

Summary of Workshop on Biodegradation of MTBE

February 1-3, 2000

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Prepared by:

Eastern Research Group, Inc.
Lexington, MA 02421-3136

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Technology Transfer and Support Division
National Risk Management Research Laboratory
Office of Research and Development
U.S. Environmental Protection Agency
Cincinnati, OH 45268

Notice

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Foreword

The U.S. Environmental Protection Agency is charged by Congress with protecting the Nation's land, air, and water resources. Under a mandate of national environmental laws, the Agency strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, EPA's research program is providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The National Risk Management Research Laboratory is the Agency's center for investigation of technological and management approaches for reducing risks from threats to human health and the environment. The focus of the Laboratory's research program is on methods for the prevention and control of pollution to air, land, water and subsurface resources; protection of water quality in public water systems; remediation of contaminated sites and ground water; and prevention and control of indoor air pollution. The goal of this research effort is to catalyze development and implementation of innovative, cost-effective environmental technologies; develop scientific and engineering information needed by EPA to support regulatory and policy decisions; and provide technical support and information transfer to ensure effective implementation of environmental regulations and strategies.

This publication has been produced as part of the Laboratory's strategic long-term research plan. It is published and made available by EPA's Office of Research and Development to assist the user community and to link researchers with their clients.

E. Timothy Oppelt, Director
National Risk Management Research Laboratory

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1.0 OVERVIEW

A workshop on biodegradation of methyl *tert*-butyl ether (MTBE)-contaminated soils and groundwater was held in Cincinnati, OH, on February 1-3, 2000, and was sponsored by the U.S. Environmental Protection Agency's (EPA) National Risk Management Research Laboratory (NRMRL) and the American Petroleum Institute (API). Researchers in academia, industry, and government agencies were invited to attend and present current research. The goals of the workshop were:

- ! To gain an understanding of the types of MTBE research that various organizations are conducting and of the conclusions that this research is generating.
- ! To identify the remaining research needs on MTBE biodegradability.
- ! To understand what research is being planned for the future and to identify potential opportunities for collaboration.

The following sections present information discussed during the Biodegradation of MTBE Workshop and present a summary of the authors' written and oral presentations. These sections include:

- ! Scope of the problem (Section 2.0)
- ! Current research (Section 3.0)
- ! Research needs (Section 4.0)
- ! Collaborative efforts (Section 5.0).

Numerous presentations are summarized in this report. The presentations referenced throughout the report are those made at the February 2000 workshop.

2.0 SCOPE OF THE PROBLEM

MTBE has become the subject of significant attention in recent years due to public focus on several sites where MTBE plumes are very large and are impacting drinking water sources. The attention has been particularly acute in California where gasoline usage is the highest in the U.S. and the population density and water usage results in increased potential for contaminant migration into drinking water wells. MTBE production and usage in the U.S. has risen steadily since 1982, resulting in potential contamination in many more areas (Bauman, 2000).

The National Water-Quality Assessment Program (NAWQA) of the U.S. Geological Survey (USGS) has assessed the extent of MTBE contamination in the U.S. and the role of non-point MTBE sources on distribution. These studies have shown that MTBE is widely distributed in the hydrosphere, and that significant regional patterns are present. In a national study of 2,948 wells during the period 1985-1995, approximately 20% of mixed well types located in areas using MTBE as the principal fuel oxygenate contained detectable concentrations of MTBE ($>0.2 \mu\text{g/L}$). However, in areas where MTBE use was not widespread, less than 5% of wells contained measurable MTBE concentrations. This general pattern of MTBE distribution in groundwater was confirmed by more localized studies (Chapelle, 2000).

At present, there is inadequate health effects data for the USEPA to set an oral reference dose for MTBE. However, because MTBE has a very unpleasant taste and odor, the EPA has issued an Advisory on MTBE in drinking water of 20-40 Fg/L. Table 1 shows the Standards, Guidelines, and Action Levels as currently set by individual states. Four states have health-based Primary Drinking Water Standards. At the time of this workshop, three states have enforceable guidelines, while twelve more have guidelines, or action levels, in place. The levels range from 5 Fg/L (CA) to 240 Fg/L (MI). The specifics of enforcement are determined by each State (Speth, 2000).

Table 1. MTBE State Drinking Water Regulations (Speth, 2000)

	Concentration <u>Fg/L</u>	<u>Type</u>
Primary Drinking Water Standards		
Maine	35	Health Based
New Jersey	70	Health Based
New York	50	Health Based
South Carolina	20-40	Health Based
Enforceable Guidelines		
California	5	Aesthetically Based
Michigan	240	Health Based
West Virginia	20-40	EPA Advisory
Guideline or Action Level		
Arizona	35	Health Based
California	13	Health Based
Connecticut	70	Health Based
Illinois	70	Health Based
Kansas	20-40	EPA Advisory
Maryland	10	Aesthetically Based
Massachusetts	70	Health Based
New Hampshire	15	Aesthetically Based
Pennsylvania	20-40	EPA Advisory
Rhode Island	20-40	EPA Advisory
Vermont	40	EPA Advisory
Wisconsin	60	Health Based

3.0 CURRENT RESEARCH

This section describes the current research on biodegradation of MTBE as described during the workshop presentations. It has been divided into four general sections including an overview of MTBE biodegradation, enhanced *in situ* bioremediation, natural attenuation, and *ex situ* bioremediation.

3.1 Overview of MTBE Biodegradation

MTBE has been shown to biodegrade under various conditions including aerobic, anaerobic, and cometabolic conditions, however it is not well understood under which geochemical conditions degradation occurs.. A summary of the research in these areas is provided in the following sections.

3.1.1 Aerobic Degradation of MTBE

Several researchers described successful mineralization of MTBE in laboratory-scale research (Cowan, 2000; Morales and Deshusses, 2000; Salanitro, 2000; Scow et al., 2000; Venosa et al., 2000; Suidan et al, 2000). Microorganisms were isolated from a variety of sources, generally from petroleum or chemical plant wastewater bioreactors.

Scow et al. (2000) and Salanitro (2000) have identified pure cultures capable of utilizing MTBE as a sole carbon and energy source. Salanitro (2000) and other researchers (Cowan, 2000; Morales and Deshusses, 2000; Venosa et al., 2000) have also developed microbial consortia capable of mineralizing MTBE under aerobic conditions. Microbial cell yields tend to be lower on MTBE than those observed for aromatic hydrocarbons (0.1-0.2 g cells/g MTBE). In addition, biodegradation rates tend to be slower than those observed for the aromatic hydrocarbons.

The microorganism, bacterial strain PM1, isolated by Scow et al. (2000) was further studied by Church and Tratnyek (2000) to determine the degradation pathway. This study confirmed the mineralization of MTBE by strain PM1 and ascertained that the degradation rates of *tert*-amyl methyl ether (TAME), ethyl *tert*-butyl ether (ETBE), di-isopropyl ether (DIPE), *tert*-

butyl alcohol (TBA), and *tert*-amyl alcohol (TAA) were of the same order of magnitude as the degradation rate of MTBE. Together with a consistency in product formation, these results suggested that similar enzyme systems are responsible for all of the reactions. The proposed aerobic degradation pathway for MTBE is shown in Figure 1.

