Aerobic Biodegradation of MTBE in Uncontaminated and Gasoline-Contaminated Aquifer Sediments

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Aerobic Biodegradation of MTBE in Uncontaminated and Gasoline-Contaminated Aquifer Sediments

by

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(ABSTRACT)

In this study, the biodegradation potential of MTBE in uncontaminated and previously contaminated aquifer sediments under aerobic conditions was investigated. Laboratory microcosms were constructed using aquifer samples collected from three different areas of a shallow gasoline-contaminated aquifer in eastern Fairfax Co., Va in the Atlantic Coastal Plain province. Uncontaminated aquifer samples were collected upgradient of the plume, and contaminated aquifer samples were collected in the source area and in an area downgradient of the source. Biodegradation of MTBE was observed in microcosms that contained previously contaminated aquifer sediments. More complete degradation was observed in aquifer sediments containing a low level of petroleum contamination than in heavily contaminated aquifer sediments. Biodegradation of MTBE appeared to be limited by a lack of oxygen in heavily contaminated soils. When degradation was discernible it appeared to follow a first order pattern with a rate constant (λ) of between 0.037 and 0.066 d^{-1}, following a lag period of 20 to 40 days. In microcosms containing lightly contaminated aquifer material, MTBE was respiked during active metabolism, and degradation occurred with no lag or acclimation period. Results indicated that little or no degradation occurred in the microcosms containing uncontaminated soil. The results of

this research suggest that the availability and level of petroleum hydrocarbon compounds influence indigenous microorganisms capable of degrading MTBE.

Keywords: MTBE, petroleum hydrocarbon compounds, acclimation, biodegradation, microcosm, concentration, indigenous microorganisms

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LITERATURE REVIEW

BACKGROUND

Methyl tert-butyl ether (MTBE) is a gasoline additive (oxygenate) that has been voluntarily used since 1979 to replace lead and increase octane in gasoline. Emission standards imposed by the Clean Air Act Amendements (CAAA) of 1992, are expected to require oxygenates in approximately 70 percent of all gasoline used in the United States by the year 2000 (Shelly and Fouhey, 1994). MTBE is the most commonly used gasoline oxygenate because of its low cost, ease of production, and favorable blending characteristics (Ainsworth 1992; Shelley and Fouhy, 1994). It is normally produced by reacting methanol with isobutylene in the liquid state, using an acidic catalyst at 100 ^{o}C (Figure 2). Manufacturers favor MTBE because it can be produced at the refinery, and the MTBE gasoline blend can be transferred through existing pipelines (Squillance, 1997). Over 10 billion kg of MTBE was used in gasoline in the United States in 1996, and that number is sure to increase over the next few years (USEPA, 1998a).

PROPERTIES OF MTBE

Methyl tertiary-butyl ether (MTBE) is an ether with the chemical formula $C_5H_{12}O$ and the structure shown in Figure 1. Table 1 summarizes the chemical and physical properties of MTBE. Although MTBE is highly volatile in the free-product phase, it has a relatively low Henry's Law constant, which indicates that it partitions readily into water. Of particular environmental significance is the high solubility of MTBE, which allows the compound to dissolve quickly in groundwater and be transported large distances. For this reason, it is pertinent to compare the solubility of MTBE to other compounds in gasoline. Table 2 compares the maximum solubility of MTBE with benzene (the most soluble BTEX compound) in groundwater contaminated with gasoline containing 0, 5, and 10 percent MTBE. For a gasoline 10 % MTBE by weight, the maximum solubility of MTBE is 3650 mg/L, whereas the maximum solubility of benzene is 60 mg/L.

METHODS FOR DETERMINATION OF MTBE

There are several methods that can be used for the detection of MTBE in water. The most reliable methods use purge-and-trap capillary column gas chromatography/mass spectrometry (GC/MS). The GC/MS methods typically provide positive identification of specific constituents in gasoline, eliminating the problem of false identification of coeluting constituents (USEPA, 1998; USGS, 1998). Another method that involves gas chromatography with direct aqueous injection (DAI) onto a polar column, coupled with detection by MS has been described by Church et al. (1997),. The results of the DAI-GC/MS method were in excellent agreement with conventional purge-and-trap methods for MTBE. Sequential purging with static headspace analysis has been proven to be effective in quantifying MTBE and other gasoline compounds (Lacy et al., 1995). Other methods which are useful for the detection of MTBE are GC/ photoionization detector (PID), and GC/flame ionization detector (FID) (USEPA, 1998a).

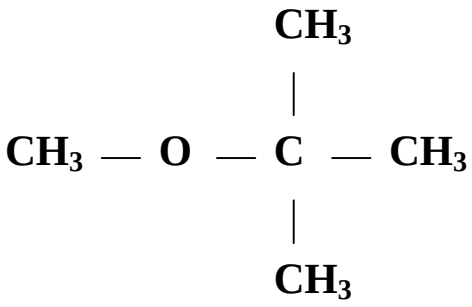

Figure 1. Chemical Structure of Methyl tertiary-butyl ether (MTBE)

TABLE 1. Chemical and Physical Properties of MTBE [a]

Characteristic/Property	***Data / Value***
CAS registry No.	1634-04-4
Molecular Formula	$C_5H_{12}O$
Physical State	Colorless Liquid
Molecular weight (g/mol)	88.15
Melting Point (at 760 mm Hg)	-109 ^oC
Boiling Point (at 760 mm Hg)	55.2 ^oC
Vapor Pressure (mm Hg at 25 ^oC)	245
Vapor Density (air=1)	3.1
Density (g/ml at 25 ^oC)	0.7404
Aqueous Solubility (g/L at 25 ^oC)	51.26
Henry's Law Constant (Atm-m^3) / (g-mole) (Dimensionless)	 0.000528 to 0.003000 0.022 to 0.120
Log K_{oc}	0.55 to 0.91
Log K_{ow}	0.94 to 1.30
Odor Threshold (mg/m^3)	0.32 - 0.47

[a] Values obtained from USEPA (749-F-94-017a), 1994; USEPA (600/R-98-048), 1998

TABLE 2. Maximum solubility of MTBE and Benzene in Groundwater. Water to gasoline ratio is 10 to 1 [a]

Percent MTBE in Gasoline	Solubility of MTBE (mg/l)	Solubility of Benzene (mg/l)
0	NA	65
5	1755	60
10	3650	60

[a] After Bauman, 1997

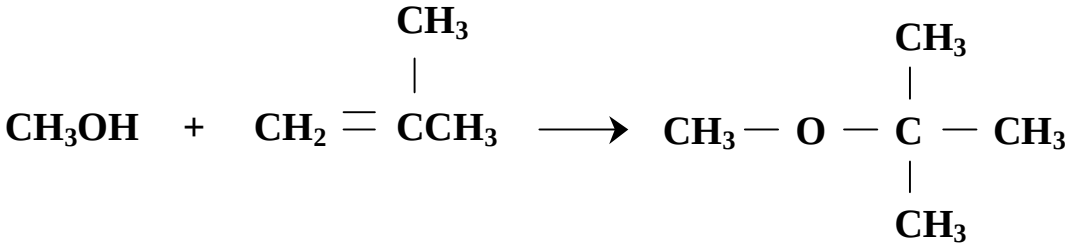

Figure 2. Formation of MTBE through the reaction of methanol and isobutylene

ENVIRONMENTAL SIGNIFICANCE OF MTBE

Occurrence in soil and groundwater

Several instances of localized water contamination by MTBE have been reported since the early 1980's, but the first report to suggest that MTBE contamination may be occurring on a widespread basis was a result of the United States Geological Survey (USGS) National Water Quality Assessment (NAWQA) program (USEPA, 1998a). The NAWQA program was designed to assess the status and trends in the quality of ground and surface waters in the United States, and began sampling in 1993 (USEPA, 1998a). MTBE was the second most frequently detected volatile organic chemical (VOC) in shallow groundwater samples collected in 1993-1994 (Squillance et al. 1996). Of 210 wells and springs sampled, 56 (27%) contained MTBE at a minimum reporting level of 0.2 μg/L (Squillance et al., 1997).

Releases of MTBE into soil and groundwater can occur during manufacture, distribution, storage, and use from point sources such as undergound storage tanks (USTs), above ground storage tanks (ASTs), pipelines, and refueling facilities (USEPA, 1998; Squillance et al., 1997). The highest concentration of MTBE in groundwater occurs from such point sources, and the most common point source is leaking underground storage tanks (LUSTs). Since 1988, 330,000 confirmed releases from regulated USTs have been reported to the EPA, and it is believed that the actual number of LUSTs is much larger (USEPA, 1998a).

