difference in start-up times at the two sites has been investigated and can be explained in several ways. First, the California reactor was inoculated with 20 lbs of carbon from the Nevada reactor, presumably adding microorganisms to the California reactor with the metabolic pathway already expressed for MTBE degradation. Preliminary results from recent studies

suggest that a cometabolic mechanism is responsible for the degradation of MTBE in both reactors (Stringfellow et al. 2000). An examination of influent groundwater entering the two reactors demonstrates that the influent has a complex character that could support cometabolic degradation (Table 3). Establishment of the cometabolizing population occurred more rapidly in the California reactor because more easily degradable hydrocarbons are available at that site (Table 3). Current evidence suggests that volatile alkanes, measured as purgable TPH (total petroleum hydrocarbons), may be serving as co-metabolites for MTBE biodegradation in the reactor.

*Conclusions*

Recent investigations suggest that ex-situ biological treatment of MTBE contaminated groundwater is technologically feasible. Experience at the Sparks site in Nevada demonstrates that biological reactors can be operated at high efficiencies and can deliver low effluent MTBE concentrations over extended periods (Table 4). Results from the California site demonstrate that a long start-up time is not an intrinsic character of MTBE biological treatment systems. The operation of such reactors could potentially benefit from a better understanding of the nature of MTBE biodegradation.

*Ex-situ suspended growth cometabolic degradation (Pitre & Steffan 1999)*

Leakage from an MTBE storage tank in southeast Texas resulted in groundwater contamination in the tank vicinity. Measurements of MTBE concentration in the groundwater from January 1996 to April 1996 revealed concentrations ranging from 13,000 mg/L to 42,000 mg/L MTBE. A study was conducted in 1996 to evaluate a pilot scale membrane bioreactor (MBR) system for the treatment of MTBE-impacted groundwater from this site. In this study, groundwater was fed from an equalization tank into a 1,000-gallon continuous-flow, well-mixed bioreactor vessel with a suspension of ENV425, a microbial strain reported to degrade MTBE and TBA. The bioreactor system was equipped with a diffused aeration system designed to provide airflows up to 600 $ft^3$ per hour, a mechanical mixer, an automated pH control system, and nutrient and co-substrate feed ports. Effluent from the bioreactor was processed through an ultrafiltration system with ceramic membrane filters designed to separate the biomass from the treated water. The treated water or permeate from the filters was either recirculated to the bioreactors to keep reactor volumes between 700 and 900 gallons, or discharged to another tank for storage and/or subsequent disposal to a sewer line. The concentrate or mixed liquor from the filters was recycled to the bioreactor.

Pilot testing was conducted in two phases, batch and continuous operation. The objectives of the batch operation were to promote the cometabolic growth of ENV425 in the bioreactor, confirm MTBE degradation, and acclimate the microbial strain to increasing MTBE concentrations. During batch operation, the MBR was inoculated with a concentrated suspension of ENV425. To increase the mixed liquor suspended solids (MLSS) concentration in the bioreactor, MTBE concentrations in the influent stream were gradually increased from 40 to 115 mg/L over a 40-day period. Envirogen's patented co-substrate approach with *iso*-propanol was applied on an intermittent basis to effect cometabolism. Using this method, MLSS concentrations were increased from 357 mg/L to 1,430 mg/L.

Once the microorganisms had acclimated to high concentrations of MTBE, continuous operation was initiated. Groundwater was diluted to controlled concentrations of MTBE (100 to 2,400 mg/L) in the equalization tank and fed continuously at varying flow rates to the bioreactor. The MLSS concentration varied from 940 mg/L at the beginning of the test to a maximum of 4,400 mg/L.

No solids were intentionally removed during the testing; however, large quantities of foam with suspended microorganisms were generated on several occasions and some of this foam was lost due to overflowing. Because of these losses, the solids mass could not be accurately quantified and the sludge retention times were not reported. The pH in the bioreactor varied between 5.4 and 7.3. During the test, attempts were made to provide excess nitrogen and phosphorus to promote cell growth. $NH_3$-N concentrations ranged from 1.3 mg/L to 156 mg/L while *ortho*-phosphate concentrations ranged from 10 mg/L to 45 mg/L in the effluent water. *Iso*-propanol was added periodically to the reactor to sustain cell reproduction. The highest loading rate achieved during the test period was 0.271lb MTBE/lb MLSS/day. At this loading rate, approximately 98% of the MTBE in the groundwater was removed. The average MTBE removal efficiency over the continuous operation phase of the test was 96.2% resulting in treated water MTBE concentrations between 1.6 to 120 mg/L.

