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Aerobic MTBE biodegradation: an examination of past studies, current challenges and future research directions

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Abstract

With the current practice of amending gasoline with up to 15% by volume MTBE, the contamination of groundwater by MTBE has become widespread. As a result, the bioremediation of MTBE-impacted aquifers has become an active area of research. A review of the current literature on the aerobic biodegradation of MTBE reveals that a number of cultures from diverse environments can either partially degrade or completely mineralize MTBE. MTBE is either utilized as a sole carbon and energy source or is degraded cometabolically by cultures grown on alkanes. Reported degradation rates range from 0.3 to 50 mg MTBE/g cells/h while growth rates (0.01–0.05 g MTBE/g cells/d) and cellular yields (0.1–0.2 g cells/g MTBE) are generally low. Studies on the mechanisms of MTBE degradation indicate that a monooxygenase enzyme cleaves the ether bond yielding *tert*-butyl alcohol (TBA) and formaldehyde as the dominant detectable intermediates. TBA is further degraded to 2-methyl-2-hydroxy-1-propanol, 2-hydroxyisobutyric acid, 2-propanol, acetone, hydroxyacteone and eventually, carbon dioxide. The majority of these intermediates are also common to mammalian MTBE metabolism. Laboratory studies on the degradation of MTBE in the presence of gasoline aromatics reveal that while degradation rates of other gasoline components are generally not inhibited by MTBE, MTBE degradation could be inhibited in the presence of more easily biodegradable compounds. Controlled field studies are clearly needed to elucidate MTBE degradation potential in co-contaminant plumes. Based on the reviewed studies, it is likely that a bioremediation strategy involving direct metabolism, cometabolism, bioaugmentation, or some combination thereof, could be applied as a feasible and cost-effective treatment method for MTBE contamination.

Abbreviations: BTEX compounds (benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene and *p*-xylene); DIPE (diisopropyl ether); ETBE (ethyl *tert*-butyl ether); HIBA (2-hydroxyisobutyric acid); MHP (2-methyl-2-hydroxy-1-propanol; also known as 2-methyl-1,2-propanediol); MTBE (methyl *tert*-butyl ether); ORC (oxygen release compounds); PHS (peat humic substances); TAME (*tert*-amyl methyl ether); TBA (*tert*-butyl alcohol).

Introduction

History of problem

Fuel oxygenates, including methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME), diisopropyl ether (DIPE), *tert*-butyl alcohol (TBA), methanol and ethanol (Zogorski et al. 1997), are added to gasoline to increase combustion efficiency and to reduce air pollution. These compounds are used worldwide in quantities that vary by season and country. Among fuel oxygenates, MTBE is most commonly used because of its high octane level, low production cost, ease of blending with gasoline, and ease of transfer and distribution (Nakamura 1994; Piel & Thomas 1990). MTBE was

initially added to gasoline in low quantities to replace lead as an octane enhancer in the late 1970's. In the United States, the addition of MTBE to gasoline significantly increased following the Clean Air Act Amendments of 1990 which mandated the use of reformulated and oxygenated gasoline in certain urban regions to reduce air pollution from motor vehicles. Starting in 1992, oxygenated fuels were used during the wintertime in carbon monoxide non-attainment areas and, more recently, throughout the year in metropolitan areas severely impacted by photochemical smog. In addition, some cities have freely elected to market oxygenate-blended fuels to enhance air quality (EPA 1998). Currently, MTBE-blended fuels make up 30% of the total gasoline consumed domestically with MTBE representing 11% of this volume on average (Andrews 1998). In 1995, the estimated production of MTBE in the United States was 18 billion pounds (Zogorski et al. 1996), most of which was used for fuel oxygenation.

Like most other gasoline components, MTBE is introduced into various environmental compartments during the production, distribution, use and storage of oxygenate-blended fuels. MTBE has been detected in urban air (Backer et al. 1997; Grosjean et al. 1998; Vainiotalo et al. 1998), surface water (Reuter et al. 1998), storm water (Zogorski et al. 1997) and groundwater (Church et al. 97; Landmeyer et al. 1997, 1998; Lince et al. 1998; Squillace et al. 1995). While the half-lives of MTBE in the atmosphere and in surface water are relatively short (on the order of days), a long half-life (on the order of years) has been estimated in subsurface systems (Howard 1991). In fact, MTBE has been shown to persist in aquifers, and MTBE plumes have been shown to migrate at rates comparable to groundwater velocities (Shaffer & Urchin 1997). The mobility of MTBE in the subsurface is due in part to its high aqueous solubility, low octanol water partition coefficient and chemical structure which is relatively resistant to microbial attack.

MTBE detection in the environment is frequent in areas with high oxygenate-blended fuel use. In California, for example, approximately 13 000 sites were shown to have hydrocarbon-impacted groundwater and greater than 10 000 of these sites were estimated to be impacted by MTBE (Happel et al. 1998). A recent study by the United States Geological Survey identified MTBE as the second most common volatile organic compound detected in wells monitored in urban areas nationwide between 1985 and 1995 (Squillace et al. 1995 & 1996).

Because the problems associated with the contamination of drinking water resources by MTBE have evolved fairly recently, a comprehensive database on effective MTBE treatment technologies is not yet available (Brown et al. 1997). While both conventional and innovative remediation methods are expected to work for MTBE, its high solubility and biological recalcitrance result in lower efficiencies and higher costs in comparison to the removal of other gasoline hydrocarbons from water (Creek & Davidson 1999). Moreover, the low taste and door thresholds of MTBE will necessitate stringent clean-up levels that are based on consumer acceptance rather than potential health effects further increasing the cost of remediating MTBE-contaminated water. In addition, the role of previously accepted passive remediation technologies such as natural attenuation for gasoline-impacted sites has to be re-evaluated due to the persistence of MTBE in the subsurface. As a result, the biodegradability of MTBE has received considerable attention recently and the bioremediation of MTBE-impacted groundwater has become an increasingly active area of basic and applied research. While there is no convincing evidence to date that the biodegradation of MTBE occurs rapidly in the field under natural conditions, several studies have demonstrated the biodegradability of MTBE in laboratory settings.

Scope of paper

The primary objective of this paper is to present a review of the current state of research on the biodegradation of MTBE under aerobic conditions. Studies that have addressed the utilization of MTBE by microbial cultures as a primary or cometabolic substrate are examined in detail. Secondary objectives of this paper include an examination of likely MTBE degradation mechanisms, an assessment of the potential for the accumulation of MTBE metabolic intermediates, and an evaluation of the effect of co-occurring gasoline components on MTBE degradation rates. Finally, the challenges of using enhanced bioremediation as a treatment technology for MTBE are discussed and future research needs are identified.

Biodegradation of MTBE by laboratory cultures

Although the detection of MTBE in groundwater was reported as early as 1986 (Garrett et al. 1986), until recently there were few studies conducted to evaluate

its biodegradability. While early studies suggested that MTBE is resistant to microbial attack under both aerobic and anaerobic conditions (Fujiwara et al. 1984; Jensen & Arvin 1990; Suflita & Mormile 1993; Yeh 1992), recent studies have revealed that bacterial and fungal cultures from various environmental sources are capable of degrading MTBE either as a primary source of carbon and energy or cometabolically following growth on another substrate (Bradley et al. 1999; Eweis et al. 1997; Fortin & Deshusses 1999a, b; Garnier et al. 1999; Hanson et al. 1999; Hardison et al. 1997; Mo et al. 1997; Park & Cowan 1997a, b; Salanitro et al. 1994; Steffan et al. 1997; and others). These studies include reports of either the partial degradation of MTBE to dead-end metabolic intermediates or its mineralization to CO_2. A summary of key results from these studies is presented in Table 1 and a synopsis of each study is given below.

Direct metabolism

Mixed and pure microbial cultures from a number of environmental sources have been shown to metabolize MTBE under controlled laboratory conditions. The first report of a culture capable of MTBE degradation involved a heterotrophic microbial consortium, designated BC-1. This culture was enriched from an activated sludge unit treating industrial chemicals (Salanitro et al. 1994). BC-1, which is thought to consist of up to 6 different species, was maintained on MTBE in a chemostat with cell recycle and was shown to degrade up to 200 mg MTBE/L at rates up to 34 mg/g cells/h. However, MTBE removal rates declined with MTBE concentrations over 5 mg/L and mineralization rates decreased at concentrations over 20 mg/L. While this culture was able to utilize MTBE as a primary growth substrate, growth rates (0.01/d) and cellular yields (0.21–0.28 g cells/g MTBE) were low. The low growth rate on MTBE was speculated to be due to either the slow metabolism of a rate limiting intermediate or to the action of MTBE as a metabolic inhibitor. TBA was formed as a transient metabolic intermediate during the breakdown of MTBE with TBA degradation occurring mostly after MTBE was completely utilized (Salanitro & Wisniewski 1996). The degradation rate of TBA was slower (14 mg/g cells/h) than that of MTBE and TBA was not utilized effectively by BC-1 for growth. When this culture was used to seed bioreactors treating MTBE-contaminated water, the activity of BC-1 decreased at temperatures less than 20 °C and at dissolved oxygen concentrations less than 1 mg/L (Sun et al. 1996; Tang & Sun 1997). It was suggested that low influent flows and long cell residence times are needed to maintain effective MTBE mass removal rates using this culture.