Environmental behavior of MTBE

The behavior of MTBE in the subsurface is determined by how it partitions between water, gasoline, and subsurface solids. The high solubility of MTBE (see table 1), combined with its high concentration in gasoline, can result in high concentrations of

MTBE in groundwater contaminated by point sources (Squillance et al., 1997). The water solubility of MTBE from oxygenated gasoline is approximately 50 times greater than that of Benzene, the most soluble BTEX compound (see table 2). When partitioning from the free-phase into the vapor phase, MTBE is 3 times more volatile than benzene. However, the Henry's law constant for MTBE is about ten times less than that for benzene, which means that MTBE is ten time less volatile than benzene when moving from the dissolved phase to the vapor phase (USEPA, 1998b). Dissolved phase concentrations of MTBE near point sources can reach 10,000 to 50,000 μg/l at the extreme end (Odencrantz, 1998).

MTBE has a lower K_{oc} value than BTEX compounds, which means that it is much less likely to sorb to subsurface material (USEPA, 1998b; Squillance et al., 1997; Odencrantz, 1998). For example, for an aquifer containing soil with an organic carbon content of 0.1 percent and a concentration of 3.0 μg/L, it is estimated that approximately 8 percent of the total MTBE present would sorb to aquifer material, whereas about 40 percent of the total benzene would sorb to aquifer material (Squillance et al., 1997). The high solubility, low Henry's law constant, and low K_{oc} value cause MTBE to partition into the aqueous phase, and migrate at the same rate as groundwater. MTBE plumes often migrate well past BTEX plumes, and can reach thousands of feet in length (Landmeyer et al., 1998; Odencrantz, 1998).

Health effects associated with the use of MTBE

Health benefits

The oxygenated fuels program was designed to reduce carbon monoxide emissions, and fight against polluted air. There are numerous health benefits to humans associated with the reduction of carbon monoxide in the urban atmosphere (Andersen, 1993). In a study by Erdal et al. (1996) researchers estimated potential public health benefits from ozone (O_3) pollution reduction attributable to the use of MTBE in gasoline, by comparing O_3 dose-response estimates from the biomedical literature with model

estimates of O_3 reduction. The study concluded that even small MTBE-associated reductions in peak O_3 levels should yield considerable public health benefits.

Detrimental health effects

Most of the research done on the toxicity of MTBE has focused on the effects of inhaled MTBE in laboratory animals and human volunteers. Little research on the effects of ingested MTBE on humans has been performed (USEPA, 1998a). Costantini (1993) reported that MTBE has a relatively low toxicity in rodents, and that no specific target organs were identified except the male rat kidney, and possibly the female rat lung and liver. Costantini did not rule out the possibility that MTBE may be toxic to humans at high doses, due to the lack of data on primates. Mennaer (1997) reported that MTBE induced neopplasms in laboratory rat and mice. However, because of the intense taste and odor of MTBE, humans will not tolerate either air or water concentrations sufficient to induce neoplasms (Mennear, 1997). In a summary of several studies, Stern and Kneiss (1997) reported that MTBE at levels added to gasoline does not pose an added risk to human health.

Despite the numerous studies on laboratory animals that conclude MTBE does not pose a significant health threat to humans, there is some epidemiological evidence that states otherwise. In many areas where MTBE is in use, there have been widespread complaints of non-specific health threats attributed to its presence in gasoline (Balter, 1996). Complaints of headaches, eye irritation, nose and throat irritation, cough, nausea, dizziness and spaciness were recorded in Alaska following the introduction of the oxygenate in the fall of 1992 (Begley, 1993). A study by Moolenaar et al. (1994) tested the blood of workers in Fairbanks, Alaska during the oxygenated fuel program and after the program was suspended. The study concluded that blood MTBE levels were measurably higher during the oxygenated fuel program than after the program was suspended.

A related concern, is the foul tastes and odors MTBE causes in drinking water. MTBE levels that are not considered to be a human health hazard can still produce taste and odor problems in water supplies. Many people can detect its terpentine-like odor as

concentrations as low as 15 μg/L. At 135 μg/L the presence of MTBE is obvious and generally distasteful to most water drinkers (Malinowski, 1998).

Regulatory issues

Currently there are no primary or secondary drinking water regulations for MTBE (USEPA, 1998a). The EPA has issued an advisory to drinking water suppliers regarding the presence of MTBE. In general, EPA advises water suppliers to ensure that MTBE levels do not exceed 20 to 40 μg/L, a level most likely to avert unpleasant taste and odors (Malinowski, 1998). In addition, the EPA has classified the compound as a possible human carcinogen, and has issued a draft lifetime health advisory of 20 to 200 μg/l (USEPA, 1994). The EPA has placed MTBE on the drinking water Contaminant Candidate List (CCL). The CCL is a list of currently unregulated contaminants targeted for consideration for the Agency's drinking water program (USEPA, 1998a). MTBE is also regulated by the Resource Conservation and Recovery Act (RCRA) and the Comprehensive Environmental Response, Compensation, and Liability Act (CERCLA).

REMEDIAL STRATEGIES FOR MTBE

Removal from soil and groundwater

Two strategies that have been listed as successful for remediating MTBE in a survey of 48 state leaking underground fuel tank (LUFT) programs ar soil vapor extraction and air sparging (Stocking, 1999; Hitzig, et al. 1998). Soil vapor extraction (SVE) is commonly used to remove gasoline contaminants from the vadose zone, by extracting vapor from above the unsaturated zone. Air sparging involves the injection of air below the water table, and the subsequent removal of contaminants by air stripping and oxygen-enhanced bioremediation. The low Henry's law constant of MTBE may limit the use of air sparging and SVE, because of the tendency for MTBE to remain dissolved in water. In situ oxidation is a strategy that may be effective in the removal of MTBE from the subsurface. Yeh and Novak (1995) found that hydrogen peroxide in the

presence of iron was able to chemically oxidize MTBE through Fenton's reaction. Chen et al. (1998) and other researchers later confirmed these results. Because MTBE does not readily adsorb to soil, various "pump and treat" methods should also be effective in removing MTBE from groundwater. The potential for in situ bioremediation of MTBE is still being studied, and the results of such studies are discussed in a later section.

Removal from drinking water

Several strategies are being considered for the removal of MTBE from drinking water supplies. Two smaller-scale strategies that are being considered for the point-of-use (POU) residential water treatment industry are granular activated carbon (GAC) filtration and reverse osmosis (RO) (Malinowski, 1998). GAC is generally not considered cost-effective for large-scale removal because of the low adsorption capacity of MTBE (USEPA, 1998a). RO units have been proven reasonably effective, but the size and surface area of the membranes limits it to small-scale removal. Four other strategies are considered more applicable to the removable of MTBE from drinking waters on a larger scale: air-stripping, biological treatment, oxidation, and heated air stripping (Malinowski, 1998). More research needs to be performed before a cost-effective removal process can be determined.

BIODEGRADATION OF MTBE

There is an urgent need to remediate polluted soil and groundwater supplies throughout the United States. Remedial strategies often involve pumping contaminants from the subsurface for aboveground treatment. For many contaminants, in situ bioremediation offers a potentially more effective and economical cleanup through partial or complete destruction of the compounds. For many chemicals such as MTBE, our limited understanding of the factors controlling biotransformation pathways and reaction

rates makes establishing the utility of in situ bioremediation an important scientific and engineering problem (Bouwer, 1992)

Pathways for MTBE biodegradation

It is believed that the biodegradation of MTBE in the aqueous phase is controlled through the cleavage of the ether bond (Yeh, 1992). The most thoroughly studied degradation pathway is atmospheric oxidation, where attack of the hydroxyl radical yields tert-butyl formate (TBF) (Church et al., 1997). In the aqueous phase, accumulation of TBF is not observed because it is readily hydrolized to tert-butyl alcohol (TBA). The most promising indicator of degradation is TBA, because it is (1) common to most pathways, (2) a demonstrated product of biodegradation, and (3) sufficiently resistant to further degradation so that it may accumulate as an intermediate (Church et al., 1997, Yeh and Novak, 1994; Salanitro et al., 1994). The use of TBA as an indicator of in situ biodegradation is limited, because TBA is difficult to measure at low concentrations in water (Church et al., 1997). TBA is subject to further biodegradaton to acetone, 2-propanol, formate, and ultimately CO_2 (Church et al., 1997; Steffan, 1997). Figure 3 presents the initial transformation pathways of MTBE.