A new strain, which presumably utilizes MTBE as a primary substrate, is currently being tested for

*Table 5.* Summary of MTBE case studies

| Type of remediation | Culture | MTBE half life | Comments | Reference |
|---|---|---|---|---|
| Ex-situ @ Sparks, Nevada | Mixed culture-fixed film on GAC | HRT = 0.2 hr; 83% average removal | Long (400 days) start up period | This reference |
| Ex-situ @ San Diego, California | Mixed culture-fixed film on GAC | HRT = 1.4 hr; 95% average removal | Short (20 day) start up period. Suspected cometabolism with alkanes | This reference |
| Ex-situ cometabolic | Pure culture (ENV425) suspended growth reactor | HRT = 3.3–5 days; 96.2% average removal | *Iso*-propanol added to increase cell growth | Pitre and Steffan 1999 |
| In-situ natural attenuation @ gas stations | Indigenous microbial population | Similar to benzene | 89% of MTBE plumes decreasing in size | IST 1999 |
| In-situ natural attenuation @ controlled release | Indigenous microbial population | ~756 days (10% MTBE in 150 feet of release) | Significant TBA formation and destruction | Barcelona and Jaglowski 1999 |
| In-situ bioaugmentation @ Port Hueneme | Mixed culture (BC-4) | 693 days in control plot<br>99 days in $O_2$-only plot<br>18 days in BC-4/$O_2$ plot | 2–8 mg $L^{-1}$ initial concentrations of MTBE | Salanitro 1999 |

use in scale-up bioreactors. Preliminary tests using an 85-liter reactor indicate that this strain is capable of degrading MTBE from 1,100 mg/L to 50 $\mu$g/L with a 3-day hydraulic retention time (R Steffan, pers. comm.).

*Conclusions*

This ex-situ field study confirms that MTBE can be removed from groundwater using a suspended growth bioreactor. The cell yield of microorganisms was low, and as a result, intermittent injections of *iso*-propanol were used to sustain high cell density. The microorganism population present was able to reduce MTBE concentrations in the bioreactor from approximately 2,400 mg/L to as low as 1.6 mg/L, with an average removal rate of 96.2% over the course of the study.

*In-situ natural attenuation (1ST 1999)*

Fuel release information from 149 BP gas stations in Florida was recently reviewed (Reid et al. 1999; 1ST 1999). Eighty sites were selected for detailed study based on the period of groundwater monitoring, the concurrent presence of MTBE and benzene, the absence of pure phase hydrocarbons, and a minimum number of monitoring wells (3) with detectable concentrations of MTBE. The study objectives included the comparison of MTBE and benzene plume lengths and plume areas, assessment of hydrogeologic controls, matrix-chemical interactions, intrinsic controls on plume behavior, and derivation of empirical attenuation rates. Plume lengths and areas were established based on hand contouring MTBE and benzene isopleths on site maps.

The mean detection frequencies and concentrations of MTBE and benzene were similar (38.9% and 317 $\mu$g/L MTBE, and 42.8% and 365 $\mu$g/L benzene). The mean lengths of the 10 $\mu$g/L MTBE and benzene plumes were 140 feet and 115 feet, respectively which is similar to the results from the LLNL plume study (Happel et al. 1998). The mean areas of the 10 $\mu$g/L MTBE and benzene plumes were 11,985 $ft^2$ and 7,910 $ft^2$, respectively. Based on well-specific time-series analysis, it was suggested that 89% of the MTBE plumes studied were decreasing in size. Study results indicated that the average extent of MTBE migration relative to benzene at Florida BP gas stations was less than that reported in previous MTBE plume studies completed outside of Florida.

Empirically derived mean attenuation rate constants for MTBE (at 137 wells) and benzene (at 146

wells) were also similar. It was concluded that the similarity in rate constants could reflect related biological controls on each compound. Moreover, it was hypothesized that benzene plumes lagged behind MTBE plumes because benzene was the preferred carbon source for microorganisms and because MTBE biodegradation did not initiate until MTBE migrated beyond the benzene plume.

The estimated high rate of MTBE attenuation was consistent with environmental factors unique to Florida. Florida is influenced by subtropical climate, relatively flat hydraulic gradients, shallow water tables, high rates of infiltration and recharge, and moderately to highly permeable aquifers. In comparison to other regions of the U.S., these conditions promote lower groundwater flow rates, more rapid flushing and groundwater oxygenation, and higher groundwater volatilization rates.