The abilities of several cultures related to BC-1 to degrade MTBE were later reported (Salanitro et al. 1998 & 1999). These cultures included consortia designated BC-4 and MC-100, and a pure culture, designated SC-100, which was identified as a *Rhodococcus*. Inoculation of soil and groundwater microcosms with laboratory cultures showed that up to 80 mg/L of MTBE were degraded to non-detectable limits in 16 weeks.

A mixed culture enriched from petroleum refinery activated sludge was also shown to degrade MTBE and TBA, and to utilize both as sole sources of carbon and energy (Park & Cowan 1997a, b). The maximum specific growth rates of the culture on MTBE and other ether oxygenates were low (0.015–0.06/h) but in the range of previously reported rates while cellular yields were moderate (0.33–0.43 mg cells COD/mg substrate COD at 30 °C). A similarity to previously reported results by Salanitro et al. (1994) was that TBA degraded slower than MTBE resulting in the transient accumulation of TBA. The consortium was also shown to be sensitive to decreases in temperature with maximum specific growth rates declining from 0.043 to 0.012/h and cell yields declining from 0.41 to 0.33 mg cells COD/mg MTBE COD when temperatures decreased from 30 to 20 °C. In addition, at dissolved oxygen concentrations below 0.9 mg/L, the rate and extent of MTBE degradation were substantially reduced.

Mo et al. (1997) isolated three pure cultures, *Methylobacterium mesophilicum* (ATCC 700107), *Arthrobacter ilicis* (ATCC 700109) and a *Rhodococcus* sp. (ATCC 700108) from activated sludge samples, the fruit of a Gingko tree, and soil surrounding the tree, using MTBE as the enrichment substrate. The Gingko tree was chosen since it is thought to produce tertiary butyl groups in its fruit. The three cultures were shown to biotransform 29% of 200 mg/L MTBE in less than 2 weeks with 8% of the MTBE mineralized to CO_2 by the end of the first week (inoculum densities $= 5 \times 10^5$–5×10^7 CFU/mL). MTBE degradation rates decreased significantly in the presence of other carbon sources including TBA, TBF, *iso*-propanol, acetone and pyruvate. Slow growth rates on MTBE were observed leading the researchers to conclude that MTBE was either a poor growth substrate or that one of its metabolites might inhibit culture growth.

Table 1. Representative maximum MTBE biodegradation rates by a range of mixed and pure cultures

Culture	Source	Metabolism type	Rate of MTBE degradation	Ref.
BC-1 mixed culture	Activated sludge treating industrial chemicals	Primary substrate Complete metabolism	34 mg/g cells/h	Salanitro et al. 1994
Methylobacterium mesophilicum (ATCC 700107) *Rhodococcus* sp. (ATCC 700108) *Arthrobacter ilicis* (ATCC 700109)	Activated sludge Gingko tree fruit and surrounding soil	Incomplete metabolism	0.18 mg/L/h[a]	Mo et al. 1997
Mixed culture	MTBE-degrading biotrickling filter	Primary substrate Complete metabolism	10 mg/g cells/h	Fortin & Deshusses 1999a, b
Rubrivivax sp. PM1	Consortium enriched from MTBE-degrading biofilter	Primary substrate Complete metabolism	50 mg/g cells/h	Deeb 1999 Deeb et al. 2000a
Graphium sp. ATCC 58400	*n*-butane and propane-oxidizing culture	Cometabolic Partial degradation	0.92 mg/g cells/h	Hardison et al. 1997
Xanthobacter sp *Alcaligenes eutrophus* *Mycobacterium vaccae*	*n-/iso*-alkane oxidizing cultures	Cometabolic Complete degradation	43.6 mg/g cells/h	Hyman et al. 1998 Hyman & O'Reilly 1999
ENV425 ENV421	Pure propane-oxidizing cultures	Cometabolic Complete degradation	26.7 mg/g cells/h	Steffan et al. 1997
Pseudomonas aeruginosa	Mixed pentane-oxidizing culture from gasoline-contaminated soil	Cometabolic Complete degradation	11.3 mg/g cells/h	Garnier et al. 1999
PEL-B201	Pure benzene-oxidizing culture	Cometabolic	0.33 mg/g cells/h	Koenigsberg et al. 1999

[a] Inoculation density = 5×10^5–5×10^7 CFU/mL.
Values were obtained from the literature and reported in mg MTBE/g cells/h using the following assumptions: 0.55 mg protein/mg cells (Brock & Madigan 1991); cell dry weight/wet weight = 0.3 (Brock & Madigan 1991); spherical bacterial cell shape with 2 μm diameter; density of cellular materials = 1 g/mL.

In studies by Fortin et al. (1997) and Fortin and Deshusses (1998, 1999a, b), a mixed culture was enriched from two MTBE-degrading biotrickling filters. This consortium was shown to degrade high concentrations of MTBE and TBA (up to 550 and 800 mg/L, respectively) in batch reactors. Initially, peat humic substances (500 mg PHS/kg MTBE) were added to assist in establishing the culture. It was suggested that the humic acids might form complexes with MTBE therefore improving its microbial uptake rates. At concentrations of 50 to 800 mg/L, MTBE and TBA were degraded at rates of 1–3 mg/L/h. The formation of TBA during the biodegradation of MTBE was not noted suggesting that TBA was degraded faster than MTBE. Specific growth rates (0.023–0.026/d) and cell yields (0.09–0.12 g cells/g MTBE) on MTBE were low but consistent with values obtained from other MTBE studies.

Eweis et al. (1997) reported the removal of MTBE in a biofilter at the Los Angeles County Joint Water Pollution Control Plant. These researchers enriched a mixed culture from the biofilter material, consisting primarily of commercial compost, that was able to utilize MTBE for growth. The activity of the consortium was found to be a strong function of pH with decreasing activities outside a pH range of 6.5 to 7.8. MTBE degradation rates in liquid culture increased from 0.031 to 0.062 mg/L/d with increases in biomass; however, the amount of biomass was not quantified in this study. The culture was shown to also degrade

TBA, toluene and methanol. Under optimum conditions, the rate of TBA degradation was slightly higher than that of MTBE, and no accumulation of TBA was observed during the biodegradation of MTBE. Two pure cultures capable of degrading MTBE were isolated from this consortium (Hanson et al. 1999). One of these cultures, designated PM1, has been found to be a *Rubrivivax*, a member of the *Leptothrix* branch of the *β-Proteobacteria*. PM1 has been shown to have rapid MTBE degradation rates (50 mg/g cells/h) at MTBE concentrations up to 500 mg/L following growth on MTBE (Deeb et al. 2000a). PM1 was also capable of growth on TBA as sole carbon and energy source. TBA accumulation was not observed during the biotransformation of MTBE presumably due to a TBA degradation rate that is higher than that of MTBE (Church et al. 2000; Deeb et al. 2000b).

Cometabolism

Studies on the cometabolic degradation of MTBE have reported the ability of mixed and pure cultures to degrade MTBE to TBA or to CO_2 following growth on either alkanes or aromatic compounds. To begin with, Hardison et al. (1997) reported the ability of a filamentous fungus, *Graphium* sp. strain ATCC 58400, to partially degrade MTBE to TBA following growth on propane or *n*-butane. When cells were grown on *n*-butane and exposed to 18 mg/L of MTBE, a maximum biodegradation rate of 0.92 mg MTBE/g cells/h was observed. In this study, TBF was identified as the first metabolic intermediate of MTBE degradation. TBF was subsequently converted to TBA by a combination of biological and non-biological processes but further degradation was not observed. The degradation of MTBE was inactivated by *n*-alkane enzyme inhibitors including acetylene, ethylene and other unsaturated hydrocarbons suggesting that MTBE and gaseous *n*-alkanes are oxidized by the same enzyme, a putative cytochrome P-450. In addition, MTBE degradation rates decreased in the presence of *n*-butane presumably due to competitive inhibition.