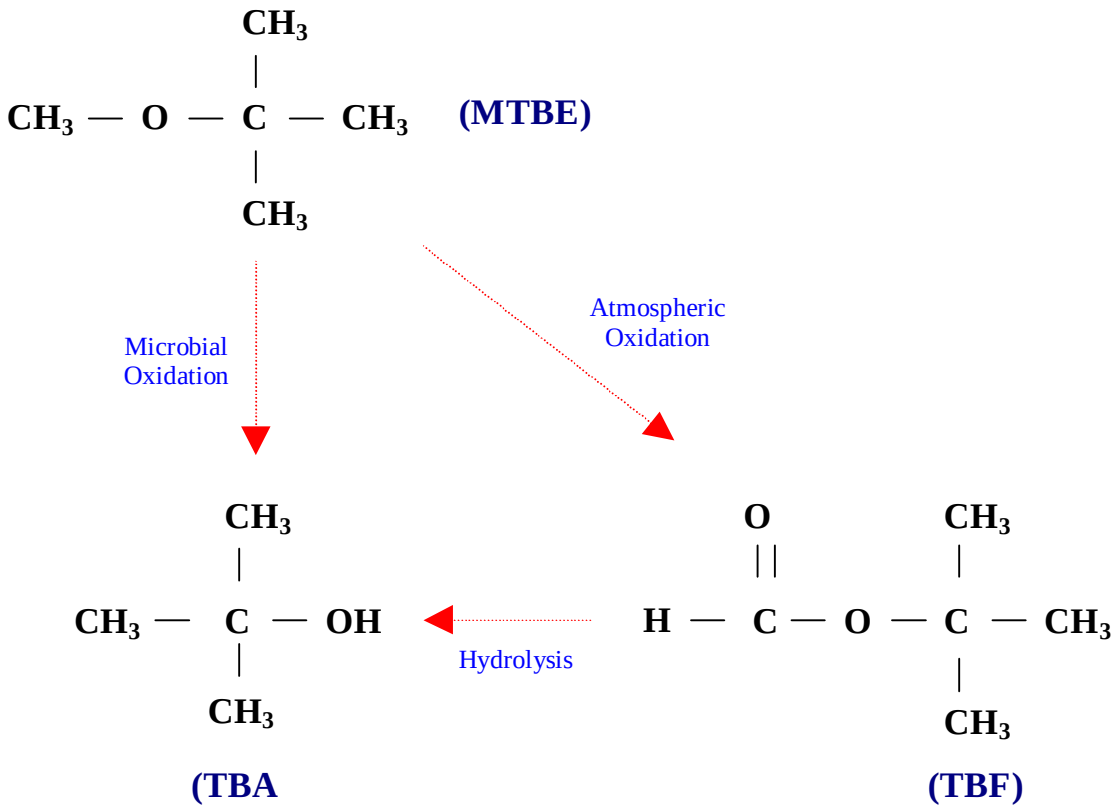

Figure 3. Pathways for initial transformation of MTBE[a]

[a] After Curch et al., (1997)

Biodegradation by pure and mixed enrichment cultures

To date, the most rapid and complete degradation of MTBE has been reported in experiments involving the use of pure or mixed enrichment cultures. Steffan et al. (1997) investigated the ability of propane-oxidizing bacteria to degrade MTBE and other gasoline oxygenates. Both a laboratory strain and natural isolates were able to degrade MTBE after growth on propane. The initial oxidation of MTBE resulted in the nearly stoichiometric production of tert-butyl alcohol (TBA). The methyl group of MTBE was oxidized to formaldehyde and ultimately CO_2. TBA was further oxidized to 2-methyl-2-hydroxy-1-propanol and then 2-hydroxy isobutyric acid. Rates of MTBE degradation by propane-oxidizing strains ranged from 3.9 to 9.2 nmol/min/mg of cell protein.

Horan and Brown (1994) evaluated aerobic biodegradation under aerobic conditions by adding MTBE to carbon-limited microbial consortia. Results indicated increased oxygen consumption when small amounts of MTBE were added as the sole carbon source. However, when MTBE concentration reached approximately 10 ppm, the enrichment culture stopped using oxygen. Researchers were unable to stabilize any microbial consortia with MTBE as the sole carbon source (Horan and Brown, 1994).

Cowan and Park (1997) investigated the aerobic biodegradation of MTBE and other gasoline oxygenates by a set of undefined mixed microbial cultures enriched from a petroleum refinery wastewater activated sludge. Results from monitoring of MTBE concentration and oxygen uptake indicated that MTBE was mineralized as the sole source of carbon and energy by the enriched cultures. Results also indicated the formation of TBA as an intermediate during the biodegradation of MTBE.

Salanitro et al. (1994) was able to isolate a mixed bacterial culture capable of degrading MTBE. The mixed culture named BC-1 was developed from seed microorganisms present in a chemical plant biotreater sludge. The enrichment culture was maintained in continuous culture treating concentrations of MTBE from 120 to 200 mg/L. BC-1 metabolized radiolabeled $^{14}CH_3O$-MTBE to $^{14}CO_2$. Cell suspensions of BC-1 were capable of degrading MTBE to TBA.

Later work by Salanitro et al. (1999) demonstrated enhanced in situ MTBE (EMB) bioremediation at the field scale. This process involved an in situ biobarrier of MTBE-degraders (BC-4), a network of O_2 injection wells near the seeded transect, and an

array of monitoring wells upstream and downstream of the treatment zone. In plots containing O_2 injection, but not seeded with BC-4, some MTBE degradation to the intermediate TBA was observed. Very rapid degradation rates were observed in plots containing BC-4 and O_2 injection. In addition, degradation beyond TBA was seen in plots containing BC-4 and O_2 injection. Researchers also concluded that MTBE degrading organisms must be grown on a specific media, because growth on MTBE would be too slow to maintain a culture in the subsurface (Salanitro et al. 1999).

Cometabolic biodegradation

Several recent studies have addressed the potentiality of cometabolic biodegradation of MTBE. Hyman and O'Reilly (1999) reported that only certain alkane-utilizing microorganisms are capable of oxidizing MTBE. The ability of certain alkane-utilizing bacteria to oxidize MTBE is directly related to the oxygenase enzymes responsible for initiating alkane oxidation in these microorganisms. Researchers concluded that aerobic cometabolic MTBE-degrading activity is most consistently predicted by the ability of microorganisms to grow on simple branched hydrocarbons (Hyman and O'Reilly, 1999).

Hardison et al. (1997) studied the cometabolism of MTBE by a Filamentous Fungus. Results indicated that gaseous n-alkane grown *Graphium* mycelia can cometabolically degrade MTBE. Degradation of MTBE was completely inhibited by acetylene, ethylene, and other unsaturated hydrocarbons and was strongly influenced by n-butane. Two intermediates of MTBE degradation, TBA and tert-butyl formate (TBF) were detected.

Garnier et al. (1999) reported cometabolic biodegradation of MTBE by a microbial consortium enriched from polluted soils. Researchers investigated the effect of the presence of several compounds present in gasoline on the biodegradation of MTBE. Complete substrate utilization for benzene, toluene, and xylenes but no MTBE utilization was observed. Complete mineralization was also found for pentane, hexane, and heptane. Pentane favored the highest MTBE utilization rate (0.20 mg/day). A pentane-degrading organism (*Psuedomonas aeruginosa*) was isolated, and a MTBE degradation rate of 0.530 mg/day was found, suggesting that this was the key organism in the consortium.

Intrinsic biodegradation

The ability of indigenous microorganisms to degrade MTBE in the subsurface is a topic of significant environmental and economic interest. Successful intrinsic bioremediation involves a reduction of risk through stabilization of the plume and partial or complete destruction of the contaminants. An understanding of the processes that effect the natural attenuation of BTEX and MTBE plumes is essential for the design of successful bioremediation strategies. Several studies have addressed the biodegradation of MTBE by naturally occurring microorganisms, under a variety of environmental conditions.

In research performed by Yeh and Novak (1994), anaerobic biodegadation of several gasoline oxygenates was studied in static soil/water microcosms. Results indicated that TBA biodegraded most readily, while MTBE was the most recalcitrant. No degradation of MTBE occurred in unamended soils under anaerobic conditions after an incubation time of 250 days (Yeh and Novak, 1994).