*Conclusions*

While not conclusive, this study suggested that natural attenuation rates in Florida for MTBE were sufficiently rapid to stabilize MTBE plumes. The most likely explanation for these rapid attenuation rates is the high volume of groundwater recharge leading to well oxygenated groundwater which potentially stimulated biological activity in the aquifer.

*In-situ controlled release (Barcelona & Jaglowski 1999)*

An in-situ natural gradient reactive tracer test was recently conducted on the grounds of the former Wurtsmith AFB in Oscoda, MI. The objective of the test was to evaluate the fate and transport of MTBE and BTEX compounds, including the contributions of common transport loss mechanisms. Originally, the aquifer was uncontaminated and aerobic with average DO concentrations of 7.7 mg/L. To simulate the impact of alkanes and alkenes which were not present at this site but are thought to contribute to anoxic conditions, a reducing barrier was placed upgradient from the test lane to create suboxic (<2.0 mg/L) conditions within the aquifer. A 660 L solution containing MTBE (4,500 $\mu$g/L), benzene (1,700 $\mu$g/L), and other aromatic compounds was pumped into a well at the top of the water table (13–16 ft below ground surface (bgs)). The solution also included bromide, sulfur hexafluoride, and perfluorinated benzene and toluene which were used as biologically inert tracers to account for the effects of dispersion, volatilization, and sorption, respectively. An oxidizing barrier utilizing Oxygen Release Compound (ORC) was placed within the test lane to evaluate its potential for use in in-situ MTBE treatment. The site was monitored for a period of eight months following injection.

The results of the tracer study indicated reasonably well-behaved transport conditions over the course of testing. Over 11,000 measurements were taken at over 3,500 locations and times during the 8 months of operation. As expected, $Br^-$, $SF_6$, and MTBE, traveling at an average groundwater velocity of 1.3 ft/day, eventually separated from the more retarded compounds such as BTEX compounds and the perfluorinated constituents. TBA concentrations of 1–10 $\mu$g/L appeared 7 ft downgradient from the injection well within a week of injection. TBA was also detected at concentrations up to 70 $\mu$g/L at 20 and 40 ft downstream of the injection well 3 to 8 weeks after injection. Analyses of the tracer solution and background water samples revealed the absence of TBA at detectable levels.

Based on preliminary analysis utilizing peak observed concentrations, apparent mass losses were of most significance for $SF_6$ followed by MTBE and bromide. Relative to bromide, an estimated 10% of the injected MTBE was lost due to a combination of mechanisms. It was speculated that the mass of TBA formed could account for the majority of the MTBE mass lost; however, a more refined analysis of the data is required to further elucidate this issue. Dissolved oxygen concentrations immediately downgradient from the ORC barrier were measured at 20–30 mg/L while in-flow concentrations were less than 4 mg/L. It was not determined whether the appearance of 296 $\mu$g/L TBA immediately downgradient from the ORC barrier occurred via biotic or abiotic means. These results suggested that MTBE transformation rates were increased relative to conditions in the anoxic aquifer upgradient from the barrier. However, the limited MTBE mass loss prevents one from making significant conclusions regarding the effectiveness of the ORC barrier in promoting MTBE biodegradation. This uncertainty is confounded by the high groundwater velocity which creates a short residence time for MTBE within the ORC barrier and more rapid dispersion of dissolved oxygen concentrations produced by the barrier. Regardless, the appearance of TBA in the absence of introduced or background sources is an indication that MTBE degradation could occur under suboxic conditions. Degradation is expected to account for the largest fraction of mass loss

due to MTBE's low expected volatilization and adsorption potential. TBA mass loss was also observed although it was unclear whether it occurred due to biodegradation or to some other mechanism.

*Conclusions*

Under well-controlled testing conditions, this field study suggests that some indigenous organisms might be able to degrade MTBE. While MTBE mass loss was not overwhelming (approximately 10% beyond bromide mass loss), it was accompanied with an increase in mass of TBA. Finally, it appears that the transformation of MTBE to TBA occurred under anaerobic conditions within the plume and aerobic conditions immediately downgradient from the ORC barrier.