In later studies, the abilities of *Xanthobacter, Alcaligenes eutrophus* and *Mycobacterium vaccae* strains to cometabolize MTBE following growth on either simple straight-chain or branched alkanes were reported (Hyman et al. 1998; Hyman & O'Reilly 1999). MTBE oxidation rates ranged from 17 to 44 mg MTBE/g cells/h depending on the growth substrate and the culture (Hyman & O'Reilly 1999). With all three cultures, TBF and TBA were the major MTBE degradation products. TBA was further degraded, albeit slowly, by the *Xanthobacter* (7.6 mg MTBE/g cells/h) and *M. vaccae* strains.

Steffan et al. (1997) reported the ability of several propane-grown pure cultures to cometabolize MTBE. One strain, designated ENV425, was shown to mineralize MTBE. Stoichiometric amounts of TBA were produced during the biodegradation of MTBE. In fact, in nearly every case, TBA degradation did not occur until MTBE was completely removed from the reactors. In addition, TBA had lower degradation rates (4.4–5.9 mg/g cells/h) than MTBE (11.3–26.7 mg/g cells/h). The rates of MTBE and TBA biotransformation by ENV425 were comparable to those observed with other propane-grown strains tested in this study.

In a recent study by Garnier et al. (1999), a mixed culture from gasoline-contaminated soil that was enriched on pentane was shown to degrade MTBE only in the presence of pentane. A pure culture (*Pseudomonas aeruginosa*) isolated from the mixed culture was reported to metabolize MTBE up to concentrations of 900 mg/L following growth on pentane. The degradation rates of MTBE and TBA by the mixed and pure cultures were reported to be 2.3 and 10.2 mg substrate/g cells/h, respectively.

Finally, MTBE cometabolism was reported with a benzene-grown culture designated PEL-B201 (Koenigsberg et al. 1999). PEL-B201 was shown to degrade approximately 3.4 mg/L of MTBE in 12 hours following growth on benzene (inoculation density $= 2 \times 10^8$ cells). MTBE degradation was severely inhibited in the presence of benzene at concentrations as low as 0.15 mg/L presumably due to competitive inhibition. In the presence of 0.3 mg/L of benzene, the time required to degrade 3.4 mg/L of MTBE increased from 12 to 50 hours. Accordingly, increasing concentrations of MTBE were shown to inhibit benzene degradation. Preliminary results from this study suggested that MTBE degradation can also take place by cultures grown on cyclohexanone, *o*-xylene or camphor.

Bioreactor studies

Several studies have addressed the use of fixed-film bioreactors for the removal of MTBE from air and water streams. Such bioreactors are capable of retaining slow-growing bacteria that might otherwise be washed out from suspended-growth reactors. Given the low cellular yields of MTBE-degraders, fixed-film biore-

actors would be expected to be effective in enriching and retaining MTBE-degrading cultures.

The biodegradation of MTBE was recently evaluated in biotrickling filters (Fortin & Deshusses 1999a, b). Groundwater and aquifer materials from two field sites were used to inoculate two laboratory-scale biotrickling filters. MTBE in an air stream was introduced as the sole source of carbon and energy. Following a 6-month acclimation period, the removal of MTBE in both bioreactors proceeded and reached a maximum of 50 g/m^3 reactor volume/h, a value comparable to removal rates of BTEX compounds in similar systems. Most of the MTBE was mineralized (97%) and no biodegradation intermediates were detected in either the liquid or gas phase. A specific MTBE degradation rate of 5 to 10 mg MTBE/g cells/h was reported. Cell yields (0.09–0.12 g MTBE/g cells) and cellular growth rates (0.023–0.026/d) were similar to those reported for laboratory-grown mixed and pure cultures. The removal of MTBE in the bioreactors was reportedly limited by biological rather than mass transfer processes.

In studies by Eweis et al. (1997 & 1998), a compost biofilter at the Joint Water Pollution Control Plant of the Los Angeles County Sanitation District that was treating air streams containing MTBE and other hydrocarbons started removing MTBE after a lag phase of over 1 year. Concentrations of up to 200 ppb MTBE in the gas phase were removed at an average efficiency of 90%. The removal rate of MTBE was estimated to be 6 to 8 g/m^3 reactor volume/h. Mixed and pure cultures derived from the biofilter material were shown to grow on MTBE as sole carbon and energy source (see previous section). A pilot-scale biofilter containing an inert ceramic matrix was subsequently inoculated with the mixed culture and operated with inlet air concentrations of 30 to 40 ppm MTBE. After an acclimation phase of 10 weeks, the removal efficiency of MTBE in this bioreactor was reported to be 95%.

Overall, the results of these studies suggest that attached growth might be a key factor in obtaining and maintaining active MTBE-degrading populations in engineered reactors.

Biodegradation of MTBE in environmental samples

There is little information on the occurrence of MTBE degraders in the environment (Salanitro & Wisniewski 1996). A summary of a number of studies evaluating MTBE degradation in microcosms and at field sites is presented below.

A number of microcosm studies have reported the biodegradation of MTBE in soil and/or groundwater samples obtained from MTBE-impacted sites. In studies using groundwater samples from two wells at the Sparks Terminal Site in Nevada, MTBE at concentrations of 5 to 80 mg/L was degraded following lag periods of 2 to 3 weeks at rates of 0.01 to 0.32/d (Salanitro et al. 1998). In soil and groundwater samples from a site in New Jersey, MTBE was degraded at a rate of 0.28 mg/L/d with a half-life of 21 days (Salanitro et al. 1998). A survey conducted to evaluate the presence of naturally occurring MTBE-degraders at 10 gasoline-contaminated sites located throughout the United States indicated that populations of MTBE-degrading microorganisms were low in subsurface environments with concentrations that were typically below 10^3/g soil or 100/L groundwater (Salanitro et al. 1998).

In aerobic microcosms prepared using soil from a gasoline-contaminated site in North Carolina, MTBE concentrations were shown to decrease from 2.1 to 1 mg/L following a 20-day lag period (Borden et al. 1997; Daniel 1995). No further MTBE degradation was observed. During the degradation of MTBE, a compound speculated to be TBA was shown to accumulate reaching levels as high as 0.35 mg/L. In a parallel field study, MTBE concentrations in groundwater plumes were shown to decrease near the contaminant source, but not downgradient from the source, with a first order decay rate of 0.001/d.

In studies by Church et al. (1999), the biodegradation of MTBE was evaluated in aerobic columns packed with aquifer materials from four different sites. Preliminary results from this study revealed that MTBE was degraded in each of the aquifer columns but only in the absence of BTEX compounds. TBA concentrations increased in column effluents with decreasing MTBE and oxygen concentrations. However, no evidence of TBA degradation was observed in any of the columns.

The mineralization of MTBE was recently demonstrated in microcosm studies using stream-bed sediments from two gasoline-contaminated groundwater sites (Bradley et al. 1999). Both sites had previous exposure to measurable concentrations of MTBE, TBA and BTEX compounds. The fraction of MTBE mineralized in microcosms with sediments from both sites ranged from 30 to 70% in 105 days. MTBE degradation was lower at the site with the higher organic

content presumably due to competitive inhibition by alternative carbon substrates. The mineralization of TBA was more rapid than that of MTBE with 70% of the TBA recovered as CO_2 in 27 days.

In a field study at the Port Hueneme Naval Base Site, California, approximately 2 to 8 mg/L of MTBE were degraded in aquifer zones that were amended with oxygen over a period of 260 days suggesting that naturally-occurring MTBE degraders are present at some sites (Salanitro et al. 1999). The degradation of MTBE was reportedly stimulated after a lag period of 2 months when oxygen levels were increased from 1 mg/L up to a maximum of 20 mg/L. The degradation of the TBA (0.05–0.25 mg/L) present in the MTBE plume was not initially observed in the oxygen amended zone but later took place following a lag phase.

In a field study conducted to monitor the long term attenuation of MTBE in subsurface environments, groundwater amended with MTBE, a number of other gasoline hydrocarbons and a conservative tracer was injected below the water table at the Borden Aquifer in Canada (Hubbard et al. 1994). While the concentrations of BTEX compounds decreased rapidly over time during the first 16 months, the initial mass of MTBE introduced into the aquifer persisted. Almost eight years later, a mass balance on the MTBE remaining in the groundwater revealed that 97% of the initial mass was lost (Schirmer & Barker 1998; Schirmer et al. 1998). Based on the well-characterized properties of this site, sorption, abiotic degradation and volatilization were ruled out as significant MTBE loss mechanisms and it was suggested that the majority of the missing MTBE was biodegraded. In support of this hypothesis, the biodegradation of MTBE was evaluated in microcosm and column batch studies using groundwater and aquifer materials from the site (Church et al. 1997; Schirmer et al. 1999). Results from the laboratory studies confirmed the presence of indigenous aquifer microorganisms with the ability to degrade MTBE (Schirmer et al. 1999).