Sufflita and Mormile (1993) studied the anaerobic biodegradation of known and potential gasoline oxygenates in aquifer/groundwater slurries. Results indicated that unlike the alcohol and ester oxygenates, the ether oxygenates were relatively persistent to anaerobic destruction. After an incubation time of at least 182 days, no evidence for the anaerobic destruction of MTBE was obtained. Later studies also attested to the recalcitrance of MTBE under anaerobic conditions, except for the partial destruction of MTBE to tert-butyl alcohol (TBA) (Mormile et al., 1994).

Hurt et al. (1999) presented evidence for anaerobic biodegradation of MTBE in a contaminated aquifer. MTBE was monitored as part of a field-scale, natural attenuation study. Some probe locations showed significantly lower levels of MTBE than in surrounding areas. In addition, the wells containing lower MTBE levels contained higher levels on TBA, a common intermediate. Further, these wells also exhibited high levels of methane, suggesting that MTBE was biodegrading under methanogenic conditions. However, no laboratory results were presented to support the results of the field studies.

Barker et al. (1990) performed a natural gradient tracer test to investigate the influence of methanol and MTBE on the behavior of monoaromatic hydrocarbons in groundwater. Results indicated that MTBE and methanol moved at approximately the same rate as groundwater, while BTEX was somewhat slowed. BTEX compounds were biodegraded when alone, and in the presence of MTBE. Researchers tentatively concluded that MTBE was recalcitrant based on field and laboratory microcosm results (Barker et al., 1990).

Jensen et al. (1990) studied the water solubility and biodegradability of MTBE and gasoline in laboratory batch experiments. Experiments were performed under aerobic conditions in batch reactors containing a sandy aquifer material, topsoil, and activated sludge. Results indicated 100 percent degradation of aromatic hydrocarbons such as BTEX, and 0 percent degradation of MTBE in all three types of inoculation material.

Borden et al. (1997) used a mass flux approach to estimate field scale first-order decay coefficients of MTBE and BTEX compounds in a gasoline-contaminated aquifer. Results indicated rapid loss of toluene and ethyl-benzene, with slower loss of m-, p-, o-xylene, and benzene. In addition, first-order decay coefficients were higher near the contaminant source, than further downgradient. Field monitoring results also indicated MTBE decay near the source, but no degradation was observed downgradient. Effective first-order decay coefficients varied from 0 to 0.001 d^{-1} for MTBE. Field results were supported by aerobic laboratory microcosms that showed limited biodegradation near the source, but no MTBE biodegradation further downgradient.

Landemeyer et al. (1998) studied the fate of MTBE relative to benzene in a gasoline-contaminated aquifer. Field results showed little MTBE degradation, other than the possible transformation to TBA in some wells. Laboratory microcosms containing aquifer material incubated with uniformly labeled ^{14}C-MTBE under aerobic and anaerobic (Fe(III)-reducing) conditions, indicated a low but measurable biodegradation potential (<3% ^{14}C-MTBE as $^{14}CO_2$) after an incubation time of seven months. Researchers concluded that mg/L to µg/L decreases in MTBE concentrations were caused by the natural attenuation processes of dilution and dispersion, rather than biodegradation at that point source gasoline release site (Landmeyer et al., 1998).

Factors limiting biodegradation

There are numerous factors that can effect the biodegradation of organic chemicals in the subsurface including but not limited to: solubility, bioavaiablity, nutrient supply, electron acceptor supply, interactions between multiple substrates, temperature, toxins, and other inhibitors. Hickman et al. (1989) investigated the effects of site variations on subsurface biodegradation potential. Researchers concluded that biodegradation rates differed significantly from site to site. Characteristics of soils with a faster biodegradation rate were higher flux of water and nutrients. Biodegradation rates were also increased by the addition of MoO_4^{2-}.

Some recent research has specifically identified factors that may increase or inhibit MTBE biodegradation. Yeh and Novak (1994) reported that MTBE degradation was inhibited by the addition of readily degradable organic compounds in organically poor soils. Hardison et al. (1997) reported that degradation of MTBE by a filamentous fungus was completely inhibited by acetylene, ethylene, and other unsaturated hydrocarbons.

The presence of oxygen available as an electron acceptor may be an important factor in the biodegradation of MTBE. In a field study be Salanitro et al. (1999), researchers demonstrated that MTBE degradation was higher in regions where oxygen was injected, than in areas where it was not. Park and Cowan (1997) also investigated the effects of oxygen the biodegradation of MTBE. Results indicated that the rate and extent of MTBE degradation was impaired at dissolved oxygen concentrations between 0.3 and 0.9 mg/L. Park and Cowan (1997) also demonstrated that the rate of MTBE degradation increased, with increasing temperature.

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AEROBIC BIODEGRADATION OF MTBE IN UNCONTAMINATED AND GASOLINE-CONTAMINATED AQUIFER SEDIMENTS

by

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ABSTRACT

In this study, the biodegradation potential of MTBE in uncontaminated and previously contaminated aquifer sediments under aerobic conditions was investigated. Laboratory microcosms were constructed using aquifer samples collected from three different areas of a shallow gasoline-contaminated aquifer in eastern Fairfax Co., Va in the Atlantic Coastal Plain province. Uncontaminated aquifer samples were collected upgradient of the plume, and contaminated aquifer samples were collected in the source area and in an area downgradient of the source. Biodegradation of MTBE was observed in microcosms that contained previously contaminated aquifer sediments. More complete degradation was observed in aquifer sediments containing a low level of petroleum contamination than in heavily contaminated aquifer sediments. Biodegradation of MTBE appeared to be limited by a lack of oxygen in heavily contaminated soils. When degradation was discernible it appeared to follow a first order pattern with a rate constant (λ) of between 0.037 and 0.066 d^{-1}, following a lag period of 20 to 40 days. In microcosms containing lightly contaminated aquifer material, MTBE was respiked during active metabolism, and degradation occurred with no lag or acclimation period. Results indicated that little or no degradation occurred in the microcosms containing uncontaminated soil. The results of this research suggest that the availability and level of petroleum hydrocarbon compounds influence indigenous microorganisms capable of degrading MTBE.

Keywords: MTBE, petroleum hydrocarbon compounds, acclimation, biodegradation, microcosm, concentration, indigenous microorganisms

INTRODUCTION

Methyl tert-butyl ether (MTBE) is a gasoline additive (oxygenate) that has been voluntarily used since 1979 to replace lead and increase the octane in gasoline. The Clean Air Act Amendments (CAAA) of 1990 required that gasoline oxygenates be blended into reformulated gasoline (RFG) to reduce carbon monoxide emissions. MTBE is the most commonly used oxygenate because of its low cost, ease of production, and favorable blending characteristics (Ainsworth, 1992; Shelley and Fouhy, 1994). RFG contains from 11 to 15% MTBE by volume, and is most commonly used in areas that do not meet National Ambient Air Quality Standards (Squillance et al., 1996). Today, MTBE is one of the most widely produced and used chemicals in the United States (Malinowski, 1998). As of 1994, 32 areas in a total of 18 states participate in the RFG program, and RFG accounts for over 30 percent of gasoline nationwide. The Oxygenated Fuels Association estimates that 70 % of all gasoline in the United States contains MTBE at varying concentrations (USEPA, 1998).

While MTBE reduces health risk from exposure to carbon monoxide emissions, potential health risks to humans exist from exposure to vapors and MTBE dissolved in water. Complaints of headaches, eye irritation, nose and throat irritation, cough, nausea, dizziness and spaciness from exposure to MTBE vapors were recorded in Alaska following the introduction of the oxygenate in the fall of 1992 (Begley, 1993). Animal studies have shown that MTBE is rapidly absorbed following oral or inhalation exposures (USEPA 1994). MTBE is generally considered safe at low doses, but little concrete information is available regarding human health effects, and more studies are needed (Costantini, 1993). EPA has classified the compound as a possible human carcinogen, and has issued a draft lifetime health advisory of 20 to 200 μg/l (USEPA, 1994). The health advisory is the maximum concentration in drinking water that is not expected to cause any adverse non-carcinogenic effects over a lifetime of exposure (Squillance, 1997). A further issue with MTBE in water is the pungent odor and taste that can be detected at levels as low as 15 μg/l (Malinowski, 1998). For this reason the EPA has issued a drinking water advisory of 20 to 40 μg/l. Some states have recently issued drinking water standards, with others soon to follow. California has issued a public

health limit of 14 ppb of MTBE in drinking water, and Maine and New York have each issued a state drinking water standard of 50 ppb. Maine is one of the first states considering a ban on the use of MTBE in reformulated gasoline.