*In-situ bioaugmentation (Salanitro et al. 1999)*

A field-scale demonstration of enhanced MTBE bioremediation using a mixed culture enriched on MTBE, designated as BC-4, was recently completed (Salanitro et al. 1999). The study was conducted at the USN Port Hueneme, CA NEX Service Station, where an MTBE plume extended over 4,000 ft in length, 400 ft in width, and 10 ft in thickness. The study area (120′ × 60′) was initially suboxic (DO < 1 mg/L) with considerable concentrations of MTBE (2,000 to 8,000 $\mu$g/L) and TBA (50 to 250 $\mu$g/L). The aquifer was approximately 10 feet below grade surface (bgs) and 10 ft thick. The upper and lower portions of the aquifer consisted of silty loam and fine to medium sand, respectively. Apparent groundwater velocities ranged from approximately 0.1 to 0.3 ft/day at 10 to 15 feet bgs and approximately 0.3 to 0.5 ft/day at 15 to 20 feet bgs.

Three plots were evaluated in this study: a control plot where no treatment was performed, a plot supplemented with $O_2$ only, and a plot supplemented with $O_2$ and augmented with BC-4. $O_2$ was injected in the treatment zone (10 × 20 ft) at approximately 15 ft and 20 ft bgs. A one-time addition consisting of nine kilograms of the culture (dry weight) was injected across the width of the third treatment plot over a 10 ft vertical distance, 10 to 20 ft bgs. The cell concentration of the injected solution was approximately 2,000 mg/L; of the suspended culture, less than or equal to 0.1% of the microorganisms were expected to be able to degrade MTBE. $O_2$ was injected intermittently over the course of testing to maintain aerobic conditions (>2 mg/L) in the treatment zones. MTBE concentrations, DO concentrations, and microbial activity were monitored every 30 to 60 days while TBA concentrations were occasionally monitored over a test period of approximately nine months. Because significant fluctuations in MTBE concentrations were observed in the control plots after 29 days, only results up to this time are discussed here.

*Control plot.* In the control plot, the DO concentrations at 15 ft remained stable at approximately 1 mg/L over the duration of the experiment. However, DO concentrations at 20 ft bgs varied from 1 mg/L to 20 mg/L indicating that the neighboring $O_2$ injection plot influenced the environment in the control plot. MTBE concentrations decreased from approximately 5–7 mg/L to 2 mg/L in the shallow monitoring wells and 8 mg/L to 1 mg/L in the deep monitoring wells after 129 days.

*$O_2$-only plot and $O_2$ + BC-4 plot.* Oxygen concentrations in the $O_2$ plot and the $O_2$ + BC-4 plot treatment zones were consistently maintained above 2 mg/L and in many cases exceeded 20 mg/L over the course of testing. Initial MTBE concentrations in the two treatment plots were similar and varied between 2 to 9 mg/L. Decreases in MTBE concentrations were observed in the $O_2$ + BC-4 plots 67 days after seeding and continuous $O_2$ injection. In the immediate vicinity of the BC-4 injection, greater than a 90% decrease in MTBE concentrations was observed while less dramatic decreases were observed downstream of this region. In contrast, no significant changes were observed in the $O_2$-only and control plots after 67 days.

Between 67 days and 129 days after BC-4 seeding (173 days after initial $O_2$ injection), MTBE concentrations continued to decline downgradient of the biobarrier. By this time, MTBE was no longer detected in many of the samples collected from the closest down-gradient monitoring points, and several of the downgradient samples had concentrations as low as 0.01 to 0.05 mg/L. MTBE concentrations were also shown to decline in upgradient locations close to the injection site. In this time period, declines in MTBE concentrations were also observed in the $O_2$-only plot and in areas of the control plot impacted by $O_2$ injection. Although MTBE losses were significantly less (approximately 1.5 to 2 orders of magnitude) than those observed in the $O_2$ + BC-4 plots, it was hypothesized that $O_2$ injection may have stimulated the activity of naturally-occurring MTBE degraders in the $O_2$-only plot.

Measurements at 15 ft bgs after 129 days revealed that TBA was not detected (0.1 $\mu$g/L detection level) in the treatment zone of the $O_2$ + BC-4 plot. In contrast, TBA was detected consistently across the $O_2$-only plot after 129 days.

*Conclusions*

The results of this study provide evidence of successful bioenhancement with $O_2$ injection alone; however, $O_2$ injection was shown to be less effective than the combined effect of $O_2$ and BC-4 injection. Furthermore, a longer lag time was observed when $O_2$ was used exclusively. An in-situ barrier using BC-4 appeared to be capable of reducing MTBE levels to <5 $\mu$g/L without the accumulation of TBA as a byproduct. Furthermore, this study showed that the injected culture remained active up to 261 days under the conditions tested. In light of these findings, bioaugmentation with BC-4 combined with $O_2$ injection appears to be a feasible in-situ MTBE treatment option and warrants further investigation.