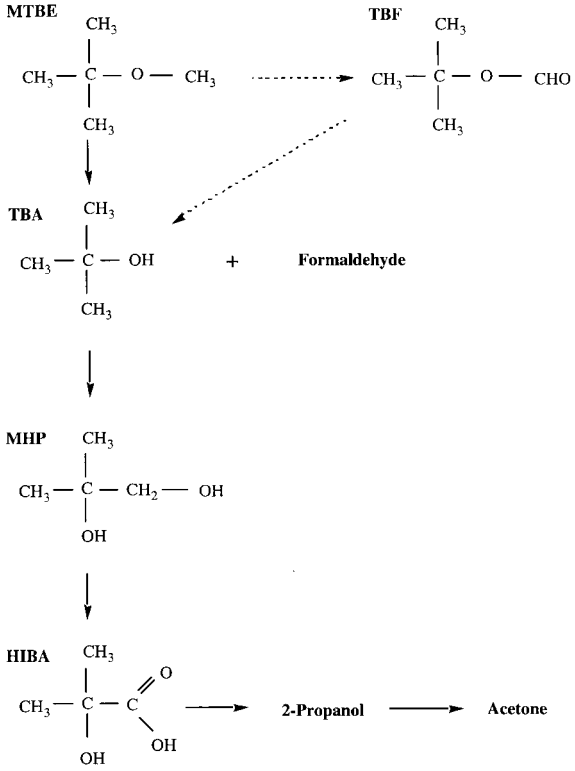

Figure 1. A demonstrated pathway for the degradation of MTBE under aerobic conditions. Adapted from Hardison et al. (1997), Salanitro et al. (1999) and Steffan et al. (1997).

Mechanisms of MTBE biodegradation

Proposed metabolic pathways

Although several pure and mixed cultures have been reported to degrade MTBE, the pathway(s) responsible for MTBE degradation have not been fully elucidated. Results from several of the above discussed studies suggest that the ether bond of MTBE is enzymatically cleaved yielding TBA and formaldehyde as the predominant detectable metabolic intermediates (Figure 1) (Salanitro et al. 1999; Steffan et al. 1997). Some studies, however, suggest that TBF is the first metabolite of MTBE degradation (Hardison et al. 1997) and that due to its rapid hydrolysis rate, it is quickly converted to TBA and is unlikely to accumulate at detectable levels (Church et al. 1997). TBA has been shown to further biodegrade to 2-methyl-2-hydroxy-1-propanol (also known as 2-methyl-1,2-propanediol, MHP) and 2-hydroxyisobutyric acid (HIBA) (Steffan et al. 1997). Speculated downstream intermediates include 2-propanol, acetone and hydroxyacteone (Church et al. 2000; Deeb et al. 2000b; Salanitro et al. 1998; Steffan et al. 1997).

All of the above reports suggest that a monooxygenase enzyme is involved in the initial biotransformation of MTBE to TBA under aerobic conditions (Hardison et al. 1997; Salanitro et al. 1999; Steffan et al. 1997). In some cases, a complex monooxygenase, cytochrome P-450, has been implicated in

catalyzing this reaction (Hardison et al. 1997). Cytochrome P-450 enzymes are common to both prokaryotic and eukaryotic cells and are a group of integral membrane proteins. Cytochrome P-450 hydroxylates organic compounds at the expense of one oxygen atom from molecular oxygen and a reducing equivalent (ASM 1994; Brock & Madigan 1994). This type of enzyme has been shown to be responsible for the transformation of MTBE and ETBE to TBA in rat and mouse tissues and in human liver microsomes (Hong et al. 1997 & 1999).

The metabolism of both MTBE and TBA in animal and human tissues has been the subject of a number of studies. The results of these studies revealed that, in most cases, the intermediates of MTBE metabolism by eukaryotic cells are the same intermediates associated with its prokaryotic metabolism. For example, TBA has long been known to be the major product of MTBE metabolism in tissues of mice and rats (Johanson et al. 1995; Miller et al. 1997) and more recently in human tissues (Nihlen et al. 1998).

Similar results have been observed in studies with animal subjects including mice and rats. Previously, TBA was thought to be inert in tissues and was heavily used as a solvent and perfume carrier in cosmetics. Later, studies revealed that TBA has potentially harmful effects and that it can be metabolized in tissues to formaldehyde and acetone (Cederbaum & Cohen 1980; Cederbaum et al. 1983; CIREP 1989). Recent studies with improved analytical methods revealed that MTBE can be rapidly metabolized to TBA in animal tissues, and that the major metabolites recovered in urine samples of animal subjects were also MHP and HIBA (Miller et al. 1997). In a study where rats were exposed to MTBE by inhalation and to TBA orally, TBA conjugates, MHP, HIBA and acetone were detected as metabolites (Bernauer et al. 1998).

Studies that have evaluated the metabolism of MTBE by humans showed that each of the major detectable intermediates were MPH and HIBA with traces of formaldehyde and acetone (Nihlen et al. 1999). Each of these four compounds has also been detected as an intermediate of MTBE metabolism by bacterial cells (Steffan et al. 1997).

Potential accumulation of rate limiting intermediates

The results from a number of the studies discussed in previous sections suggest that low growth rates on MTBE could be due to the slow metabolism of a rate limiting intermediate. The proposed major intermediates of MTBE degradation include formaldehyde, TBA, MHP, HIBA, 2-propanol and acetone (Figure 1). While the biodegradability of formaldehyde, 2-propanol and acetone is well documented, not much information is available on the biodegradation potentials of MHP and HIBA. However, based on the chemical structures of these two compounds, both should be expected to degrade at faster rates than MTBE and TBA. In fact, the accumulation of significant concentrations of MHP and HIBA during the biodegradation of MTBE and TBA has not been observed in laboratory studies (Steffan et al. 1997). On the other hand, many studies have documented the accumulation of TBA during the biodegradation of MTBE (Hardison et al. 1997; Park & Cowan 1997a, b; Salanitro et al. 1994; Steffan et al. 1997). In some of these studies, MTBE was incompletely degraded to TBA as a dead-end product (Hardison et al. 1997). In other studies, TBA was shown to accumulate until MTBE was significantly degraded (Salanitro et al. 1994; Steffan et al. 1997). Perhaps one of the most interesting questions regarding the enzymatic mechanisms involved in the degradation of MTBE by prokaryotic cells is whether MTBE monooxygenase is also responsible for TBA degradation. It is unclear from previous studies whether the enzymatic attack on MTBE or TBA is the rate limiting step for the complete degradation of MTBE. In fact, the observed range of results with respect to the accumulation of TBA during MTBE degradation suggests that the kinetics may differ significantly among different species. The purpose of this section is to give an overview of TBA degradation reports, to compare MTBE and TBA degradation rates by MTBE-degrading cultures, and to determine whether the available information suggests that the metabolism of TBA is the rate limiting step for MTBE degradation.

To begin with, a comprehensive summary of results from studies reporting both the degradation of MTBE and TBA is presented in Table 2. In some cases, cultures were capable of degrading TBA but not MTBE. For example, Fayolle et al. (1999) demonstrated the mineralization of TBA, but not MTBE, by activated sludge consortia. A pure culture (*Pseudomonas* sp. IFP 2003), isolated from a consortium, was shown to utilize TBA as the sole carbon and energy source. While not being capable of MTBE degradation, the culture rapidly mineralized TBA at a rate of 18 mg/g cells/h. In another study, TBA at a concentration of 3 mg/L was completely biodegraded after 200 days in microcosms using soil from a gasoline-

contaminated site while no biodegradation of MTBE took place following incubation periods up to 1 year (Zenker et al. 1999). In a third study, TBA was degraded with a half-life of 16 days in aquifer soils from a gasoline-impacted site while MTBE was not (Salanitro et al. 1998). These observations are not surprising since the degradation of TBA has been widely reported (Hickman & Novak 1989; Hickman et al. 1989; Novak et al. 1985; Yeh & Novak 1991, 1994, 1995). Novak et al. (1985) investigated the biodegradation potential of TBA and reported its disappearance in microcosms prepared using soils from three subsurface sites. Hickman and Novak (1989) reported the degradation of TBA in a range of subsurface soils at rates of 0.1 to 0.3 mg/L/d. TBA degradation has also been shown to take place under anaerobic conditions in a range of soils at rates of 0.05 to 0.15 mg/d/g soil (Yeh & Novak 1994) suggesting that naturally occurring TBA degraders are indigenous to subsurface environments.