Environmental releases of MTBE can occur through both point and non-point sources, but the highest concentrations in water are attributed to point sources. Gasoline spills to the land surface and releases from aboveground and underground storage tanks (ASTs and USTs) are the most common examples of point sources. As of July 1994, roughly 22% of the 1.2 million petroleum USTs at 500,000 sites in the United States have been reported to leak. As part of the USGS National Water Quality Assessment Program, MTBE was the second most frequently detected chemical in shallow groundwater samples collected in 1993-1994 (Squillance et al., 1996).

The high solubility of MTBE allows for the rapid dissolution into water and therefore the rapid spread through groundwater systems. The solubility of pure MTBE in water is approximately 51,200 mg/l, whereas benzene (the most soluble BTEX compound) has a maximum solubility of around 1,800 mg/l at 25 °C. For a gasoline containing 15% MTBE by weight, the maximum solubility of MTBE is approximately 5000 mg/l compared to 60 mg/l for benzene. The recurrence of leaking USTs combined with the high solubility of MTBE provides the potential for MTBE contamination at these sites. Dissolved phase concentrations near point sources have been reported to reach 10,000 to 50,000 μg/L (Odencrantz, 1998). In addition, MTBE sorbs very poorly to soil, therefore transport of MTBE plumes will not be significantly retarded as groundwater flows through the aquifer. MTBE has been shown to travel large distances (sometimes over 1000 ft) from the source area (Odencrantz, 1998).

The recent health concerns over MTBE combined with its widespread use and occurrence in groundwater systems has focused increased attention on feasible remediation strategies. Several treatment options are being considered including air stripping, oxidation, carbon filtration, reverse osmosis, soil vapor extraction (SVE) and ex-situ biological treatment (Malinowski, 1998). In situ bioremediation, in which the soil is not removed from the field or groundwater is not pumped for aboveground treatment, offers a potentially more effective and economical clean up technique through partial or complete destruction of the contaminants (Bouwer, 1992; Alexander, 1994).

Several recent studies have addressed the biological fate of MTBE. Most research has suggested that MTBE is recalcitrant, or very slow to degrade under aerobic (Barker et al., 1990; Jensen, 1990; Landmeyer et al., 1998; Zenker et al., 1999) and anaerobic conditions (Suflita and Mormile, 1993; Yeh and Novak, 1994; Landmeyer et al., 1998). However, other recent research has presented evidence for MTBE biodegradation by pure and mixed isolated cultures under aerobic conditions (Mo et al., 1997; Salanitro et al.,1994; Salanitro et al., 1999; Cowan and Park, 1997). MTBE degradation through cometabolism with n-alkanes has also been reported (Hardison et al., 1997; Garnier et al., 1999). Hyman et al., (1999) reported that the only certain alkane-utilizing organisms are capable of oxidizing MTBE. Recent field studies have shown some indication of MTBE biodegradation under anaerobic (methanogenic) conditions (Hurt et al., 1999).

Evidence for intrinsic biodegradation of MTBE in a gasoline contaminated aquifer near the contaminant source, has recently been presented in field and laboratory experiments (Borden et al., 1997). Borden et al. (1997) used a mass flux approach to estimate field scale first-order decay coefficients under mixed aerobic-denitrifying conditions. Mass flux results indicated rapid loss of toluene and ethyl-benzene, with slower loss of m-, p-, o-xylene, and benzene. In addition, first-order decay coefficients were higher near the contaminant source, than further downgradient. Field monitoring results also indicated MTBE biodegradation near the source, but no biodegradation of MTBE was observed downgradient. Field results were supported by aerobic laboratory microcosms that showed limited biodegradation in near-source core microcosms, but no MTBE biodegradation in downgradient core microcosms. The results of Borden et al. (1997) indicate that indigenous aerobic microorganisms are capable of degrading MTBE to some extent, but the factors controlling biodegradation are not well understood.

The potential for intrinsic bioremediation of MTBE is of particular environmental and economic significance, and is the foundation of this study. The primary purpose of this research was to compare the biodegradation potential of indigenous aerobic microorganisms in contaminated and uncontaminated aquifer samples. Experiments were designed based on the hypothesis that aerobic microorganisms capable of degrading MTBE will preferentially utilize readily-degradable petroleum compounds (such as

BTEX) and will later biodegrade MTBE after the readily biodegradable hydrocarbon compounds have been removed. Further, the impact of antecedent conditions on the microbial response of MTBE was evaluated. Three different aquifer sediment samples were obtained from a gasoline-contaminated site: (1) uncontaminated, upgradient of the contaminant source area, (2) contaminated, near the source area, and (3) contaminated, downgradient of the source area. Aquifer material was then used to construct laboratory microcosms in which MTBE biodegradation potential was evaluated for each of the three sampling locations. In addition, first-order biodegradation rates were calculated from microcosm data when applicable.

MATERIALS AND METHODS

Site Description

The study area is in eastern Fairfax County, Virginia , in the Atlantic Coastal Plain province. The geology of the area consists of tertiary and quaternary age upland terrace deposits identified as part of the Bacons Castle Formation which overlies the Potomac Formation (Mixon et al., 1989). Gasoline from underground and aboveground storage tanks has contaminated soil and groundwater at the U.S. Army Garrison Fort Belvoir, VA (Fig. 1). During previous site investigations, several engineering/consulting firms installed a series of monitoring wells. The drilling logs from these wells were used to model the stratigraphy of the site (Law Engineering, 1998a). The stratigraphy of the area is complicated, consisting of fill material and interbedded clay, sand and silt layers. Water levels in these wells were measured, and utilized with drilling logs to determine the local hydrogeology. The hydrogeology is complex consisting of a series of perched aquifers and aquitards. Water level measurements indicate a general flow to the northeast in the uppermost perched aquifer (Fig. 1). Groundwater samples collected from these wells in January/February of 1998 indicated BTEX contamination (Law Engineering, 1998b). The highest levels of BTEX contamination were reported in well W1124-4 (deep) and W1124-5S (shallow) (~2000 μg/L and 600 μg/L total BTEX compounds, respectively). The highest MTBE concentration (88.4 μg/L) was also reported in

W1124-5S (no MTBE data was available for W1124-4). This contamination was expected because of the close proximity of the wells to the source and the general direction of groundwater flow.

Sample Collection

Soil samples were collected in three different locations based on the experimental matrix. Upgradient, source area, and downgradient soil borings are labeled SB-1, MLS-1, and MLS-2 respectively (Fig. 1). Boreholes were drilled until reaching the top of the water table. The depths to the water table for borings SB-1, MLS-1, and MLS-2 were 15, 16, and 28 feet, respectively. Split spoon samples of saturated aquifer material were collected below the water table. Samples were collected in $1^{3}/_{8}$" x 4" stainless steel split tube samplers inserted inside the split spoons. All sampling tubes and equipment were flame sterilized in the field, prior to contact with aquifer material. Sampling tubes were immediately sealed with plastic caps upon removal from the split spoon sampler. These tubes were then sealed inside airtight mason jars, packed on ice, and transported back to the laboratory. All soils were stored at 4 °C in the dark until utilized for analysis or microcosms construction. Multi-level samplers (MLS) were installed at the source-area and downgradient locations (MLS-1 and MLS-2, respectively).

Groundwater Characterization

Groundwater samples were collected from several wells in October, 1998 and analyzed for dissolved oxygen, sulfate, nitrate, ferrous iron, pH, and MTBE. Dissolved oxygen was measured in the field using the azide modification of the Winkler titration method (Hach, 1995). Temperature and pH were measured using a portable electrode probe (Hach, 1995). Fe (II) was measured in the field, using a calorimetric method (Hach, 1995). Sulfate and nitrate were analyzed in the laboratory using a Dionex 2010i ion chromatograph. MTBE and petroleum hydrocarbon contamination were analyzed in the laboratory using headspace gas chromatography (method is identical to that described in Microcosm Construction and Analysis section). The level of petroleum hydrocarbon contamination was quantified by comparing the total chromatographic area to a standard curve created with unleaded gasoline.