## Conclusions

The purpose of this paper was to discuss bioremediation strategies within the context of MTBE contamination. In this paper, key parameters for optimizing MTBE biodegradation and potential limitations that may impede successful bioremediation were identified. MTBE has been shown to biodegrade under many field and laboratory conditions. Most successful bioremediation strategies for MTBE employ some form of bioenhancement including the addition of oxygen, nutrients, or co-substrates. In addition, bioaugmentation in combination with bioenhancement has been shown to be effective for in-situ bioremediation of MTBE. While MTBE appears to degrade most easily under aerobic conditions, there is evidence that anaerobic degradation could naturally occur, albeit very slowly in the field. Cell yields on MTBE and culture growth rates are low relative to BTEX compounds; however, this limitation could potentially be advantageous for ex-situ fixed film treatment applications. Practical implementation of in-situ bioremediation for MTBE is in the developing stages with more field studies needed to verify laboratory results; however, initial studies of bioaugmentation and bioenhancenient at Port Hueneme and Vandenberg Air Force Base suggest that bioremediation has a strong potential for success. The available literature and the case studies discussed above suggest that MTBE biodegradation may naturally occur given sufficient dissolved oxygen concentrations and relatively low groundwater velocities (e.g., less than 1 ft/day).

## References


Alexander M (1994) Biodegradation and bioremediation. Academic Press, Inc., San Diego, CA

Andrews C (1998) MTBE-A long-term threat to ground water quality. Ground Water 36: 705–706

Barcelona MJ & Jaglowski DR (1999) Subsurface fate and transport of MTBE in a controlled reactive tracer experiment. In Proceedings of the 2000 Petroleum Hydrocarbons and Organic chemicals in Ground Water: Prevention, Detection, and Remediation (pp 123–137). National Ground Water Association, Westerville, OH

Barker JF, Schirmer M & Butler BJ (1998) Fate and transport of MTBE in groundwater – Results of a controlled field experiment in light of other experience. Paper presented at the Southwestern Regional Conference of the National Ground Water Association June 3–4, Anaheim, CA

Betts KS (1999) Growing evidence of widespread GMO contamination. Environ. Sci. Technol. 33: 484A

Borden RC, Daniel RA & LeBrun LE (1997) Intrinsic biodegradation of MTBE and BTEX in a gasoline-contaminated aquifer. Water Resour. Res. 33: 1105–1115

Bradley PM, Landmeyer JE & Chapelle FH (1999) Aerobic mineralization of MTBE and *tert*-butyl alcohol by stream bed sediment microorganisms. Environ. Sci. Technol. 33: 1877–1879

California Department of Health Services (DHS) Division on Drinking Water and Environmental Management Drinking Water Program MTBE in California Drinking Water (1999) <http://www.dhs.cahwnet.gov/org/ps/ddwem/ publications/chemical s/mtbe/mtbsummary.htm>. Viewed in August, 1999

Cho JS & Wilson JT (1999) Hydrocarbon and MTBE removal rates during natural attenuation application. Proceedings of The Fifth International In: Alleman BC & Leeson A (Eds). In Situ and On-Site Bioremediation Symposium (5: 109–114). Battelle Press, Columbus

Dybas MJ, Tatara GM, Knoll WH, Mayotte TJ & Criddle CS (1995) Niche adjustment for bioaugmentation with *Pseudomonas* sp. strain KC. In: Hinchee RE, Fredrickson J & Alleman BC (Eds) Bioaugmentation for Site Remediation (pp 77–84). Battelle Press, Columbus

Eweis JN, Chang DP, Schroeder ED, Scow KM, Morton RL & Caballero RC (1997) Meeting the challenge of MTBE biodegradation. In Proceedings of the 90th Annual Meeting and Exhibition of the Air and Waste Management Association, June 8–13, Toronto, Canada

Forsyth JV, Tsao YM & Bleam RD (1995) Bioremediation: When is augmentation needed? In: Hinchee RE, Fredrickson J & Alleman BC (Eds). Bioaugmentation for Site Remediation (pp 1–14). Battelle Press, Columbus

Fortin NY & Deshusses MA (1999a) Treatment of methyl *tert*-butyl ether vapors in biotrickling filters. 1. Rector startup, steady-state performance, and culture characteristics. Env. Sci. Technol. 33: 2980–2986