The inconsistency of the summarized results in Table 2 suggests that the degradation mechanisms of MTBE and TBA could be culture dependent. A better understanding of the monooxygenase enzymes responsible for MTBE and TBA degradation by different cultures will facilitate predictions of when TBA oxidation is the rate-limiting step for MTBE degradation. While it seems that both MTBE and TBA are initially attacked by monooxygenase enzymes, it is unclear whether the same enzyme could be responsible for the degradation of both compounds. In a study with a cometabolizing culture, the enzyme inhibitor, acetylene, was shown to inactivate the oxidation of propane, MTBE and TBA suggesting that MTBE and TBA were both oxidized by propane monooxygenase (Hyman et al. 1998). In a study with a culture that grows on MTBE, MTBE-grown cells degraded TBA rapidly while TBA-grown cells did not degrade MTBE suggesting that the monooxygenase enzymes induced by MTBE and TBA were different (Deeb et al. 2000b).

As the problems associated with MTBE contamination continue to emerge and magnify, interest in the fate and transport of TBA in the subsurface is likely to intensify. To begin with, TBA can be present in MTBE-blended gasoline as an impurity and may therefore appear in groundwater at gasoline spill sites. In addition, microbial oxidation and hydrolysis of MTBE in aquatic environments is expected to produce TBA. As a result, the detection of TBA in subsurface environments has been suggested as an indicator of MTBE bioattenuation (Church et al. 1997).

The effect of co-contaminants on MTBE degradation rates

The biodegradation of MTBE can either be enhanced or repressed by the presence of other organic contaminants in groundwater plumes. That is, the biodegradation of MTBE can be stimulated by a cometabolic substrate as was discussed in a previous section. On the other hand, MTBE biodegradation can be inhibited by the presence of more readily degradable compounds in subsurface environments. The inhibition of MTBE degradation by organic contaminants can be caused by a number of phenomena including specific inhibition of the MTBE-oxidizing enzyme, preferential utilization of hydrocarbons by MTBE-degrading cultures, or growth of non-MTBE-degraders that can outcompete the slow-growing MTBE-degraders for available electron acceptors and nutrients.

Following a spill involving an MTBE-blended gasoline, MTBE is typically the first compound to be detected in groundwater downgradient from the spill (Shaffer & Urchin 1997). This is because MTBE has the highest solubility and polarity, and the lowest sorption potential among gasoline hydrocarbons. While the aliphatic components of gasoline are relatively immobile due to their low solubility in water, groundwater contaminated with MTBE-blended gasoline can be expected to contain the monoaromatic components of gasoline together with MTBE. Key physical and chemical properties of MTBE are presented in Table 3 and are contrasted to properties of BTEX compounds, the major aromatic components of gasoline. BTEX compounds are used to enhance the octane rating of gasoline and to reduce knocking in combustion engines. When leaded gasoline was phased out, the fraction of BTEX compounds in gasoline increased from about 26 to 34% on a volume basis (Cline et al. 1991). More recently, depending on the source of crude oil and the refining method used, the total aromatic fraction in gasoline has been as high as 40% but in some cases limited to a maximum of 25% when fuel oxygenates are added (Poulsen et al. 1992; Thomson et al. 1999). Therefore, while MTBE is likely to dominate at the leading edge of gasoline-impacted groundwater plumes, it can be expected to co-exist with BTEX compounds near contaminant sources and at the lagging edge of contaminant plumes. A recent survey reported the co-occurrence of MTBE and BTEX compounds at a large number of gasoline-contaminated sites in California (Happel et al. 1998). Once the point source was eliminated, and given time and distance,

Table 2. Comparative summary of MTBE and TBA degradation rates by cultures from a number of environmental sources

Reference	Notes	MTBE degradation rate	TBA degradation rate
Salanitro et al. 1994	Mixed culture (BC-1) MTBE was used for growth TBA was not effective as a growth substrate TBA accumulated (transient) during MTBE degradation	34 mg/g cells/h	14 mg/g cells/h
Park & Cowan 1997a, b	Mixed culture MTBE and TBA were used for growth TBA accumulated (transient) during MTBE degradation	MTBE > TBA NR[a]	TBA < MTBE NR[a]
Fortin et al. 1997	MTBE and TBA were used for growth TBA did not accumulate during MTBE degradation	1.1–2.6 mg/L/h[b]	1.8–2.8 mg/L/h[b]
Church et al. 2000 Deeb et al. 2000a, b Hanson et al. 1999	Pure culture (PM1) MTBE and TBA were used effectively for growth TBA did not accumulate during MTBE degradation	2.16×10^{2} mM/h [b]	2.96×10^{-2} mM/h[b]
Hyman et al. 1998	Pure culture (*Xanthobacter* sp.) MTBE and TBA were degraded cometabolically	5.9–15 nmol/min/mg protein	3.1 nmol/min/mg protein
Steffan et al. 1997	Pure cultures (ENV425 and ENV 421) MTBE and TBA were degraded cometabolically	2.1–9.2 nmol/min/mg protein	1.3–2.4 nmol/min/mg protein
Garnier et al. 1999	Pure culture (*Pseudomonas aeruginosa*) MTBE and TBA were degraded cometabolically	3.9 nmol/min/mg protein	0.95 nmol/min/mg protein
Bradley et al. 1999	Stream-bed sediments from gasoline-contaminated sites MTBE and TBA were mineralized	30–70% mineralized in 105 d	70% mineralized in 27 d
Salanitro et al. 1998	Groundwater from Southbridge site	ND[c]	Half-life = 16 d
Fayolle et al. 1999	Pure culture (*Pseudomonas* sp. IFP 2003) Cometabolic	ND[c]	18 mg/g cells/h
Zenker et al. 1999	Soil from gasoline-contaminated site	ND[c]	3 mg/L MTBE in 200 d

[a] NR = not reported.
[b] Inoculation density not reported.
[c] ND = not degraded.

dissolved plumes of MTBE were shown to separate from BTEX plumes causing detection frequencies of concurrent BTEX and MTBE to decrease in monitoring wells at these sites from 80 to 60% over a period of three years.

Because aromatic compounds are ubiquitous in the environment, a variety of microorganisms have evolved that are capable of exploiting them as sources of carbon and energy (Smith 1990; Zylstra 1994). The widespread distribution of soil bacteria capable of metabolizing BTEX compounds is well documented (Gibson & Subramanian 1984). As a result, biodegradation is recognized as the major attenuation mechanism for BTEX compounds in groundwater aquifers (Rice et al. 1995; Salanitro 1993). It is therefore important to determine whether the presence of MTBE could negatively impact BTEX attenuation rates leading to the elongation of BTEX plumes, or whether the ease of degradability of BTEX compounds could cause the repression of MTBE degradation in mixed contaminant plumes.

Limited studies have evaluated the effect of substrate interactions on the biodegradation rates of MTBE and BTEX compounds in contaminant mixtures. In most of the reported studies, the presence of MTBE at concentrations comparable to those reportedly detected in groundwater at gasoline-contaminated sites did not have a negative effect on BTEX degradation rates suggesting that the presence of MTBE does not affect the cell viability and activity of BTEX-degrading cultures (Deeb & Alvarez-Cohen 2000; Jensen & Arvin 1990; Salanitro et al. 1999). The results from studies that have evaluated the effect of BTEX compounds on MTBE biodegradation rates are not as clear or consistent. In one case, the cometabolic degradation of MTBE by cultures grown

Table 3. Selected physical and chemical properties of benzene (B), toluene (T), ethylbenzene (E), *o*-xylene (*o*-X), *m*-xylene (*m*-X), *p*-xylene (*p*-X) and methyl *tert*-butyl ether (MTBE)

	Benzene	Toluene	Ethylbenzene	*o*-xylene	*m*-xylene	*p*-xylene	MTBE
Molecular weight [g/mol]	78.1	92.1	106.2	106.2	106.2	106.2	88.2
Specific gravity	0.88	0.87	0.87	0.88	0.86	0.87	0.74
Boiling point [°C]	80.1	110.6	136.3	144.4	139.3	137–138	53.6–55.2
Water solubility [mg/L]	1780	535	161	175	146	156	43 000–54 300
Vapor pressure [mm Hg] @ 25 °C	76–95	28	9.53	6.6	8.3	8.7	245–256
Log K_{oc}^{a}	1.5–2.1	1.6–2.2	2.0–3.0	1.7–1.8	2.2–3.2	2.1–3.1	1.0–1.1
Log K_{ow}^{b}	1.6–2.2	2.1–2.8	3.2	2.8–3.1	3.2	3.1–3.3	1.2

[a] K_{oc} is the organic carbon based partition coefficient.
[a] K_{ow} is the octanol water partition coefficient.
Adapted from Howard (1991), Mackay & Shiu (1981), Mackay et al. (1992), Merck Index (1989), Verschueren (1996) and Zogorski et al. (1997).

on benzene or *o*-xylene was severely retarded by low concentrations (0.15 mg/L) of benzene (Koenigsberg et al. 1999).