The degradation pathway shown in Figure 1 contains some hypothesized steps in the pathway. Clearly, aerobic biodegradation of MTBE is demonstrable. Additional research is necessary to clarify the microorganisms involved in the process, factors that impact cell yield and biodegradation rates, and the degradation pathway.

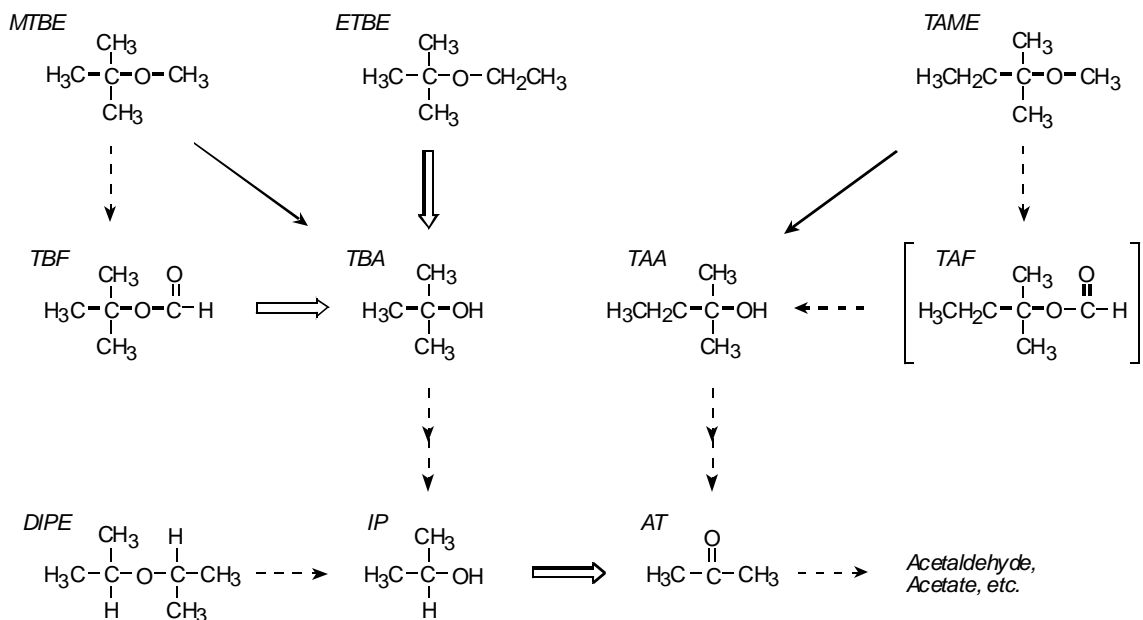


Figure 1. Proposed Degradation Pathway of MTBE by Bacterial strain PM1 (Church and Tratnyek, 2000)

3.1.2 Anaerobic Degradation of MTBE

The majority of researchers have investigated MTBE biodegradation under aerobic conditions, and in fact, Morales and Deshusses (2000) and other researchers were unable to demonstrate any MTBE biodegradation under anaerobic conditions. However, Finneran and Lovley (2000) and Kropp et al. (2000) have demonstrated biodegradation of MTBE under anaerobic conditions.

In the study by Finneran and Lovley (2000), several sediments were investigated for MTBE and TBA biodegradation potential. Results varied among sediments, with the most success occurring when Fe (III) oxide and humic substances were added to the serum bottles. Radiolabeled [14C] MTBE was added during investigation of two of the sediments and conversion to carbon dioxide and methane was observed, although at low levels. TBA was observed to biodegrade much more rapidly than MTBE under iron-reducing and methanogenic conditions. Anaerobic TBA degradation is relatively rapid and extensive. Rates are comparable to those seen for aerobic TBA degradation. Sediment adapted to degrade TBA converts 50% of the added uniformly labeled [C-14] TBA to both [C-14] CO₂ and [C-14] CH₄ in 45 days.

Kropp et al. (2000) conducted a similar study in which sediment slurries were investigated for anaerobic biodegradation of MTBE and other alternative gasoline oxygenates such as methanol, ethanol, and isopropanol as well as several of the ethers such as TAME, ETBE, and DIPE. Kropp et al. (2000) found that the simple alcohols were susceptible to anaerobic biodegradation, but the effect of increased branching, as seen with TBA, was increased recalcitrance to anaerobic decay. This same observation (that increased branching tends to cause recalcitrance to anaerobic decay) was also seen with MTBE and its isomer butyl methyl ether. In general, while Kropp et al. (2000) found definite evidence for anaerobic degradation of MTBE and other ether oxygenates under methanogenic conditions, the phenomenon was not widespread. Kropp et al. concluded that MTBE should be considered as a compound for which anaerobic biodegradation is extremely difficult.

Information on the pathway of anaerobic MTBE has not yet been investigated. Investigation of the anaerobic biodegradation of MTBE is still in the early stages and more research is necessary to fully understand this process.

3.1.3 Microbial Cometabolism of MTBE

Hyman (2000) provided a review of microbial cometabolism of MTBE. A summary of this review is provided in this section.

Several aerobic microorganisms, including bacteria and fungi, have been identified that are capable of cometabolically-degrading MTBE. There are also several primary substrates that have been identified that can be used to stimulate MTBE biodegradation, including alkanes, aromatics, and cyclic compounds. In general, MTBE cometabolism appears to be associated most strongly with microorganisms that grow aerobically on the short chain alkanes (<C8).

Of particular interest is the ability of microorganisms, in particular *Mycobacterium vaccae* JOB5, to grow on iso-alkanes. This is of interest for two reasons:

- ! Hyman (2000) hypothesizes that the ability of microorganisms to cometabolically degrade MTBE is consistently found in strains that are predisposed to catabolize structural analogs of MTBE such as simple branched alkanes; and
- ! Simple branched alkanes are important components of gasoline and compounds such as iso-pentane and can represent up to 10% v/v of fresh gasoline. Therefore, gasoline contains many important primary substrates for organisms that are capable of cometabolically oxidizing MTBE. Treatments designed to maximize gasoline hydrocarbon degradation at point sources of gasoline contamination may fortuitously result in the concurrent cometabolic degradation of MTBE.

Current research indicates that the initial step in the aerobic cometabolism of MTBE is catalyzed by oxygenase enzymes, specifically a cytochrome P-450 monooxygenase. While inhibition studies have supported the role of this enzyme in fungal systems, it is less clear that this enzyme plays a key role in bacterial systems. Further research is also required into the substrate range of these oxygenase enzymes. This research is essential to determine the potential effect on MTBE biodegradation caused by inhibition by other substrates.

The pathway for cometabolic MTBE biodegradation requires further study. The production of tert-butyl formate (TBF) prior to TBA accumulation has been demonstrated; however, the remaining steps in the process are largely unknown. It appears likely though that substantial oxidation does occur.