Fortin NY & Deshusses MA (1999b) Treatment of methyl *tert*-butyl ether vapors in biotrickling filters. 2. Analysis of the rate-limiting

step and behavior under transient conditions. Env. Sci. Technol. 33: 2987–2991

Fujiwara Y, Kinoshita T, Sato H & Kojima I (1984) Biodegradation and bioconcentration of alkylethers. Yugagaku 33: 111–114

Garnier P, Auria R, Auger C & Revah S (1999) Cometabolic biodegradation of methyl t-butyl ether by *Pseudomonas aeruginosa* grown on pentane. Appl. Microbial. Biotechnol. 51: 498–503

Gerhardt P, Murray RGE, Wood WA & Krieg NR (1994) Methods for general and molecular bacteriology. American Society for Microbiology. Washington, DC

Hanson JR, Ackerman CE & Scow KM (1999) Biodegradation of methyl *tert*-butyl ether by a bacterial pure culture. Appl. Environ. Microbiol. 65: 4788–4792

Happel AM, Bechenbach, FH & Halden RU (1998) An evaluation of MTBE impacts to California groundwater resources. Lawrence Livermore National Laboratory. UCRL-AR-1 30897

Hardison LK, Curry SS, Ciuffetti LM & Hyman MR (1997) Metabolism of diethyl ether and cometabolism of methyl *tert*-butyl ether by a filamentous fungus, a *Graphium* sp. Appl. Environ. Microbiol. 63: 3059–3067

Hurt KL, Wilson JT, Beck FP & Cho JS (1999) Anaerobic biodegradation of MTBE in a contaminated aquifer. In: Alleman BC & Leeson A (Eds) Proceedings of The Fifth International In Situ and On-site Bioremediation Symposium (5: 103–108). Battelle Press, Columbus

Hyman M & O'Reilly K (1999) Physiological and enzymatic features of MTBE-degrading bacteria. In: Alleman BC & Leeson A (Eds) Proceedings of The Fifth International In Situ and On-site Bioremediation Symposium (5: 7–12). Battelle Press, Columbus

Hyman M, Kwon P, Williamson K & O'Reilly K (1998) Cometabolism of MTBE by alkane-utilizing microorganisms. In: Wickramanayake GB & Hinchee RE (Eds). Proceedings of the First International Conference on Remediation of Chlorinated and Recalcitrant Compounds (1: 321–326). Battelle Press, Columbus

Integrated Science and Technology (1ST) (1999) Comparative MTBE versus benzene plume behavior – BP Oil Company Florida facilities. 1349 Old Highway 41, Suite 225, Marietta, GA 30060

Javanmardian M & Glasser HA (1997) In-situ biodegradation of MTBE using biosparging. Division of Environmental Chemistry. Proceedings from 213th ACS National Meeting, p 424

Jensen HM & Arvin F (1990) Solubility and degradability of the gasoline additive MTBE, methyl-*tert*-butyl-ether, and gasoline compounds in water. In: Arendt F, Hinsenveld M & van den Brink WJ (Eds). Contaminated Soil '90 (pp 445–448). Kluwer Academic Publishers, Dordrecht, The Netherlands

Johnson P (1998) Assessment of contributions of volatilization and biodegradation to in-situ air sparging performance. Environ. Sci. Technol. (32)2: 276–281

Kampbell D, Wiedemeier T & Hansen J (1995) Intrinsic bioremediation of fuel contamination in groundwater at a field site. J. Hazard. Mat. 49: 197–204

Koenigsberg S, Sandefur C, Mahaffey W, Deshusses M & Fortin N (1999) Peroxygen mediated bioremediation of MTBE. In: Alleman BC & Leeson A (Eds). Proceedings of The Fifth International In Situ On-Site Bioremediation Symposium (5: 13–18). Battelle Press, Columbus

MacDonald TR, Kitanidis PK, McCarty PL & Roberts PV (1999) Mass-transfer limitations for macroscale bioremediation modeling and implications on aquifer clogging. Ground Water 37: 523–531

Mace RE, Fisher S, Welch DM & Parra SP (1997) Extent, mass, and duration of hydrocarbon plumes from leaking petroleum storage tanks sites in Texas. Bureau of Economic Geology, The University of Austin, Texas