All wells contained measurable levels of dissolved oxygen (1.7 – 4.2 mg/L) except W1124-5S (below detection (BDL)), which is located in the vicinity of the source area (Fig. 1 and Table 1). Groundwater collected from W1124-5S contained a significantly lower level of sulfate (~13 mg/L) than other wells (> 20 mg/L), suggesting that sulfate reduction may be occurring. Toluene and ethyl-benzene concentrations in W1124-5S were 0.04 and 0.27 mg/L respectively, compared to less than 0.004 mg/L in other wells. The highest level of MTBE (1.60 mg/L) and petroleum hydrocarbon contamination (22.54 mg/L) was also found in W1124-5S. Groundwater was also collected from the bottom two ports of MLS-1 (depths of 18 and 20 ft) in December of 1998. The level of MTBE in these samples ranged from 1.69 to 2.49 mg/L, which is consistent with the contamination found in the nearby well, W1124-5S.

Characterization of Aquifer Sediments

Aquifer samples were characterized in terms of MTBE contamination, level of petroleum hydrocarbons, moisture content, absorbed water content, total organic carbon, and particle size distribution. Contamination was measured by extracting 5 g of aquifer sample with 10 ml of water to simulate the aquifer sediment/groundwater system. The mass of MTBE was measured using headspace gas chromatography. The mass of MTBE measured is not the total mass in the sample, but the amount available to water in a aquifer sediment/water system. The level of petroleum hydrocarbon contamination was quantified by comparing the total chromatographic area to a standard curve created with unleaded gasoline. The moisture content was determined using an ASTM procedure (ASTM D2216 / ASTM, 1989). A sample was weighed, dried at 110 °C and weighed again. The moisture content (in percent) is the difference in weight between the initial and oven-dried sample divided by initial weight. Absorbed water content was measured using the procedure described by McCloskey (1995). A sample was air dried for 24 hours, weighed, oven dried at 110°C for 24 hours and weighed again. The absorbed water content (in percent) is the difference in weight between the air-dried and oven-dried sample divided by the weight of the air-dried sample. Percentage of total organic carbon (TOC) was measured using the loss of ignition test (McCloskey, 1995). A sample was weighed, dried at 110°C for 24 hours and weighed again. The sample was then baked at

430°C for 24 hours and weighed again. The TOC (in percent) is the difference between the oven-dried and baked sample, divided by the initial weight. Particle size distribution was determined by a standard method using a mechanical sieve analysis and a hydrometer analysis (ASTM D422/ ASTM, 1989).

Microcosm Construction and Analysis

Biodegradation of MTBE by indigenous microorganisms was evaluated in static aquifer sediment/water microcosms. Five grams of aquifer material was aseptically transferred to 20 ml headspace vials, and saturated with 10 ml of deionized water. MTBE and other compounds were added by injecting a known mass of contaminant using a sterilized syringe. All procedures related to microcosm construction were performed at 4 °C to minimize losses due to volatilization. Microcosms were capped with 22 mm aluminum crimp seals containing rubber septa. All microcosms were incubated upside down (to prevent volatilization), in the dark at 20 °C prior to being sampled. Microcosms were hand shaken approximately twice per week to distribute the contaminants and oxygen throughout the system.

The initial concentration of MTBE added to microcosms that contained uncontaminated aquifer material was 5 mg/l. The initial concentration in microcosms containing contaminated aquifer material varied depending on the level of contamination. The objective was to add enough MTBE to raise the concentration to 5 mg/l. This was a difficult task, due to the highly variable levels of contamination throughout the sample. Toluene and ethyl-benzene were added to selected microcosms to evaluate the impact of these compounds on MTBE degradation. Selected microcosms were also amended with nutrients (50 ppm NH_4^+-N and 10 ppm PO_4^{-2}-P), to determine if nutrient addition might enhance MTBE biodegradation. All microcosms were initially aerobic, with ambient DO present in the deionized water, and oxygen transfer also occurring from the headspace into the aqueous phase.

Abiotic controls were prepared for each soil condition, and used to observe losses due to volatilization and sorption. Control microcosms contained soils that were autoclaved 1-2 times per day for at least ten days at 132 °C and 15 psi for 30 minutes.

Autoclaved soil was aseptically transferred to 20 ml headspace vials and autoclaved one more time. Controls were constructed in the same manner as live microcosms. The initial MTBE concentration was 5 mg/l for all controls.

Live microcosms were sacrificed in triplicate using headspace gas chromatography. MTBE and other petroleum compounds were extracted from the headspace using a HP 19395A automatic headspace analyzer. The oil bath temperature in the headspace analyzer was 65 °C. Headspace analysis was performed with a HP 5880 gas chromatograph equipped with a flame ionization detector. MTBE and other compounds were separated on a 2 m x 2 mm glass column packed with 5% SP-1200 / 1.75 % Bentone® on a 100/120 supelcoport support. The carrier gas was Nitrogen (30 ml/min), and the oven temperature was ramped from 60 °C to 123 °C. The injection port temperature was 130°C and the detector temperature was 250 °C. MTBE concentration was quantified by comparing MTBE peak area to a linear standard curve. Petroleum hydrocarbon compounds were quanitified by comparing the total gas chromatographic area to a linear standard curve created with unleaded gasoline. The presence of tert-butyl alcohol (TBA), a common intermediate of MTBE degradation, was also monitored throughout the experiments. Abiotic controls were sacrificed in duplicate using the same procedure as live microcosms. Dissolved oxygen (DO) was periodically measured in selected microcosm sets, using a YSI model 57 DO meter.

Oxygen Utilization

Assuming that the aerobic biodegradation of MTBE is complete to carbon dioxide and water, the stoichiometric equation is:

(MTBE) $C_5H_{12}O + 7.5\ O_2 \rightarrow 5\ CO_2 + 6\ H_20$

O_2 Consumption: 2.72 mg O_2 per mg $C_5H_{12}O$

Based on the approximate volume of headspace (8 mL) in each microcosm, and the solubility of oxygen in water, the approximate mass of oxygen in each microcosm is 2.3 mg. The mass of MTBE in each microcosm is less than 0.05 mg, therefore oxygen

supply should not limit MTBE degradation, when only MTBE is present. When other readily degradable petroleum hydrocarbon compounds are present at a high concentration, oxygen may be completely consumed.

Evaluation of MTBE Degradation

After microcosms were constructed, live and control microcosms were destructively sacrificed, and MTBE concentrations (as well as toluene and ethyl-benzene when applicable) were measured over periods of up to 140 days. The average MTBE concentration and standard deviation of 3 samples (2 samples for controls) was plotted versus time on a log scale. Petroleum hydrocarbons were plotted as relative concentration versus time when applicable. First-order rate constants were applied to the microcosm data where a visible pattern of MTBE degradation was apparent. Results from biodegradation studies often showed or indicated an acclimation phase in which no substrate loss was evident (Alexander, 1994). First-order degradation rate constants were calculated from the slope of the log concentration – time plots, where active degradation was occurring (acclimation phase excluded).

The general first-order equation for loss of substrate is

$$\frac{dC}{dt} = R_{bio} = -\lambda C$$

where t = Time [T]; C = Substrate Concentration [$M L^{-3}$]; R_{bio} = Rate of biological reaction [$M L^{-3} T^{-1}$]; and λ = first-order rate constant [T^{-1}].

RESULTS AND DISCUSSION

Characterization of Aquifer Sediments

Table 2 summarizes the results of the characterization tests for the three different aquifer samples. The objective was to obtain three samples with similar chemical and physical characteristics, but differing levels of contamination. SB-1, MLS-1, and MLS-2 aquifer sediments contained similar percentages of total organic carbon (3.10 – 3.47

%). The upgradient sample (SB-1) contained greater than 70 % clay, while the source area (MLS-1) and downgradient (MLS-2) samples consisted of over 90 % sand. SB-1 aquifer samples contained a moisture content of over 30 %, while MLS-1 and MLS-2 samples contained moisture contents of approximately 14 % and 18 % respectively. SB-1 aquifer samples contained an absorbed water content of over 4.17 %, while MLS-1 and MLS-2 samples contained absorbed water contents of 0.46 % and 0.71 % respectively. The physical and chemical differences between samples can cause variations in biodegradation potential.