Researchers are currently investigating the potential for cometabolism of MTBE at the field-scale. Stringfellow (2000) has demonstrated successful stimulation of MTBE biodegradation

with iso-pentane as a primary substrate in laboratory-scale bioreactors. Field applications of iso-pentane-degrading bacteria are currently being implemented by Stringfellow (2000). Field evidence obtained by Butler et al. (2000) strongly suggests that cometabolism of MTBE was the primary mechanism for MTBE removal from the aquifer.

3.2 Enhanced *In Situ* Bioremediation

The information in this section discusses the enhanced *in situ* bioremediation techniques. This section is divided into bioaugmentation studies and studies in which indigenous microorganisms were stimulated.

3.2.1 Bioaugmentation

Two studies were presented in which MTBE-degrading microbial cultures were introduced into the subsurface (Salanitro, 2000; Scow et al., 2000). Scow et al. (2000) worked with the bacterial strain PM1. The objectives of this study were to determine, both in laboratory and field experiments, if strain PM1 was effective at removal of MTBE from a contaminated groundwater aquifer at the Port Hueneme Naval Facility in Oxnard, CA. Microcosm studies were first conducted to determine whether MTBE biodegradation by strain PM1 would occur in site sediments. MTBE biodegradation was significantly higher in those microcosms inoculated with strain PM1 than in those microcosms without inoculation. Initial concentrations of MTBE were removed within 5 days, and subsequent concentration spikes were removed more rapidly. Nutrient addition appeared to have no impact on biodegradation rates.

The field study was initiated in November 1999 and currently, the system has not operated for a sufficient period of time to determine the effectiveness of the process. The field study consists of two test plots located 610 m downgradient from the source of MTBE. Both plots are aerated using an oxygen generator from which oxygen is injected into seven 20-gallon tanks associated with each plot. Plot A receives only oxygen and Plot B receives oxygen and was inoculated with strain PM1 (density of approximately 10⁹ cells per ml in the final injection solution).

Salanitro (2000) has demonstrated the use of biobarriers, also at the Port Hueneme Naval Facility in Oxnard, CA and at a site in Tahoe City, NV. Salanitro (2000) worked with a mixed culture, MC-100, and examined its MTBE biodegradation potential first in laboratory studies using

site groundwater. MTBE biodegradation was much more rapid when microcosms were inoculated with MC-100 than in uninoculated microcosms. MTBE concentrations of 10 to 12 mg/L were degraded to below detection limits within two weeks. MTBE (70-80 mg/L) and BTEX (45 mg/L) in groundwater with high concentrations of gasoline (700 mg/L) were also completely degraded in microcosms inoculated with MC-100.

The field studies at Port Hueneme consisted of creating three test plots: one with oxygen injection only; one with oxygen injection augmented with MC-100; and one control (no treatment). The experiment was conducted for one year. In the control test plot, no significant decline in MTBE concentrations was observed. In the oxygen-injection-only plot, MTBE degradation appeared to occur after a lag time of approximately 260 days. However, TBA was not degraded in this test plot. In the inoculated test plot, MTBE biodegradation occurred soon after inoculation and was non-detectable after 260 days. TBA was not detected in this test plot. Similar results were obtained at a different field site in Tahoe City.

The results from both of these studies indicate that bioaugmentation has merit and warrants further research. Additional research is needed to verify results and to determine the effectiveness of bioaugmentation under different operating conditions and under different hydrogeologies.

3.2.2 Stimulation of Indigenous Microorganisms

Stimulation of indigenous microorganisms was investigated by Mackay et al. (2000). Laboratory and field experiments were conducted at an MTBE plume at Vandenberg Air Force Base, CA. Microcosm studies with site sediments suggested that native aerobic MTBE-degrading bacteria were present in the site sediments and could be stimulated to degrade MTBE solely by adding oxygen (Wilson et al., 1999). In two separate field tests, dissolved oxygen was released into the MTBE plume by diffusion through the walls of tubing pressurized with oxygen and in contact with the groundwater flowing through unpumped well screens or permeable walls. Upgradient concentrations of MTBE ranged from 100-400 µg/L. In both field tests, significant reductions in MTBE concentrations (<5-100 µg/L) were measured downgradient of the diffusive oxygen release systems in repeated sampling events, suggesting that oxygen release led to stimulation of *in situ* biodegradation of MTBE. Appearance of TBA also indicated the activity of MTBE-degrading microorganisms.

Results from this research indicate that oxygen addition alone may be sufficient at some sites to effect the biodegradation of MTBE. Further research is needed to optimize the process and to determine factors that may affect the process.

3.3 Natural Attenuation Several field analyses of natural attenuation of MTBE were presented. Some of the studies showed that natural attenuation of MTBE was possible, but the degree of attenuation varied greatly from site to site. Evidence of biodegradation in groundwater was demonstrated by Borden (2000), Butler et al. (2000), Landmeyer (2000), and Wilson (2000); and by Baehr et al. (2000) in the vadose zone. In contrast, Hunter (2000) and Weaver (2000) found no evidence of biodegradation; however, both of these studies were conducted in areas with high groundwater velocity and, at Weaver's sites, high recharge rates. Happel et al. (2000) presented preliminary results that also indicated fairly slow attenuation of MTBE as compared to BTEX (two orders of magnitude lower).

Related to natural attenuation is the development of a new method for monitoring petrochemical biodegradation as described by Mills and Haines (2000). In this method, the isotopic composition of biodegradation products was analyzed. This method may allow the differentiation between degradation of gasoline components, MTBE, and natural organic matter, thereby offering the potential for more conclusive evidence of MTBE biodegradation.

Borden (2000) described an extensive three-dimensional field characterization that was conducted to define the horizontal and vertical distribution of BTEX, MTBE, and indicator parameters in a shallow coastal plain aquifer. Field-scale degradation rates were highest near the source and declined further downgradient. Laboratory microcosm studies conducted under aerobic and denitrifying conditions showed an identical pattern of biodegradation with high biodegradation rates near the source and lower rates further downgradient. Mathematical modeling studies using BIOPLUME II and a three-dimensional analytical solution showed that: 1) the field data could not be adequately fitted using a spatially uniform first-order decay rate; and 2) use of a spatial uniform first-order decay rate would substantially underestimate contaminant concentrations and risks to downgradient receptors. Therefore, while biodegradation was occurring in the aquifer, current models were inadequate to predict MTBE natural attenuation accurately.

Schirmer et al. (2000) conducted a natural gradient experiment in the Borden Aquifer, CFB Borden, Ontario. MTBE was injected in 1988 in a 2,800 L slug at a concentration of 270 mg/L plus 19 mg/L BTEX and 515 mg/L Cl. It was found that approximately 3% of the initial MTBE mass remained after eight years. MTBE was found where expected based on modeling, but it was found sporadically and at concentrations much lower than predicted. The nature of the aquifer, the characteristics of the contaminant, and the fact that the slug was introduced 1.5 m below the water table, indicated that the processes of sorption, abiotic degradation, and volatilization were not significant contributors to the observed MTBE attenuation (Butler et al., 2000). The most likely explanation appears to be biodegradation. Additional laboratory studies by Butler et al. (2000) demonstrated biologically-catalyzed MTBE degradation in the Borden aquifer; however, this result appeared to be incidental and difficult to predict. Cometabolism was easily initiated in laboratory microcosms and this may be the more likely mechanism for MTBE biodegradation in the Borden aquifer.