Mackay DM, Finarson MD, Wilson RD, Fowler B, Scow K, Hyman M, Naas C, Schirmer M & Durrant GD (1999) Field Studies of In-Situ Remediation of an MTBE Plume at Site 60, Vandenberg Air Force Base, California. In Proceedings of the 2000 Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection, and Remediation (pp 145–157). National Ground Water Association, Westerville, OH

Maine Department of Human Services, Maine Department of Environmental Protection, and Maine Department of Conservation (1998) "The presence of MTBE and other gasoline compounds in Maine's drinking water, a preliminary report, October 13, 1998", available at www.state.me.us/dhs/boh

Mo K, Lora CO, Wanken AF, Javanmardian M, Yang X & Kulpa CF (1997) Biodegradation of methyl *t*-butyl ether by pure bacterial cultures. Appl. Microbial. Biotechnol. 47: 69–72

Moran MJ, Zogorski JS & Squillace PJ (1999) MTBE in ground water of the United States – Occurrence, potential sources, and long-range transport. In: Proceedings of the Water Resources Conference, American Water Works Association, Norfolk, VA, Sept. 26–29, 1999. American Water Works Association

National Research Council (NRC) (1993) In situ bioremediation: When does it work? National Academy Press, Washington, DC

National Research Council (NRC) (1994) Alternatives for Ground Water Cleanup. National Academy Press, Washington, DC

Office of Science and Technology Policy (OSTP) (1997) Executive Office of the President. National Science and Technology Council. Committee on Environmental and Natural Resources. Interagency Assessment of Oxygenated Fuels. Washington, DC

Park K & Cowan RM (1997) Effects of oxygen and temperature on the biodegradation of MTBE. In: Preprints of Extended Abstracts, Proceedings of the 213th ACS National Meeting, San Francisco, CA. (37)1: 421–423

Pitre MP & Steffan R (1999) Biotreatment of MTBE-contaminated groundwater in membrane bioreactor. Envirogen Report

Reid JB, Reisinger HJ, Bartholomae PG, Gray JC & Huilnian AS (1999) A comparative assessment of the long-term behavior of MTBE and benzene plumes in Florida, USA. In: Alleman BC & Leeson A (Eds) Proceedings of The Fifth International In Situ and On-site Bioremediation Symposium (5: 97–102). Battelle Press, Columbus

Reisch MS (1994) Top 50 chemicals production rose modestly last year. Chem. Eng. News 72: 12–15

Rice DW, Grose RD, Michaelsen JC, Dooher BP, MacQueen DH, Cullen SJ, Kastenberg WF, Evertt LE & Marino MA (1995) California Leaking Underground Fuel Tank (LUFT) Historical Case Analyses. Lawrence Livermore National Laboratory, UCRL-AR-122207

Salanitro JP, Diaz LA, Williams MP & Wisniewski HL (1994) Isolation of a bacterial culture that degrades methyl t-butyl ether. Appl. Environ. Microbiol. 60: 2593–2596

Salanitro JP (1995) Understanding the limitations of microbial metabolism of ethers used as fuel octane enhancers. Curr. Opin. Biotechnol. 6: 337–340

Salanitro JP & Wisniewski HL (1996) Observations on the biodegradation and bioremediation potential of methyl *t*-butyl ether. Paper presented at the 17th Annual Meeting of the Society of Environ. Toxicol. Chem.

Salanitro JP, Chou CS, Wisniewski HL & Vipond TE (1998) Perspectives on MTBE biodegradation and the potential for in situ aquifer bioremediation. Paper presented at the Southwestern Regional Conference of the National Ground Water Association

Salanitro JP, Spinnier GE, Neaville CC, Maner PM, Steams SM, Johnson PC & Bruce C (1999) Demonstration of the enhanced MTBE bioremediation (FMB) in situ process. In: Alleman BC & Leeson A (Eds) Proceedings of The Fifth International In Situ and On-site Bioremediation Symposium (5: 37–46). Battelle Press, Columbus

Schirmer M & Barker JF (1998) A study of long-term MTBE attenuation in the Borden aquifer, Ontario, Canada. Ground Water Monit. Remediat. 18: 113–122

Shaffer KL & Uchrin CG (1997) Uptake of methyl tertiary butyl ether (MTBE) by groundwater solids. Bull. Environ. Contam. Toxicol. 59: 744–749

Shuler ML & Kargi F (1992) Bioprocess Engineering: Basic Concepts. PTR Prentice-Hall Inc., Englewood Cliffs, NJ