The objective was to obtain uncontaminated, petroleum hydrocarbon and MTBE contaminated, and MTBE only contaminated samples for SB-1, MLS-1, and MLS-2 respectively. As expected, sample SB-1 was completely free of MTBE and other petroleum contamination. The near-source aquifer sediments (MLS-1) contained MTBE ranging from 1.06 mg/kg to 56.89 mg/kg and petroleum hydrocarbons from 0.53 mg/kg to 27.37 mg/kg. The downgradient aquifer sediments (MLS-2) contained MTBE ranging from below detection (BDL) to 1.01 mg/kg and petroleum hydrocarbons from BDL to 2.63 mg/kg.. The highly variable levels of petroleum contamination observed in the aquifer sediment characterization tests made it to difficult to distinguish the antecedent contamination between aquifer samples MLS-1 and MLS-2.

Source area microcosms (MLS-1)

Source area microcosms were constructed with aquifer material obtained from MLS-1 soil boring. Upon sacrificing, it was observed that the level of petroleum hydrocarbon contamination was significantly less than the soil used for downgradient (MLS-2) microcosms. MLS-1 microcosms contained petroleum hydrocarbons from BDL to approximatley 2 mg/L. These results are in contrast to the results of the aquifer sediment characterization and groundwater analysis, and can be attributed to the highly variable contamination of the aquifer samples.

One set of MLS-1 microcosms was constructed in which MTBE was added at an initial concentration of 2.5 ppm, with no other petroleum hydrocarbon compounds or nutrients. Fig. 2 is a plot of MTBE concentration versus time for both live microcosms and controls. MTBE concentration in live microcosms began to decline after an

acclimation period of approximately 20 days. After an incubation time of 78 days, the average MTBE concentration fell to below 0.276 ppm. One data point (0.009 mg/L @ t= 68 days) that fell below the scale (< 0.1 ppm) was plotted on the 0.1 ppm axis. The pattern of degradation from t=20 to t=78 days appears to follow first-order kinetics, and the first-order degradation rate constant was calculated to be 0.037 d^{-1} (see Table 3).

At t=80 days, all remaining live microcosms were respiked with MTBE to a level of 5 ppm. The MTBE concentration declined to below 0.192 ppm after an incubation time of 52 days, with no acclimation period. From t=80 to t=132 days, MTBE follows a first-order degradation pattern, and the first-order degradation rate constant was calculated to be 0.063 d^{-1} (see Table 3). The disappearance of the second addition of MTBE occurred more rapidly than the first, with no distinguishable acclimation period. Other biodegradation studies have shown that the loss of a second addition of a chemical occurs faster than the first, with little or no acclimation phase (Alexander, 1994). It is presumed that the pre-existing petroleum hydrocarbons are responsible for acclimatizing MTBE degrading microorganisms. MTBE in the controls remained relatively constant, indicating that little or no biodegradation occurred (Fig. 2).

A second set of microcosms was constructed in which 5 ppm of MTBE was added along with 1 ppm of toluene and ethyl-benzene. The reasons for adding toluene and ethyl-benzene were to determine if the indigenous microorganisms will preferentially utilize toluene and ethyl-benzene, and to determine if the presence of these readily degradable compounds might stimulate MTBE biodegradation.

Fig. 3 is a plot of MTBE concentration versus time for both live microcosms and controls. Toluene and ethyl-benzene rapidly degraded (< 10 days) in both live microcosms and controls. This indicates that we were unable to eradicate growth of toluene and ethyl-benzene degrading microorganisms. Toluene and ethyl-benzene persisted in standards prepared for each sampling event, indicating that these compounds were not lossed to volatilization. Past research has also indicated difficulty in eliminating microbial growth of BTEX-degrading microorganisms in autoclaved controls (Goldsmith, 1985).

There was a large amount of scatter in MTBE concentrations between t=11 and t=33 days, suggesting that degradation and/or abiotic losses may be occurring in some

live microcosms. Following a lag period of approximately 39 days, the average MTBE concentration in live microcosms declined to below 0.174 ppm at t= 81 days. Although there is a significant amount of scatter at t=62 and t=74 days, degradation of MTBE appears to follow first-order kinetics between t=39 and t=81 days. The first-order degradation rate constant between t=39 and t=81 days was calculated to be 0.066 d^{-1}. The longer lag period (see Fig. 2 and 3) may be the result of indigenous microorganisms preferentially utilizing toluene and ethyl-benzene prior to degrading MTBE. The MTBE concentration in controls again remained relatively constant, throughout the entire duration of the experiment (Fig. 3).

Dissolved oxygen (DO) measurements made at the end of the experiments were consistently greater than 3.0 mg/l in all MLS-1 microcosms. This indicates that while oxygen may have been utilized as an electron acceptor, DO levels were never limiting biodegradation. More than likely, the total concentration of organic carbon substrate (MTBE and other petroleum hydrocarbons), was not high enough to significantly deplete the total supply of DO in the system (see Oxygen utilization in Materials and Method section).

Although some intermediate peaks were observed in the gas chromatographic analysis, these peaks were not identified. Measurable levels of tert-butyl alcohol (TBA), a common intermediate for MTBE degradation, were not observed in the gas chromatographic analysis. The use of TBA as an indicator of MTBE degradation is limited, because TBA is difficult to measure at low concentrations in water (Church et al., 1997).

The calculated first-order biodegradation rate constants determined in MLS-1 microcosm experiments ranged from 0.037 to 0.066 d^{-1}, which are significantly higher than those (0 to 0.001 d^{-1}) reported by Borden et al. (1997). Biodegradation of MTBE in MLS-1 microcosms was near complete, from an initial concentration of 2.5 to 5.0 ppm to a final concentration of less than 0.3 ppm. In the study by Borden et al. (1997), MTBE biodegraded from an intitial concentration of 2.1 mg/L to between 1.0 and 1.5 mg/L and then remained constant. One possible reason for the discrepancy is that the rates presented by Borden et al. (1997) were calculated from a mass flux analysis in the field,

rather than from laboratory microcosm data. In addition, site variations can significantly influence biodegradation rates.

Downgradient microcosms (MLS-2)

Two sets of microcosms were constructed using aquifer sediments from the location downgradient of the source (MLS-2). One set of microcosms was constructed in which 5 ppm of MTBE was added with no other petroleum hydrocarbon compounds or nutrients. Fig. 4 is a plot of MTBE concentration versus time for both live microcosms and controls. No substantial decrease in MTBE concentration is apparent after an incubation time of 78 days. The level of petroleum hydrocarbon contamination did not significantly decline either, during the same time period. Dissolved oxygen at t= 72 days was greater than 4 mg/l, indicating that little or no aerobic degradation was occurring. One possible explanation for the high DO levels combined with the persistence of readily degradable compounds is that biodegradation was limited by a lack of nutrients. At t= 78 days all remaining microcosms were amended with nutrients (50 mg/l NH_4^+-N, and 10 PO_4^{-2}-P). From t= 79 to t= 123 days MTBE concentration declined in selected microcosms, but no degradation pattern is apparent.

The same experiment was performed again, except nutrients were added to all microcosms at t= 0 days and dissolved oxygen was measured periodically for 80 days. Fig. 5A and Fig. 5B are plots of the relative concentration (ratio of concentration to initial concentration) of petroleum hydrocarbon compounds and MTBE versus time. Dissolved oxygen concentration in live microcosms and controls is plotted as a vertical bar graph in Fig. 5C.

The initial concentration of petroleum hydrocarbons in the second set of MLS-2 microcosms was approximately 36 mg/L. The data in Fig. 5A illustrates that average petroleum hydrocarbon concentration declined to approximately 30% of the initial concentration after 20 days, but then remained relatively constant. MTBE declined to approximately 2.5 mg/L from an initial concentration of 5 mg/L following a 20 day lag period, but did not decline below this level (Fig. 5A). These results are very similar to

those presented by Borden et al. (1997), in which MTBE biodegraded from an initial concentration of 2.1 mg/L to between 1.0 and 1.5 mg/L and then remained constant.

There is an initial depletion of dissolved oxygen that is likely due to the degradation of readily degradable petroleum hydrocarbons (Fig. 5). After an incubation time of 20 days the relative concentration of petroleum hydrocarbons did not fall below 30 %. The subsequent decline in MTBE concentration appears to coincide with the depletion of the remaining dissolved oxygen.