Landmeyer (2000) conducted a study of the fate of MTBE in anaerobic aquifer sediments. Very little biodegradation was observed under anaerobic conditions over a 7-month period. However, recent evidence indicates that complete degradation of MTBE to carbon dioxide is possible under mixed anaerobic/aerobic conditions, such as those present where anaerobic groundwater discharges to aerobic surface waters. Other field evidence indicates significant uptake of MTBE by oak trees.

Wilson (2000) is in the process of conducting a survey of existing underground storage tank (UST) sites in association with BP/Amoco. Groundwater samples were analyzed for MTBE, TBA, BTEX, naphthalene, methane, iron (II), total organic carbon (TOC), oxygen, sulfate, and sulfide. Results are still being analyzed, but methane concentration doesn't appear to explain TBA or MTBE concentration. There is generally more TBA than MTBE, possibly as a biodegradation product from MTBE or possibly due to the higher solubility of TBA.

Baehr et al. (2000) conducted a study investigating the concentration of MTBE measured in the unsaturated zone. Concentrations indicated that degradation of MTBE in the unsaturated zone in southern NJ is sufficient to eliminate the atmosphere as a viable source of MTBE present in shallow ground water. This may have some implication on natural attenuation at gasoline-spill sites. Degradation of BTEX compounds within the capillary zone has been shown to greatly enhance the transport of BTEX mass from the water table to the unsaturated zone due to

volatilization and upward diffusive transport, resulting in a significant natural attenuation pathway. Given that MTBE is degraded in the vadose zone, a similar pathway may exist for MTBE natural attenuation.

Hunter (2000) presented data on a small gasoline spill (7-12 gallons of reformulated gasoline [RFG]) that contaminated bedrock drinking water wells. MTBE concentration in the reformulated gasoline was estimated to be 11% by volume. Contaminated soil was removed and households were provided with point-of-entry filtration. Otherwise, no other remedial efforts were employed. Initial MTBE concentrations were approximately 6,500 µg/L. Within two years, all wells were below the 35 µg/L health standard. It is believed that removal was due to rapid groundwater flow and dispersion rather than biodegradation.

Weaver (2000) characterized four plumes on Long Island, NY. The aquifers all had high groundwater velocities and recharge rates. In general, the MTBE plumes were thousands of feet long. All plumes were documented to “dive” into the aquifer possibly due to recharge. Inadequate site characterization would have missed the plumes if groundwater monitoring well screens were only screened across the water table.

Happel et al. (2000) conducted an analysis of compliance data from over 500 Leaking Underground Fuel Tanks (LUFT) sites in CA. Approximately 7,000 sampling events were conducted on these 500 wells. Approximately 50% of the sampling events detected MTBE. Preliminary data indicate that MTBE attenuated at a rate two orders of magnitude lower than BTEX.

These studies illustrate both the potential of MTBE natural attenuation as well as the inadequacy of natural attenuation. Natural attenuation of MTBE is highly sensitive to site characteristics and may simply not be feasible at some sites. In addition, Borden (2000) demonstrated that existing models are not adequate to predict MTBE natural attenuation and may significantly underestimate the plume size over time. In particular, these studies illustrate the need for additional research into the factors that influence natural attenuation.

3.4 *Ex Situ* Bioremediation

Several researchers are investigating the potential for *ex situ* bioremediation of MTBE. *Ex situ* bioremediation of MTBE could be applied as part of a pump-and-treat approach for

remediation of contaminated groundwater, or it may be used as part of the treatment train for drinking water. The majority of the investigations that were presented are currently at the laboratory-scale; however, Chang et al. (2000) presented data from a pilot-scale test.

Cowan et al. (2000) examined the kinetics of an MTBE-degrading microbial culture. The microbial growth rate was slower than for most heterotrophs, with maximum specific growth rates ranging from 0.017 to 0.057 h⁻¹ at 30°C. Consequently, the low growth rates limited the types of bioreactors that could be used for water treatment. Reactors that were examined included a sequencing batch reactor (SBR), a submerged attached growth air-lift (SAGAL), and a cyclically operated submerged attached growth bioreactor (COSAG). With the SBR, effluent concentrations were sustained at <20 µg/L; however, two shocks occurred to the system during the experiment and recovery times were quite long (1.5-2 months). The SAGAL reactor performed well, also sustaining effluent MTBE concentrations <20 µg/L. Variations in reactor temperature impacted the reactor performance. The COSAG bioreactor is currently in operation and data is currently being evaluated; however, results to date are promising, with no MTBE detected in the reactor effluent at a hydraulic residence time of 4.5 hours.

Venosa et al. (2000) described results from four bioreactors operated for over one year to determine MTBE biodegradation under different substrate/co-substrate conditions. The reactors used were porous pot reactors. The reactor conditions were as follows:

- ! influent MTBE concentration of 150 mg/L with MTBE the only organic carbon source;
- ! influent MTBE concentration of 75 mg/L with ethanol also added at a concentration of 75 mg/L;
- ! influent MTBE concentration of 75 mg/L with diethyl ether also added at a concentration of 75 mg/L; and
- ! influent MTBE concentration of 75 mg/L with diisopropyl ether also added at a concentration of 75 mg/L.

Results showed that at high biomass concentrations, MTBE was biodegraded in the presence or absence of other carbon sources. Mineralization of MTBE occurred, as confirmed through chemical oxygen demand (COD) and carbon analysis. Little loss occurred from the control reactor, confirming system integrity. This bioreactor design was useful for laboratory

situations, but would probably be ineffective in the field due to limited flow rates. Future research includes pilot-scale evaluations using commercially available membrane bioreactors.

In a related study by Suidan et al. (2000), the kinetics of MTBE biodegradation of cultures developed in the Venosa et al. (2000) studies were examined. Studies were conducted in batch reactors and several parameters were investigated including MTBE, TBA, total and inorganic carbon, dissolved oxygen, pH, and gaseous carbon dioxide and oxygen. MTBE was mineralized to 1 F g/L within 24 hours with initial concentrations at 5, 15 and 40 mg/L. Results indicated that biotransformation of TBA was the rate-limiting step in the mineralization of MTBE. In addition, the presence of ethanol competed with TBA biodegradation, but not MTBE degradation.

Morales and Deshusses (2000) conducted microcosm and column studies with an MTBE-degrading consortium. Biodegradation was only observed under aerobic conditions and in the presence of the MTBE-degrading consortium. No degradation was observed under anaerobic conditions or with indigenous microorganisms. In the presence of the consortium, complete degradation of 20-25 mg/L MTBE was observed in approximately 10 days. The degradation rate decreased with successive MTBE spikes, possibly due to toxic levels of nitrite. Column studies were operated for 6 months with an approximate 100% MTBE removal efficiency for loadings of 0.25 - 3 g/m³-h for columns packed with soil and 0.25 - 3 g/m³-h for columns packed with perlite. Microcosm studies showed 70% conversion of MTBE to carbon dioxide, with a lower conversion in columns. A new treatment technique Deshusses called “pump and trickle” where groundwater is brought to the surface and reinjected in an infiltration trench seeded with MTBE degrading micro-organisms was proposed.