Squillace PJ, Pankow JF Korte NF & Zogorski JS (1997) Review of the environmental behavior and fate of methyl *tert*-butyl ether. Environ. Toxicol. Chem. 16: 1836–1844

Squillace PJ, Moran MJ, Lapham WW, Prince CV, Clawges RB & Zogorski JS (1999) Volatile organic compounds in untreated ambient groundwater of the United States, 1985–1995. Environ. Sci. Technol. 33: 4176–4187

Steffan RJ, McClay K, Vainberg S, Condee CW & Zhang D (1997) Biodegradation of the gasoline oxygenates methyl *tert*-butyl ether, ethyl *tert*-butyl ether, and *tert*-amyl methyl ether by propane-oxidizing bacteria. Appl. Environ. Microbiol. (63)11: 4216–4222

Stringfellow WT (1998) Biodegradation of methyl *tert*-butyl ether by microorganisms found in a groundwater treatment system. Abstracts of the 98th Annual Meeting of the American Society for Microbiology

Stringfellow WT, Hines RD & Kilkenny ST (2000) Applying co-metabolic biological reactions for the ex-situ treatment of MTBE contaminated ground water. 219 American Chemical Society National Meeting, San Francisco, CA. 40: 286–288

Suflita IM & Mormile MR (1993) Anaerobic biodegradation of known and potential gasoline oxygenates in the terrestrial subsurface. Environ. Sci. Technol. 27: 976–978

Sun PT, Salanitro JP & Tang WT (1996) Fate and biokinetics of methyl-*t*-butyl ether in activated sludge systems and its engineering significance. In: 51st Purdue Industrial Waste Conference Proceedings (pp 507–524). Ann Arbor Press, Inc., Chelsea, MI

Tang WT & Sun PT (1997) Field evaluation of biological and non-biological treatment technologies to remove MTBE/oxygenates from petroleum product terminal wastewaters. Shell Development Company, Houston TX. American Petroleum Institute, Washington, DC. Publication number 4655

Tyner L, Brown K, Caron D, Perina T, Daftary D & Sibbett B (1998) Natural attenuation of BTFX and MTBE under complex hydrogeological conditions. In: Wickramanayake GB & Hinchee RE (Eds). Proceedings of The First International Conference on Remediation of Chlorinated and Recalcitrant Compounds (C1: 333–339). Battelle Press, Columbus.

US Environmental Protection Agency. (USEPA) (1998) Field applications of in-situ remediation technologies: chemical oxidation. Office of Solid Waste and Emergency Response (5 102G). EPA 542-R-98-008

US Environmental Protection Agency Blue Ribbon Panel. (USEPA Blue Ribbon Panel) (1999) Blue Ribbon Panel for Reviewing the Use of MTBE and Other Oxygenates in Gasoline Home Page. http ://www. epa.gov/orcdizux/consumer/fuels/oxypanel /blueribb .htm

US Environmental Protection Agency. (USEPA) (1999) Use of monitored natural attenuation at Superfund, RCRA corrective action, and underground storage tank sites. Office of Solid Waste and Emergency Response. Directive 9200.4-17P

White GF, Russell NJ & Tidswell EC (1996) Bacterial scission of ether bonds. Microbiol. Rev. 60: 216–232

Wilson J (1999) Natural attenuation of MTBE in ground water: What do we need to know?. Proceedings of the 2000 Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection, and Remediation

Wilson RD, Schirmer M, Naas CN, Smith A, Smith C, Scow KM, Hyman MR & Mackay DM (1999) Laboratory scale evaluation of in-situ aerobic MTBE biodegradation options for Vandenberg Air Force Base, California. Proceedings of the 2000 Petroleum Hydrocarbons and Organic chemicals in Ground Water: Prevention, Detection, and Remediation (pp 167–175). National Ground Water Association, Westerville, OH

Yang X, Tsao M, Javanmardian M & Glasser HA (1998) Development of cost-effective MTBE in-situ treatment technologies. Presented at the ATV Vintermode om Grundvandsforurening. Belje, Nemark: March 10–11

Yeh, CK (1992) Degradation of gasoline oxygenates in the subsurface. Thesis, Virginia Polytechnic Institute and State University

Yeh CK & Novak JT (1994) Anaerobic biodegradation of gasoline oxygenates in soils. Water Environ. Res. 66: 744–752

Yeh CK & Novak IT (1995) The effect of hydrogen peroxide on the degradation of methyl and ethyl *tert*-butyl ether in soils Water Environ. Res. 67: 828–834