In studies with a pure culture PM1, MTBE and benzene were each utilized as the sole source of carbon and energy (Deeb et al. 2000b). PM1 was not shown to degrade ethylbenzene and the xylenes at concentrations of 20 mg/L and the concurrent presence of these compounds in mixtures with MTBE completely inhibited the biodegradation of MTBE. Substrate interactions during the degradation of mixtures of 20 mg/L benzene and 20 mg/L MTBE by MTBE-grown cells were particularly distinctive. The degradation of MTBE proceeded until benzene started to degrade. Following a lag phase of approximately four hours, benzene degradation proceeded rapidly. At this point, the rate of MTBE degradation slowed significantly until all of the benzene was degraded after which the rate of MTBE degradation increased to previous levels. The lag in benzene degradation was presumably due to the induction of the enzymes necessary for its degradation. However, once the necessary enzymes were induced, sequential additions of benzene or toluene were rapidly degraded. Benzene-grown cells of PM1 degraded toluene without a lag phase whereas MTBE was degraded after a long induction period (15 hours). Results from this study suggest that MTBE and benzene degradation by PM1 takes place by two independently-induced monooxygenase-initiated pathways (Deeb et al. 2000a).

The impact of toluene on MTBE degradation was also evaluated in studies conducted with a pilot-scale biofilter that was inoculated with an MTBE-degrading consortium (Eweis et al. 1997 & 1998). After this biofilter had been treating a 30 to 40 ppm MTBE-contaminated air stream for 180 days, 8 to 25 ppm toluene were added to the MTBE-laden influent. The biofilter acclimated quickly and was able to fully remove both compounds. However, when the concentration of toluene was increased to 70 ppm, MTBE and toluene removal efficiencies dropped to less than 70% presumably due to nitrogen limitations at the higher carbon loading rate. It is unclear from this study which organisms within the consortium were responsible for the degradation of MTBE and toluene, and whether they shared a common enzymatic pathway.

In studies by Church et al. (1999), the biodegradation of MTBE was evaluated in laboratory columns packed with aquifer material from four different sites. MTBE was shown to degrade under aerobic conditions but only in the absence of BTEX compounds. The authors of this study suggested that the degradation of MTBE is not likely to occur in the presence of significant levels of readily degradable hydrocarbons. Observations from a number of field studies support the results of this study. For example, at a site in Michigan where the activity of aquifer mi-

croorganisms was stimulated using Oxygen Releasing Compounds (ORC), MTBE degradation was shown to occur but only after BTEX concentrations were significantly reduced (Koenigsberg et al. 1999). Similar trends of MTBE and BTEX disappearance were observed at other MTBE-impacted sites where ORC were used. In laboratory studies conducted in parallel with the above-mentioned field studies, the presence of xylene together with MTBE caused a 52% reduction in MTBE degradation.

The results from the above studies suggest that the bioattenuation rates of BTEX compounds might not be significantly affected by the concurrent presence of MTBE in contaminant plumes. However, it is possible that the bioattenuation of MTBE could be inhibited by BTEX compounds due to BTEX preferential utilization or to the depletion of electron acceptors and nutrients. Therefore, it is likely that even in the presence of indigenous microorganisms capable of degrading MTBE, the rate of MTBE degradation could be delayed if more easily degradable contaminants are present. Clearly, controlled field studies are needed to elucidate MTBE degradation potential in co-contaminant plumes.

Current and future research directions

As was described in previous sections, several cultures from diverse environments have been shown to efficiently degrade MTBE in shake flask experiments under controlled laboratory conditions. In addition, the successful removal of MTBE from contaminated fluids has been demonstrated in a number of pilot and field scale fixed-film or suspended-growth bioreactors. While in some instances MTBE was shown to degrade in laboratory aquifer microcosms and at gasoline-contaminated sites, there are no compelling indications that the bioattenuation of MTBE is occurring at significant rates in subsurface environments. As a result, biological removal of MTBE has yet to be widely applied in the field. In order to increase the feasibility of using *in situ* bioremediation as a treatment technology for MTBE, an active engineering approach can be implemented to enhance and optimize MTBE biodegradation processes. Engineered *in situ* bioremediation involves the stimulation of microorganisms within a subsurface aquifer to degrade contaminants of concern by active manipulation of physical, chemical or biological conditions. In most cases, stimulation of microbial populations requires the addition of electron acceptors, inorganic nutrients, or a primary substrate if the contaminant of concern is not a good source of carbon and energy (NRC 1993). In some cases, the addition of exogenous microorganisms could be needed to promote contaminant destruction.

Several studies have examined the effect of environmental conditions, particularly dissolved oxygen, in addition to temperature and pH, on the activity of MTBE-degraders. In the absence of oxygen, MTBE has been shown to be relatively resistant to microbial attack with some exceptions (Hurt et al. 1999; Yeh & Novak 1995). In the presence of oxygen, decreasing activities of MTBE-degraders have been shown to be strongly correlated to reductions in dissolved oxygen concentrations. This phenomenon has been observed in laboratory studies (Park & Cowan 1997a, b; Fortin & Deshusses 1999a, b; Koenigsberg et al. 1999; Yang et al. 1998), in pilot scale bioreactors (Sun et al. 1996; Tang & Sun 1997) and in field trials at gasoline-contaminated sites (Javanmardian & Glasser 1997; Salanitro et al. 1999). In studies by Park and Cowan (1997a, b) and Steffan et al. (1997), 10 to 15 °C increases in temperature induced up to four-fold increases in MTBE degradation rates in addition to optimizing growth rates and cellular yields of MTBE-degrading cultures. When investigated, the activity of MTBE-degrading cultures was also found to be a strong function of pH with decreasing activities outside the neutral pH range (Eweis et al. 1997).

The results from these studies are useful for developing strategies to apply engineered bioremediation for the treatment of MTBE-contaminated aquifers. To begin with, it is clear that the presence of oxygen is critical for the degradation of MTBE by most of the examined cultures. Therefore, addition of oxygen to the aquifer would be beneficial especially in the vicinity of gasoline release sources where rapid consumption of oxygen is typically observed. Next, if the indigenous microbial populations do not possess the ability to utilize MTBE as a primary substrate, a cometabolic substrate can be introduced to groundwater aquifers in order to support microbial growth and to provide energy. Several compounds, including propane, *iso*-propanol and *n*-butane, are documented cometabolic substrates for MTBE biodegradation (Hardison et al. 1997; Steffan et al. 1997). However, some studies have suggested that even when indigenous MTBE-degrading cultures are present in subsurface environments, their concentrations could be too low to sustain MTBE metabolism

(Salanitro et al. 1998). Therefore, aquifer seeding (bioaugmentation) with laboratory enriched MTBE-degrading populations could be necessary in some cases to promote MTBE degradation at levels required to adequately mitigate contaminant migration. As a result, several field studies are currently being conducted to investigate the feasibility of bioaugmentation at gasoline-contaminated sites. Preliminary results from a bioaugmentation study conducted at Port Hueneme, CA, using a mixed culture (MC-100) revealed that oxygen-amended biobarriers seeded with the laboratory culture were more effective than unseeded ones for degrading MTBE (Salanitro et al. 1999). In a second field trial, a pure bacterial culture (PM1) and oxygen are currently being injected into the MTBE-contaminated aquifer at Port Hueneme. MTBE degradation readily occurred when aquifer microcosms with sediment samples from this site were inoculated with PM1 suggesting that this site would be a good candidate for bioaugmentation (Hanson et al. 1999). Results from these studies suggest that bioaugmentation may be a promising *in situ* technology for the removal of MTBE from groundwater.

In conclusion, contrary to early reports of MTBE recalcitrance, a review of recent research on the biodegradability of MTBE suggests that MTBE is indeed degradable by a wide range of different microorganisms. Therefore, it is likely that an aerobic bioremediation strategy involving direct metabolism, cometabolism, bioaugmentation, or some combination thereof, could be applied as a feasible and cost-effective treatment method for MTBE contamination. However, a better understanding of the factors limiting MTBE biodegradation in the environment are needed to effectively design such strategies.