Chang et al. (2000) described the use of a mixed culture and bacterial strain PM1 in a biotrickling filter unit used to treat MTBE-contaminated groundwater. The biotrickling filter unit consisted of seven granular activated carbon (GAC) packed-bed columns with a diameter of 14 inches and a depth of six ft. Start-up and operation of the columns was not steady, but shut down periods were unrelated to problems with the biotrickling filter. One column was inoculated with the strain PM-1 from a pure culture grown on ethanol. Influent concentrations of MTBE ranged from 290-460 µg/L, with removal efficiencies of greater than 90%.

A pilot-scale, compost-based biofilter for treatment of MTBE vapor also has been investigated. To date, the removal efficiency approaches 100% for loading rates less than about 300 g/m³-d.

4.0 RESEARCH NEEDS

Historically, there has been concern regarding the recalcitrance of MTBE. While all researchers presented data and agreed upon the biodegradability of MTBE, it was apparent that additional research was necessary to more fully understand both the basic microbiology of MTBE biodegradation, as well as develop effective technologies for remediation of MTBE-contaminated groundwater and soils and to understand the environmental conditions under which MTBE is degraded. It is critically important that this research on MTBE biodegradation be conducted to improve the understanding and performance of MTBE remedial technologies.

The following areas appear key for further research into the microbiology of MTBE biodegradation:

- ! The influence of various environmental parameters on MTBE biodegradation, including geochemical factors and temperature should be investigated.
- ! The effect of BTEX on MTBE biodegradation in moderate to low BTEX concentrations and high BTEX concentrations needs to be understood.
- ! The by-products of MTBE biodegradation, such as TBA, should be studied since they are often detected at sites.
- ! A better understanding of the cause for low growth rates and low cell yields on MTBE should be developed. Adequate biomass must be maintained for efficient degradation of MTBE.
- ! Given that evidence has been shown for MTBE biodegradation under aerobic, anaerobic, and cometabolic conditions, identification of the microorganisms involved in these processes may provide a link between research conducted in different laboratories.

- ! Likewise, given the variety of conditions under which MTBE biodegradation has been observed, more research is needed on the mechanism of MTBE biodegradation, including pathways and regulators of MTBE metabolism under aerobic, anaerobic, or cometabolic conditions. This may have an impact on *ex situ* bioreactor performance and provide information on the potential for and predictability of *in situ* bioactivity.

Prior to implementing MTBE remedial technologies, it is also apparent that there needs to be a better understanding of the scope of the problem nationwide and the state-of-the-art for treatment technologies. These research needs are summarized as follows:

- ! Develop a database containing information on MTBE-contaminated sites nationwide representing various environmental conditions. Site data should ideally include contaminant concentrations and distribution, geochemical data, and hydrogeological information. The EPA and BP/Amoco have formed a collaborative effort to obtain this information from a number of petroleum-industry sites. Additional input from other sources would be beneficial.
- ! Assess ability of various technologies to achieve different target levels and associated costs to achieve the target level.
- ! Develop a database of technologies that are at pilot- or full-scale and may work for MTBE. As much cost and performance data as possible should be included.

Detailed suggestions for research needs on specific technologies were discussed during the workshop. The technologies under discussion could be broadly categorized by monitored natural attenuation, enhanced *in situ* treatment, and *ex situ* treatment. In addition, a number of research needs were apparent in the area of site characterization. In the following paragraphs, the research needs for these specific areas are discussed.

Site Characterization. Site characterization is a critical component of the site cleanup. If site characterization is not adequately performed, site cleanup may not be achieved and serious health and environmental impacts could occur later. Conventional site characterization strategies that have been implemented at BTEX-contaminated sites may not be adequate to delineate the MTBE plume or to identify and quantify MTBE biodegradation indicators. The following research needs have been identified:

- ! Sites must be more comprehensively characterized. Plumes may be deeper and longer than expected.
- ! Source mass should be better characterized since this impacts treatment.
- ! Understand the effect recharge has on the downward movement of an MTBE plume.
- ! Guidance in the form of a protocol should be developed on the proper site characterization methods and analytical methods.

Monitored Natural Attenuation. Monitored natural attenuation may be applicable under specific site conditions; however, a significant amount of research is still needed to fully understand the processes that impact natural attenuation of MTBE. The following research needs have been identified:

- ! Determine data needs beyond those obtained for BTEX assessments. Microbiological studies may help determine these data needs.
- ! Screen a large number of sites to better understand how prevalent MTBE biodegradation is and how significantly MTBE biodegradation contributes to natural attenuation of MTBE. This is also necessary to determine *in situ* MTBE biodegradation rates.
- ! Determine specific site conditions conducive to or inhibiting biodegradation of MTBE. This data could come from a combination of microbiological studies and assessment of a database of site data.
- ! Understand the role of groundwater/surface water interfaces.

- ! Develop a protocol for conducting natural attenuation assessments of MTBE.

Enhanced In Situ Biodegradation. Enhanced *in situ* biodegradation is being investigated in the field and promising results have been demonstrated. Additional research needs are as follows:

Conduct additional pilot-scale field trials and assess the following parameters:

- ! Life cycle costs and reliability
- ! Achievable degradation rates
- ! Biomass required and maintained
- ! Electron acceptor delivery methods
- ! Adequate methods to evaluate performance
- ! Development of techniques for effective electron acceptor delivery
- ! Study of enhanced *in situ* MTBE biodegradation under a variety of conditions including different hydrogeological conditions, different contaminant concentrations, and mixed contaminant systems
- ! Development of aggressive source area technologies. It is unknown whether enhanced biodegradation will be effective for residual nonaqueous phase liquids.
- ! Compilation of case studies of enhanced *in situ* bioremediation to find determinants of success or failure
- ! Development of techniques for determining the presence of MTBE-degrading bacteria and identify what factors may be limiting their activity. Microbiological studies would provide information to assist in this determination
- ! Development of protocols for conducting and monitoring *in situ* MTBE bioremediation technologies

Ex Situ Bioremediation. *Ex situ* bioremediation techniques have shown successful biodegradation of MTBE under a variety of conditions. In addition to some additional research at the laboratory-scale level, there are several areas of research to be explored at the field-scale. Additional research needs are as follows:

Conduct pilot-scale field trials and assess the following parameters:

- ! Life cycle costs and reliability
- ! Achievable degradation rates
- ! Biomass required and maintained
- ! Adequate methods to evaluate performance
- ! Long term performance data with shock loadings and other operational performance requirements
- ! Mechanisms and processes to control degradation in aboveground water treatment reactors
- ! Reactor performance and costs under different influent conditions, including varying MTBE concentrations, loadings, and mixed contaminants
- ! Existing GAC systems for biological activity and evaluate efficacy and cost of inoculating existing GAC reactors with MTBE-degrading cultures
- ! Biotreatment as cost competitive compared to existing technologies such as GAC treatment
- ! State-of-the-practice database providing operational information of various reactor types
- ! Protocols for conducting and evaluating *ex situ* bioremediation of MTBE

Overall. A combination of technologies is likely to be the most appropriate choice for site remediation. As such, it is important to examine the best treatment train technologies that bring MTBE concentrations to low levels (i.e. thermal destructive technologies combined with air sparging or SVE followed

by biodegradation). This is an important area of research since a treatment train approach may likely be necessary at many sites.