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References

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

Ashworth RA, Howe GB, Mullins ME & Rogers TN (1988) Air-water partitioning coefficients of organics in dilute aqueous solutions. J. Hazard. Mat. 18: 25–36

Backer LC, Egeland GM, Ashley DL, Lawryk NJ, Weisel CP, White MC, Bundy T, Short E & Middaugh JP (1997) Exposure to regular gasoline and ethanol Oxyfuel during refueling in Alaska. Environ. Health Perspect. 105: 850–855

Bernauer U, Amberg A, Scheutzow D & Dekant W (1998) Biotransformation of 12C- and 2–13C-labeled methyl *tert*-butyl ether, ethyl *tert*-butyl ether and *tert*-butyl alcohol in rats: Identification of metabolites in urine by 13C nuclear magnetic resonance. Chem. Res. Toxicol. 11: 651–658

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

Brock TD & Madigan MT (1991) Biology of Microorganisms. Prentice-Hall, Englewood Cliffs, NJ

Brown A, Devinny JS, Davis MK, Browne TE & Rodriguez RA (1997) A review of potential technologies for the treatment of methyl tertiary butyl ether (MTBE) in drinking water. In: Proceedings of the API/NGWA Conference on Petroleum Hydrocarbons and Organic Chemicals in Groundwater: Prevention, Detection and Remediation, 12–14 November, Houston, TX

Cederbaum AI & Cohen G (1980) Oxidative demethylation of *t*-butyl alcohol by rat liver microsomes. Biochem. Biophys. Res. Commun. 97: 730–736

Cederbaum AI, Qureshi A & Cohen G (1983) Production of formaldehyde and acetone by hydroxyl-radical generating systems during the metabolism of tertiary butyl alcohol. Biochem. Pharmacol. 32: 3517–3524

Church CD, Isabelle L, Pankow JF, Rose DL & Tratnyek PG (1997) Method for determination of methyl *tert*-butyl ether and its degradation products in water. Environ. Sci. Technol. 31: 3723–3726

Church CD, Tratnyek P & Scow K (2000) Pathways for the degradation of MTBE and other fuel oxygenates by isolate PM1. Preprints of Extended Abstracts, Am. Chem. Soc. 40: 261–263

Church CD, Tratnyek PJ, Pankow JF, Landmeyer JE, Baehr AL, Thomas MA & Schirmer M (1999) Effects of environmental conditions on MTBE degradation in model column aquifers. In: Proceedings of the Technical Meeting of USGS Toxic Substances Hydrology Program, Vol 3 (pp 93–101). Charleston, SC

Cline PV, Delfino JJ & Rao PS (1991) Partitioning of aromatic constituents into water from gasoline and other complex solvent mixtures. Environ. Sci. Technol. 25: 914–920

Cosmetic Ingredient Review Expert Panel (1989) Final report on the safety assessment of *t*-butyl alcohol. J. Am. Coll. Toxicol. 8: 627–641

Creek DN & Davidson JM (1999). The performance and cost of MTBE remediation technologies. In: Proceedings of the API/NGWA Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection and Remediation Conference, 17–20 November, Houston, TX (pp 560–568)

Daniel R (1995) Doctoral Thesis. North Carolina State University, Raleigh, NC

Deeb RA & Alvarez-Cohen L (2000) Aerobic biotransformation of gasoline aromatics in multi-component mixtures. Biorem. J. 4: 171–179

Deeb RA (1999) Aerobic biotransformation of BTEX compounds and MTBE in contaminant mixtures by enriched and pure cultures. PhD Thesis. University of California at Berkeley, Berkeley, CA

Deeb RA, Hu, Hu HY, Hanson JR, Scow KM & Alvarez-Cohen L (2000a) Substrate interactions in BTEX and MTBE mixtures by an MTBE-degrading isolate. Environmental Science and Technology (in press)

Deeb RA, Nishino S, Spain J, Hu HY, Scow K & Alvarez-Cohen L (2000b) MTBE and benzene biodegradation by a bacterial isolate via two independent monooxygenase-initiated pathways. Preprints of Extended Abstracts. Am. Chem. Soc. 40: 280–282

Environmental Protection Agency (1998) MTBE Fact Sheet: §1 – Overview. EPA 510-F-97-014

Environmental Protection Agency (1998) MTBE Fact Sheet: §2 – Remediation of MTBE contaminated soil and groundwater. EPA 510-F-97-015

Environmental Protection Agency (1998) MTBE Fact Sheet: §3 – Use and distribution of MTBE and ethanol. EPA 510-F-97-016

Eweis J, 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, 8–13 June, Toronto, Canada

Eweis J, Schroeder ED, Chang DP & Scow KM (1998) Biodegradation of MTBE in a pilot scale biofilter. In: Wickramanayake GB & Hinchee, RE (Eds). Proceedings of the First International Conference on Remediation of Chlorinated and Recalcitrant Compounds, 18–21 May, Monterey, CA (pp 341–346). Battelle Press, Columbus, OH

Fayolle F, Le Roux F, Hernandez G & Vandecasteele JP (1999) Mineralization of ethyl *t*-butyl ether by defined mixed cultures. In: Alleman BC & Leeson A (Eds). Proceedings of the Fifth International In Situ and On-Site Bioremediation Symposium, 19–20 April, San Diego, CA, Vol 3 (pp 25–30). Battelle Press, Columbus, OH

Fortin NY & Deshusses MA (1998) Gas phase biotreatment of MTBE. In: Proceedings of the 1998 USC–TRG Conference on Biofiltration, 22–23 October, Los Angeles, CA

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

Fortin NY, Deshusses MA, Eweis JB, Hanson JR, Scow KM, Chang DP & Schroeder ED (1997) Biodegradation of MTBE: kinetics, metabolism of degradation by-products and role of oxygen release compounds. In: Proceedings of the 1997 ACS Pacific Conference on Chemistry and Spectroscopy, 21–25 October

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

Garrett P, Moreau M & Lowry JD (1986) MTBE as a groundwater contaminant. In: Proceedings of the API/NGWA Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection and Remediation Conference

Gerhardt P, Murray RG, Wood WA & Krieg NR (Eds) (1994) Methods for General and Molecular Bacteriology. American Society for Microbiology, Washington, DC

Gibson DT & Subramanian V (1984) Microbial degradation of aromatic hydrocarbons. In: Gibson DT (Ed). Microbial Degradation of Organic Compounds (pp 361–369). Marcel Dekker, New York

Grosjean E, Grosjean D, Gunawardena R & Rasmussen RA (1998) Ambient concentrations of ethanol and methyl *tert*-butyl ether in Porto Allegre, Brazil, March 1996 to April 1997. Environ. Sci. Technol. 32: 736–742

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, Beckenbach EH & Halden RU (1998) An evaluation of MTBE impacts to California groundwater resources. Lawrence Livermore National Laboratory, Environmental Protection Department, Environmental Restoration Division, University of California. UCRL-AR-130897

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

Hickman GT & Novak JT (1989) Relationship between subsurface biodegradation rates and microbial density. Environ. Sci. Technol. 23: 525–532

Hickman GT, Novak JT, Morris MS & Rebhun M (1989) Effects of site variations on subsurface biodegradation potential. J. Water Pollut. Control Fed. 61: 1564–1575

Hong JY, Wang YY, Bondoc FY, Yang CS, Chung S, Lee M & Huang WQ (1997) Rat olfactory displays a high activity in metabolizing methyl *tert*-butyl ether and other gasoline ethers. Fundam. Appl. Toxicol. 40: 205–210

Hong JY, Wang YY, Bondoc FY, Yang CS, Gonzalez F, Pan Z, Cokonis CD, Hu WY & Bao Z (1999) Metabolism of methyl *tert*-butyl ether and other gasoline ethers in mouse liver microsomes lacking cytochrome P450 2E1. Toxicol. Lett. 105: 83–88

Hong JY, Yang CS, Lee M, Wang YY, Huang WQ, Tan Y, Patten CJ & Bondoc FY (1997) Role of cytochromes P450 in the metabolism of methyl *tert*-butyl ether in human livers. Arch. Toxicol. 71: 266–269

Howard PH (1991) Handbook of Environmental Degradation Rates. Lewis Publishers, Chelsea, MI

Hubbard CE, Barker JF, O'Hannesin SF, Vandegriendt M & Gillham RW (1994) Transport and fate of dissolved methanol, methyl-tertiary-butyl-ether, and monoaromatic hydrocarbons in a shallow sand aquifer. API Publication Number 4601

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, 18–21 May, Monterey, CA, Vol 5 (pp 103–108). Battelle Press, Columbus, OH

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, 18–21 May, Monterey, CA, Vol 3 (pp 7–12). Battelle Press, Columbus, OH

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, 18–21 May, Monterey, CA, Vol 3 (pp 321–326). Battelle Press, Columbus, OH

Javanmardian M & Glasser HA (1997) In-situ biodegradation of MTBE using biosparging. Preprints of Extended Abstracts. Am. Chem. Soc. 37: 424–426

Jensen HM & Arvin E (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

Johanson G, Nihlen A & Löf A (1995) Toxicokinetics and acute effects of MTBE and ETBE in male volunteers. Toxicol. Lett. 82–83: 713–718

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, 18–21 May, Monterey, CA, Vol 3 (pp 13–18). Battelle Press, Columbus, OH

Landmeyer J, Pankow J & Church C (1997) Occurrence of MTBE and *tert*-butyl alcohol in a gasoline-contaminated aquifer. In: Proceedings of the 213th ACS National Meeting, Vol 37 (pp 4113–4115). San Francisco, CA,

Landmeyer JE, Chapelle FH, Bradley PM, Pankow JF, Church CD & Tratnyek PG (1998) Fate of MTBE relative to benzene in a gasoline-contaminated aquifer (1993–98). Ground Water Monit. Remediat. 18: 93–102

Lince DP, Wilson LR & Carlson GA (1998) Methyl *tert*-butyl ether (MTBE) contamination in private wells near gasoline stations in upstate New York. Bull. Environ. Contam. Toxicol. 61: 484–488

Mackay D & Shiu EY (1981) Critical review of Henry's constants for chemicals of environmental interest. J. Phys. Chem. Ref. Data 10: 1175–1199

Mackay D, Shiu WY & Ma KC (1992) Illustrated Handbook of Chemical Properties and Environmental Fate for Organic Chemicals, Vol IV: Oxygen, Nitrogen and Sulfur Containing Compounds. Lewis Publishers, Boca Raton, FL

Merck Index (1989). Eleventh edition. Merck and Co., Rahway, NJ

Miller MJ, Ferdinandij ES, Klan M, Andrews LS, Douglas JF & Kneiss JJ (1997) Pharmacokinetics and disposition of methyl *t*-butyl ether in Fischer-344 rats. J. Appl. Toxicol. 17: S3–S12

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

Nakamura DN (1994) MTBE, still the best choice. Hydrocarbon Processing 73: 17

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

Nihlén A, Löf A & Johanson G (1998) Controlled ethyl *tert*-butyl ether (ETBE) exposure of male volunteers. II Acute effects. Toxicol. Sci. 46: 143–150

Nihlén A, Sumner SCJ, Löf A & Johanson J (1999) Urinary metabolites in humans. Chem. Res. Toxicol. 12: 822–830

Nihlén A, Walinder R, Löf A & Johanson G (1998) Experimental exposure to methyl tertiary-butyl ether: II. Acute effects in humans. Toxicol. Appl. Pharmacol. 148: 281–287

Novak JT, Goldsmith, CD, Benoit RE & O'Brien, JH (1985) Biodegradation of methanol and tertiary butyl alcohol in subsurface systems. Water Science Technol. 17: 71–85

Park K & Cowan RM (1997a) Biodegradation of gasoline oxygenates. In: Alleman B & Leeson A (Eds). Proceedings of the Fourth International In Situ and On-Site Bioremediation Syposium, Vol 1 (p 17). Battelle Press, Columbus, OH

Park K & Cowan RM (1997b) Effects of oxygen and temperature on the biodegradation of MTBE. Preprints of Extended Abstracts, Am. Chem. Soc. 37: 421–423

Piel WJ & Thomas RX (1990) Oxygenates for reformulated gasoline. Hydrocarbon Processing 69: 68–73

Poulsen M, Lemon L & Barker JF (1992) Dissolution of monoaromatic hydrocarbons into groundwater from gasoline-oxygenate mixtures. Environ. Sci. Technol. 26: 2483–2489

Reuter JE, Allen BC, Richards RC, Pankow JF, Goldman CR, Scholl RL & Seyfried JS (1998) Concentrations, sources, and fate of the gasoline oxygenate methyl *tert*-butyl ether (MTBE) in a multiple use lake. Environ. Sci. Technol. 32: 3666–3672

Rice DW, Dooher BP, Cullen SJ, Everett LG, Kastenberg WE, Grose RD & Marino MA (1995) Recommendations to improve the cleanup process for California's leaking underground fuel tanks (LUFTS). Lawrence Livermore National Laboratory. UCRL-AR–121762

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

Salanitro J, Spinnler G, Maner P, Wisniewski H. & Johnson, P (1999) Potential for MTBE bioremediation – *in situ* inoculation of specialized cultures. In: Proceedings of the API/NGWA Conference on Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection and Remediation Conference, 17–20 November, Houston, TX

Salanitro JP & Wisniewski HL (1996) Observations on the biodegradation and bioremediation potential of methyl *t*-butyl ether. In: Proceedings of the 17th Annual Meeting of the Society of Environmental Toxicology and Chemistry

Salanitro JP (1993) The role of bioattenuation in the management of aromatic hydrocarbon plumes in aquifers. Ground Water Monit. Remediat. 13: 150–161

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

Salanitro JP, Chou CS, Wisniewski HL & Vipond TE (1998) Perspectives on MTBE biodegradation and the potential for in situ aquifer bioremediation. In: Proceedings of the Southwestern Regional Conference of the National Ground Water Association, MTBE and Perchlorate in Ground Water, 3–4 June, Anaheim, CA

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

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

Schirmer M, Barker JF & Hubbard CE (1998) Delineation and characterization of the Borden MTBE plume: an evaluation of 8 years of natural attenuation processes. API Publication 4668

Schirmer M, Butler BJ, Barker JF, Church CD & Schirmer K (1999) Evaluation of biodegradation and dispersion as natural attenuation processes of MTBE and benzene at the Borden field site. Phys. Chem. Earth (B) 24: 557–560

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

Smith M (1994) The physiology of aromatic hydrocarbon degrading bacteria. In: Ratledge C (Ed). Biochemistry of Microbial Degradation (pp 347–378). Kluwer Academic Publishers, Dordrecht, The Netherlands

Squillace PJ, Pope DA & Price CV(1995) Occurrence of the gasoline additive MTBE in shallow ground water in urban and agricultural areas. USGS Fact Sheet FS-114-95

Squillace PJ, Zogorski JS, Wilber WG & Price CV (1996) Preliminary assessment of the occurrence and possible sources of MTBE in groundwater in the United States, 1993–1994. Environ. Sci. Technol. 30: 1721–1730

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: 4216–4222

Suflita JM & 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: Proceedings of the 51st Purdue Industrial Waste Conference Proceedings (pp 507–524). Ann Arbor Press, 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. API Publication Number 4655

Thomson BM, Finfrock DJ & McHaley CP (1999) Development of permeable barriers for groundwater remediation: air stripping of methyl tertiary-butyl ether (MTBE). J. Environ. Sci. Health, Part A, Environ. Sci. Eng. 34: 263–287

Vainiotalo S, Peltonen Y & Pfaffli P. 1998. MTBE concentrations in ambient air in the vicinity of service stations. Atmos. Environ. 32: 3503–3509

Verschueren K (1996) Handbook of Environmental Data on Organic Chemicals, 3rd edn. Van Nostrand Reinhold, New York

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

Yeh CK & Novak JT (1991) Anaerobic biodegradation of oxygenates in the subsurface. In: Proceedings of the 1991 Petroleum Hydrocarbons and Organic Chemicals in Groundwater: Prevention, Detection and Restoration, 20–22 November, Houston, Texas (pp 427–440)

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

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

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

Zenker MJ, Borden RC & Barlaz MA (1999) Investigation of the intrinsic biodegradation of alkyl and cyclic ether. In: Alleman BC & Leeson A (Eds). Proceedings of the Fifth International In Situ and On-Site Bioremediation Symposium, 19–20 April, San Diego, CA, Vol 1 (pp 165–171). Battelle Press, Columbus, OH

Zogorski J, Morduchowitz A, Baehr A, Bauman B, Conrad D, Drew R, Korte N, Lapham W, Pankow J & Washington E (1997) Fuel oxygenates and water quality coordinated by the interagency oxygenated fuel assessment, Office of Science and Technology Policy, Executive Office of the President, Washington, DC

Zogorski J, Morduchowitz A, Baehr A, Bauman B, Conrad D, Drew R, Korte N, Lapham W, Pankow J & Washington E (1996) Fuel oxygenates and water quality: current understanding of sources, occurrence in natural waters, environmental behavior, fate, and significance. Coordinated by the Interagency Oxygenated Fuel Assessment, Office of Science and Technology Policy, Executive Office of the President, Washington, DC

Zylstra GJ (1994) Molecular analysis of aromatic hydrocarbon degradation. In: Garte SJ (Ed). Molecular Environmental Biology (pp 83–115). Lewis Publishers, Boca Baton, FL