5.0 COLLABORATIVE EFFORTS

The need for collaborative efforts into investigating bioremediation of MTBE became evident during the workshop. Different research groups have different strengths, and combining these strengths would bring the most powerful approach to solving the problem of MTBE contamination. A work group comprised of government agencies (e.g., EPA and USGS), industry representatives (e.g., the American Petroleum Institute), and academia would be the most productive. Additional suggestions are as follows:

1. Many different areas of expertise were evident during the workshop. These can be grouped into three broad categories: microbiology, bioreactor design, and field expertise. The team could include a combination of these areas of expertise. The microbiology of MTBE bioremediation is not fully understood and researchers working with MTBE-degrading microbial consortia or those examining field biodegradation would benefit from the input from microbiologists. Likewise, researchers involved with bioreactor design and implementation could create a strong team if working with researchers with significant field experience.
2. Lead organizations should be aware of the need to create this combination of experts when developing new programs. The best way to create this awareness is through widespread dissemination of current research and existing research needs.
3. In order to disseminate the current information on MTBE bioremediation, workshops designed for lead organizations could be developed. Government agencies with experience conducting these types of workshops could collaborate with various experts in the field of MTBE bioremediation.
4. An organization is needed that would take the lead on disseminating information on MTBE remediation. A combination of government agencies, industry, and academia would provide an appropriate forum for this activity.

6.0 LITERATURE CITED

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- Hyman, M. 2000. Microbial Cometabolism of MTBE. Presented at the MTBE Biodegradation Workshop, Cincinnati, OH, February 1-3.
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- Speth, T. 2000. Drinking Water Issues. Presented at the MTBE Biodegradation Workshop, Cincinnati, OH, February 1-3.
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- Wilson, R.D. et al. 1999. Laboratory-Scale Evaluation of *In Situ* Aerobic MTBE Biodegradation Options for Vandenberg Air Force Base, CA. *Proceedings of the Conference "Petroleum Hydrocarbons and Organic Chemicals in Ground Water: Prevention, Detection and Remediation"*, cosponsored by the API and NGWA, November 17-19, Houston, TX.

Attachment A

MTBE Biodegradation Workshop Agenda

MTBE Biodegradation Workshop

Marriott Kingsgate Conference Center
Cincinnati, Ohio
February 1–3, 2000

Agenda

Meeting Goals

Ben Blaney, US EPA

OVERVIEW OF ISSUES

MTBE and Underground Storage Tanks

Sammy Ng, US EPA

EPA's ORD Current and Future Research on MTBE Bioremediation

Fran Kremer and Stephen Schmelling, U.S. EPA

MTBE Biodegradation: API and Industry Perspectives

Bruce Bauman API

The Distribution and Environmental Fate of MTBE in the Hydrosphere: The Approach of the U.S. Geological Survey

Francis Chapelle, USGS

EPA Region 9 Perspective on MTBE Response

Steve Linder, US EPA

PRESENTATIONS

In Situ Treatment

Transport and Fate of a BTEX and MTBE Plume: What do we know?

Bob Borden, NCSU

Bioaugmentation of MTBE-Contaminated Groundwater with Bacterial Strain PM1

Doug Mackay, University of Waterloo

Microbial Cometabolism of MTBE

Michael Hyman, North Carolina State University

Anaerobic Biodegradation of MTBE and TBA
Kevin Finneran, University of Massachusetts-Amherst

In Situ Control of MTBE Plumes with Inoculated Biobarriers
Joseph Salanitro, Equilon Enterprises

In Situ Treatment of MTBE by Biostimulation of Native Aerobic Microorganisms
Doug Mackay

Monitored Natural Attenuation

Evaluating Attenuation of MTBE: What we have Learned from LUFT Data and Laboratory Studies

Anne Happel, Lawrence Livermore Laboratory

The Fate of MTBE in the Borden
Aquifer
Barbara Butler, University of Waterloo

MTBE Attenuation Processes: Ambient and Enhanced Redox Conditions, Stream-Bed-Ground-Water Interactions, and Plant Uptake
James Landmeyer, USGS

Field Evidence for MTBE Degradation in the Unsaturated Zone at Low Concentrations
Arthur Baehr, USGS

Natural Attenuation of MTBE in the Subsurface Under Methanogenic Conditions
John Wilson, US EPA

Comparative Evaluation of MTBE Sites on Long Island
Jim Weaver, US EPA

Ex Situ Treatment

Drinking Water Issues
Thomas Speth, US EPA

MTBE Biodegradation: Kinetics, Reactor Engineering, and the Potential for Ex-Situ Treatment of Groundwater
Robert Cowan, Rutgers University

Mineralization of MTBE in Continuous Flow High Biomass Bioreactors
Albert Venosa, US EPA

Kinetics of MTBE Biodegradation
Makram Suidan, University of Cincinnati

Research in Bioremediation of MTBE at UC Riverside: Lessons from Laboratory Experiments
Marc Deshusses, University of California-Riverside

Experience with Laboratory and Field Scale *Ex-Situ* Biodegradation of MTBE
Daniel Chang, University of California-Riverside

POSTERS

The Effects of Ethanol on BTEX Natural Attenuation: Pure Culture and Aquifer Column Experiments

Pedro Alvarez, US EPA

The Borden Field Experiment - Where Has the MTBE Gone? Demonstrating In Situ Remediation - the Borden Aquifer Research Facility

Jim Barker, University of Waterloo

Process Level Investigations of the In Situ Degradation of MTBE

Clinton Church, Oregon Graduate Institute

Research in Bioremediation of MTBE at UC Riverside: Lessons from Laboratory Experiments

Marc Deshusses

Biodegradation of MTBE in Soil Monitored by IRMS

John Haines, US EPA

Natural Attenuation of MTBE at a Site where 24 Bedrock Wells were Contaminated by a 10-Gallon Gasoline Spill

Bruce Hunter, Maine Department of Environmental Protection

Anaerobic Biodegradation of MTBE and Alternative Gasoline Oxygenates

Kevin Kropp, University of Oklahoma

Using Iso-Pentane to Stimulate MTBE Biodegradation In Groundwater Treatment Systems

William Stringfellow, Lawrence Berkely National Laboratory

Structure and Behavior of the MTBE Plume at Port Hueneme, CA

John Wilson

Attachment B

MTBE Biodegradation Workshop Speaker List



MTBE Biodegradation Workshop

Marriott Kingsgate Conference Center

Cincinnati, Ohio

February 1–3, 2000

Speaker List

Arthur Baehr

Water Resources
U.S. Geological Survey
Mount View Office Park
810 Bear Tavern Road
West Trenton, NJ 08628
609-771-3978
Fax: 609-771-3915
E-mail: abaehr@usgs.gov

Michael Barcelona

Research Professor
Department of Civil &
Environmental Engineering
University of Michigan
1221 1st Building
Ann Arbor, MI 45109
734-763-6512
Fax: 734-763-6513
E-mail: mikebar@engin.umich.edu

Bruce Bauman

American Petroleum Institute (API)
1220 L Street, NW
Washington, DC 20005
202-682-8000
E-mail: bauman@api.org

Ben Blaney

Assistant Laboratory Director
for Waste Research
U.S. Environmental Protection Agency
26 West Martin Luther King Drive
Cincinnati, OH 45268
513-569-7852
Fax: 513-569-7680
E-mail: blaney.ben@epa.gov

Bob Borden

Department of Civil Engineering
North Carolina State University
Box 7908
Raleigh, NC 27695-7908
919-515-1625
Fax: 919-515-7908
E-mail: rcborden@eos.ncsu.edu

Barbara Butler

Research Associate & Adjunct
Assistant Professor
Department of Biology
University of Waterloo
200 University Avenue
Waterloo, ON N21 3G1
CANADA
519-885-1211
Fax: 519-746-0614
E-mail: bjbutler@uwaterloo.ca

Daniel Chang

Professor
Department of Civil &
Environmental Engineering
University of California, Davis
One Shields Avenue
Davis, CA 95616-5294
530-752-2537
Fax: 530-752-7872
E-mail: dpchang@ucdavis.edu

Francis Chapelle

U.S. Geological Survey
720 Gracern Road - Suite 129
Columbia, SC 29210
803-750-6116
Fax: 803-750-6181
E-mail: chapelle@usgs.gov

Joan Colson

U.S. Environmental Protection Agency
26 West Martin Luther King Drive (G75)
Cincinnati, OH 45268
513-569-7501
Fax: 513-569-7585
E-mail: colson.joan@epa.gov

Robert Cowan

Department of Environmental Sciences
Rutgers University
14 College Farm Road
New Brunswick, NJ 08901-8551
732-932-8750
Fax: 732-932-8644
E-mail: cowan@envsci.rutgers.edu

Marc Deshusses

Assistant Professor
Department of Chemical and
Environmental Engineering
University of California, Riverside
Bourns Hall B321
Riverside, CA 92521
909-787-2477
Fax: 909-787-2425
E-mail: mdeshuss@engr.ucr.edu

Carl Enfield

U.S. Environmental Protection Agency
26 West Martin Luther King Drive
Cincinnati, OH 45268
513-569-7489
E-mail: enfield.carl@epa.gov

Kevin Finneran

Department of Microbiology
University of Massachusetts - Amherst
Morrill Science Center
Amherst, MA 01003
413-545-9649
Fax: 413-545-1578
E-mail: finneran@microbio.umass.edu

Anne Happel

Lawrence Livermore National Laboratory
700 East Avenue L-542
Livermore, CA 94550-9234
925-422-1425
Fax: 925-423-7998
E-mail: happel1@llnl.gov

Michael Hyman

Department of Microbiology
North Carolina State University
Raleigh, NC 27695-7615
919-515-7814
Fax: 919-515-7867
E-mail: hymanm@mbio.ncsu.edu

Fran Kremer

U.S. Environmental Protection Agency
26 West Martin Luther King Drive (481)
Cincinnati, OH 45268
513-569-7346
E-mail: kremer.fran@epa.gov

James Landmeyer

U.S. Geological Survey
720 Gracern Road - Suite 129

Columbia, SC 29210-7651
803-750-6128
Fax: 803-750-6181
E-mail: jlandmey@usgs.gov

Steve Linder

U.S. Environmental Protection Agency
75 Hawthorne Street (WST-8)
San Francisco, CA 94105
415-744-2036
Fax: 415-744-1026
E-mail: linder.steven@epa.gov

Doug Mackay

University of Waterloo
744 Frenchman's Road
Stanford, CA 94305
650-324-2809
Fax: 650-324-2259
E-mail: d4mackay@uwaterloo.ca

Hugh McKinnon

Associate Lab Director
National Risk Management Risk
Research Laboratory
U.S. Environmental Protection Agency
26 West Martin Luther King Drive (225)
Cincinnati, OH 45268
513-569-7689
E-mail: mckinnon.hugh@epa.gov

Sammy Ng

Acting Director, Office of
Underground Storage Tanks
U.S. Environmental Protection Agency
401 M Street, SW (5401G)
Washington, DC 20460
703-603-9900
Fax: 703-603-0175
E-mail: ng.sammy@epa.gov

Kirk O'Reilly

Senior Environmental Specialist
Chevron Research & Technology

P.O. Box 1627
Richmond, CA 94802-0627
510-242-5365
Fax: 510-242-1954
E-mail: kito@chevron.com

Joseph Salanitro

Equilon Enterprises
3333 South Highway 6 - P.O. Box 1380
Houston, TX 77251-1380
281-544-7552
Fax: 281-544-8727
E-mail: jpsalanitro@equilon.com

Stephen Schmelling

National Risk Management
Research Laboratory
U.S. Environmental Protection Agency
919 Kerr Research Drive - P.O. Box 1198
Ada, OK 74821
580-436-8540
Fax: 580-436-8581
E-mail: schmelling.steve@epa.gov

Thomas Speth

U.S. Environmental Protection Agency
26 West Martin Luther King Drive (B24)
Cincinnati, OH 45268
513-569-7208
E-mail: speth.thomas@epamail.epa.gov

Makram Suidan

Water Quality Processes Program
Department of Civil and
Environmental Engineering
University of Cincinnati
P.O. Box 210071
Cincinnati, OH 45221-0071
513-556-3695
Fax: 513-556-4003
E-mail: makram.suidan@uc.edu

Albert Venosa

U.S. Environmental Protection Agency
26 West Martin Luther King Drive
Cincinnati, OH 45268

513-569-7668
Fax: 513-569-7105
E-mail: venosa.albert@epa.gov

Jim Weaver

National Exposure Research Laboratory
Ecosystems Research Division
U.S. Environmental Protection Agency
960 College Station Road
Athens, GA 30605-2700
706-355-8329
Fax: 706-355-8302
E-mail: weaver.jim@epa.gov

John Wilson

Research Microbiologist
Office of Reserch and Development
National Risk Management
Research Laboratory
U.S. Environmental Protection Agency
Kerr Research Laboratory
919 Kerr Research Drive
Ada, OK 74820
580-436-8534
Fax: 580-436-8703
E-mail: wilson.johnt@epa.gov

Attachment C

MTBE Biodegradation Workshop Poster Presenters



MTBE Biodegradation Workshop

Marriott Kingsgate Conference Center
Cincinnati, Ohio
February 1–3, 2000

Poster Presenter List

Pedro Alvarez

Civil and Environmental Engineering
University of Iowa
4116 Seamans Center
Iowa City, IA 52242-1527
319-335-5065
Fax: 319-335-5660
E-mail: pedro-alvarez@uiowa.edu

Jim Barker

Department of Earth Sciences
University of Waterloo
200 University Avenue
Waterloo, ON N2L 3G1
Canada
519-885-1211
Fax: 519-746-7484
E-mail: barker@cgrnserc.uwaterloo.ca

Clinton Church

Oregon Graduate Institute
20000 Northwest Walker Road
Beaverton, OR 97006
503-690-1651
Fax: 503-690-1273
E-mail: church@ese.ogi.edu

Marc Deshusses

Assistant Professor
Department of Chemical and Environmental
Engineering
University of California, Riverside
Bourns Hall B321
Riverside, CA 92521
909-787-2477
Fax: 909-787-242
E-mail: mdeshuss@engr.ucr.edu

John Haines

U.S. Environmental Protection Agency
26 West Martin Luther King Drive (420)
Cincinnati, OH 45268
513-569-7446
E-mail: haines.john@epa.gov

Bruce Hunter

Hydrogeologist
Maine Department of Environmental
Protection
17 State House Station
Augusta, ME 04333-0017
207-287-7672
Fax: 207-287-7826
E-mail:
bruce.e.hunter@state.me.us

Kevin Kropp

Post Doctoral Research Associate
Department of Botany & Microbiology
University of Oklahoma
770 Van Vleet Oval
Norman, OK 73019
405-325-3771
Fax: 405-325-7619
E-mail: kevinkropp@ou.edu

William Stringfellow

Research Engineer
Center for Environmental Biotechnology
Lawrence Berkeley National Laboratory
(MS-70A-3317)
Berkeley, CA 94720
510-486-7903
Fax: 510-486-7152
E-mail: wstringfellow@lbl.gov

John Wilson

Research Microbiologist
U.S. Environmental Protection Agency
National Risk Management Research
Laboratory
Kerr Research Laboratory
919 Kerr Research Drive
Ada, OK 74820
580-436-8534
Fax: 580-436-8703
E-mail: wilson.johnt@epa.gov

Attachment D

MTBE Biodegradation Workshop List of Attendees



United States
Environmental Protection
Agency
Office of Research and
Development



MTBE Biodegradation Workshop

**Marriott Kingsgate Conference
Center
Cincinnati, Ohio
February 1–3, 2000**

Attendee List

Steven Acree

Office of Research & Development
Subsurface Protection & Remediation Division
U.S. Environmental Protection Agency
Robert Kerr Environmental Research Center
P.O. Box 1198
Ada, OK 74821-1198
580-436-8609
E-mail: acree.steven@epa.gov

404-562-9464
Fax: 404-562-9439
E-mail: ariail.david@epa.gov

Gilberto Alvarez

Environmental Engineer
U.S. Environmental Protection Agency
77 West Jackson Boulevard (DU-7J)
Chicago, IL 60604-3507
312-886-6143
Fax: 312-353-3159
E-mail: alvarez.gilberto@epa.gov

John Brophy

Office of Air and Radiation
U.S. Environmental Protection Agency
401 M Street, SW (6406-J)
Washington, DC 20460
202-564-9068
E-mail: brophy.john@epa.gov

Tom Conrardy

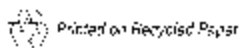
Bureau of Petroleum Storage Systems
Florida Department of Environmental Protection
2600 Blair Stone Road (MS-4530)
Tallahassee, FL 32399-2400
E-mail: tom.conrardy@dep.state.fl.us

David Ariail

Environmental Engineer
U.S. Environmental Protection Agency
Sam Nunn Federal Center
61 Forsyth Street, SW
Atlanta, GA 30303-8960

Linda Fiedler

U.S. Environmental Protection Agency
401 M Street, SW (5102-G)
Washington, DC 20460
703-603-7194
E-mail:
fiedler.linda@epa.gov



Annette Gatchett

U.S. Environmental Protection Agency
26 West Martin Luther King Drive (481)
Cincinnati, OH 45268
513-569-7697
E-mail: gatchett.annette@epa.gov

Joe Haas

Engineering Geologist
New York State Department
of Environmental Conservation
Building 40 - SUNY
Stony Brook, NY 11790-2356
631-444-0332
Fax: 631-444-0373
E-mail: jehaas@gw.dec.state.ny.us

Douglas Heath

Hydrogeologist
U.S. Environmental Protection Agency
One Congress Street - Suite 1100 (CNH)
Boston, MA 02114-2023
617-918-1585
Fax: 617-918-1505
E-mail: heath.doug@epa.gov

Ravi Kolhatkar

BP-Amoco
150 West Warrenville Road (H-7)
Naperville, IL 60563
630-420-3824
Fax: 630-420-5016
E-mail: kolhatrv@bp.com

William Kramer

Principal Hydrogeologist
Handex Environmental
P.O. Box 451
500 Campus Drive
Morganville, NJ 07751
732-536-8667
Fax: 732-536-7751
E-mail: bkramer@handexmail.com

Andrea Leeson

Research Leader
Battelle
505 King Avenue
Columbus, OH 43201
614-424-6424
E-mail: leeson@battelle.org

Ernie Lory

National Environmental
Technology Test Site Manager
U.S. Navy
NFESC - ESC 411
Port Hueneme, CA 93043
805-982-1299
Fax: 805-982-4304
E-mail: loryee@nfesc.navy.mil

Norm Novick

Technology Assessment
and Enhancement Coordination
Exxon Mobil
8280 Willow Oaks II - Room 6W117
3225 Gallows Road
Fairfax, VA 22037
703-849-4968
Fax: 703-849-5217
E-mail: norman_j_novick@email.mobil.com

Rey Rodriguez

Subcommittee Chair, MTBE Partnership
California MTBE Research Partnership
653 Michelle Street
West Covina, CA 91790
626-917-7747
Fax: 626-917-7847
E-mail: mapper3d@aol.com

Laurel Staley

Chief
Treatment & Destruction Branch
U.S. Environmental Protection Agency
26 West Martin Luther King Drive (420)
Cincinnati, OH 45268
513-569-7863
Fax: 513-569-7105
E-mail: staley.laurel@epa.gov

Curt Stanley

Senior Staff Hydrogeologist
Equilon Services
West Hollow Technology Center
P.O. Box 1380
Houston, TX 77251-1380
281-544-7675
Fax: 281-544-8727
E-mail: ccstanley@equilon.com

Hal White

Office of Underground Storage Tanks
Office of Solid Waste and Emergency
Response
U.S. Environmental Protection Agency
401 M Street, SW (5403-G)
Washington, DC 20460
703-603-7177
Fax: 703-603-0175
E-mail: white.hal@epa.gov

Richard Willey

Hydrologist
Region 1
U.S. Environmental Protection Agency
One Congress Street (HBS)
Suite 1100
Boston, MA 02114-2023
617-918-1266
Fax: 617-918-1291
E-mail: willey.dick@epa.gov