The approximate loss of MTBE is 0.025 mg, which corresponds to a DO consumption of 0.068 mg (see Oxygen utilization in Materials and Methods section). The decline in dissolved oxygen during the period of MTBE degradation is approximately 2.5 mg/L or a total mass of 0.025 mg (see Fig. 5C). The observed loss of DO is less than the required amount for MTBE biodegradation. However, there was likely some oxygen remaining in the headspace which could have been transferred into the aqueous phase and utilized by the MTBE-degrading microorganisms.

MTBE degradation appears to be limited by a lack of oxygen after an incubation time of approximately 45 days. Dissolved oxygen concentration in live microcosms steadily declined to nearly zero at t=73 days. Several studies have attested to the recalcitrance of MTBE under anaerobic conditions (Suflita and Mormile, 1993; Yeh, 1992; Yeh and Novak, 1994).

There are several possible reasons for the acclimation phase preceding MTBE degradation. The lag phase may be associated with the presence of petroleum hydrocarbon compounds. Petroleum degrading microorganisms may be able to degrade after acclimating to compounds in gasoline such as alkanes and alkenes. These compounds are more structurally similar to MTBE than ring compounds such as BTEX. There may be an inhibitory concentration of petroleum hydrocarbons at which MTBE-degrading microorganisms are not active. The lag phase could also be due to the acclimation of the microorganisms to MTBE itself. There are various other environmental factors that can affect the acclimation of a microbial community to a substrate (Alexander, 1994).

Upgradient microcosms (SB-1)

Three sets of microcosms were constructed using uncontaminated aquifer sediments from the location upgradient of the source (SB-1). One set of microcosms was assembled in which 5 ppm of MTBE was added, with no other petroleum hydrocarbons or nutrients. Fig. 6 is a plot of MTBE concentration versus time for both live microcosms and controls. MTBE concentration in live microcosms declined slightly, to between 3.0 and 4.5 ppm. We consider these losses to be abiotic, since controls also declined to approximately the same level.

A second set of microcosms from SB-1 samples was constructed in which 5 ppm of MTBE was added with 1 ppm of toluene, and 1ppm of ethyl-benzene. Fig. 7 is a plot of MTBE concentration versus time for both live microcosms and controls. Toluene and ethyl-benzene concentrations declined below detection (BDL) with no visible lag period, after an incubation time of less than 20 days. Toluene and ethyl-benzene concentrations also declined BDL in controls, after an incubation time of less than 40 days. There was a large amount of scatter in MTBE concentrations in live microcosms (between 20 and 60 days), suggesting that degradation or abiotic losses may be occurring in selected microcosms. However, overall MTBE concentrations remained relatively constant after an incubation time of 114 days, and no evidence of degradation is discernible. The presence of toluene and ethyl-benzene did not appear to stimulate biodegradation of MTBE. These results are consistent with a study by Jensen et al. (1990) in which complete degradation of BTEX and no degradation of MTBE was observed in previously uncontaminated aquifer material.

A third set of microcosms was constructed in which 5 ppm of MTBE was added with 10 ppm of toluene, and amended with nutrients (50 mg/L NH_4^+-N, and 10 mg/L PO_4^{-2}-P). The purpose of adding nutrients was to determine if degradation was "nutrient limiting" in the previous microcosms constructed with SB-1 samples. A larger concentration of toluene was added to determine if losses in controls were biotic or abiotic. Fig. 8 is a plot of MTBE concentration versus time for both live microcosms and controls. Similar to the response seen in Fig. 7, toluene completely degraded after an incubation time of less than 20 days. Toluene levels in controls also declined BDL after an incubation time of less than 40 days. Once again, MTBE concentrations remained

constant after an incubation time of 104 days, indicating that little or no degradation was occurring. The presence of toluene did not appear to stimulate biodegradation of MTBE. The addition of nutrients also did not stimulate biodegradation of MTBE in microcosms constructed with upgradient (SB-1) samples.

The reasons for the difference in biodegradation potential between uncontaminated and previously contaminated aquifer sediments are not precisely understood. The difference in physical soil characteristics (uncontaminated soil was much higher in clay content) may play an important role. One possible explanation is that MTBE degrading microorganisms are stimulated by the presence of other compounds (alkanes) in gasoline, which they can use as a growth substrate. After acclimatizing to the alkanes, the same microorganisms are then able to use MTBE as an electron donor. Several recent studies have indicated that alkane-oxidizing microorganisms are capable of biodegrading MTBE (Hyman and O'Reilly, 1999; Garnier et al., 1999).

CONCLUSIONS

In this study, static aquifer sediment/water microcosms were used to evaluate the biodegradation potential of MTBE by indigenous aerobic microorganisms. Microcosms were constructed with aquifer material collected from 3 different locations at a gasoline contaminated site: (1) uncontaminated, upgradient of the contaminant source area (SB-1), (2) contaminated, near the source area (MLS-1), and (3) contaminated, downgradient of the source area (MLS-2). The effect of adding readily degradable BTEX compounds (toluene and ethyl-benzene) was studied in microcosms constructed with SB-1 and MLS-1 aquifer samples. First-order biodegradation rate constants were calculated from the slope of MTBE concentration versus time plots, when applicable.

Biodegradation of MTBE was observed in microcosms containing aquifer sediments previously contaminated with gasoline (MLS-1 and MLS-2), but not in microcosms containing uncontaminated aquifer sediments (SB-1). In addition, biodegradation of MTBE was more complete microcosms containing slightly contaminated (< 2 mg/L) aquifer samples (MLS-1), than in microcosms containing

heavily contaminated (~ 36 mg/L) samples (MLS-2). The calculated first-order biodegradation rate constants determined in MLS-1 microcosm experiments ranged from 0.037 to 0.066 d^{-1}. The data indicate that biodegradation of MTBE in microcosms containing MLS-2 aquifer samples, may have been limited by a lack of oxygen available as an electron acceptor. Oxygen was initially depleted through metabolism of readily degradable petroleum hydrocarbon compounds. The depletion of the remaining dissolved oxygen appears to coincide with the decrease in MTBE concentration, until dissolved oxygen levels approached zero and MTBE concentration remained constant. Biodegradation of petroleum hydrocarbon compounds and MTBE also appears to be limited by a lack of nutrients under some conditions.

Toluene and ethyl-benzene rapidly degraded when added to SB-1 and MLS-1 microcosms. The presence of toluene and ethyl-benzene did not appear to stimulate biodegradation of MTBE in either type of soil condition. Biodegradation of MTBE occurred with a longer lag period when toluene and ethyl-benzene were added to MLS-1 microcosms, than when MTBE only was added.

There appear to be several unknown factors that control the biodegradation of MTBE by indigenous microorganisms. The most notable conclusion that can be made is that indigenous microorganisms exist that are capable of biodegrading MTBE under aerobic conditions. Additionally, these organisms proliferate in soils that have been previously contaminated with other petroleum hydrocarbon compounds found in gasoline.

PRACTICAL APPLICATIONS

The use of intrinsic bioremediation may not be practicable for the attenuation of MTBE plumes. The ability of MTBE to be transported further than other compounds in gasoline presents a problem, because biodegradation was only seen in previously contaminated soils. Therefore, microorganisms at the leading edge of the plume will only be exposed to MTBE and degradation will not be accelerated. Furthermore, the lack of oxygen within the gasoline plume will limit MTBE degradation in that area. However,

biodegradation of MTBE could be engineered through injection of oxygen into the gasoline and MTBE contaminated region of the plume.

Air sparging and soil vapor extraction (SVE) are two other strategies that are being considered for the remediation of MTBE plumes. Air sparging, in which air is injected below the water table, removes contaminants through air stripping and also enhances aerobic biodegradation by providing oxygen to the indigenous microorganisms. SVE involves the extraction of vapors from above the unsaturated zone and the removal of contaminants from the vadose zone. The higher vapor pressure, higher solubility, and lower adsorption of MTBE compared to BTEX indicate that air sparging and SVE may work well for remediating MTBE from groundwater. In a survey of 48 state leaking underground fuel tank (LUFT) programs, SVE and air sparging were listed as successful in remediating MTBE contaminated sites (Stocking, 1999; Hitzig et. al, 1998). One limitation may be the low Henry's law constant, indicating that MTBE tends to remain dissolved in water. Another limitation with these strategies is that they may not be feasible for large plumes, because a very immense number of wells would be needed to remediate the entire plume.

The injection of a pure or enriched microbial culture is another strategy receiving increased attention. Cultures such as BC-4 have recently been isolated by Salanitro et al. (1999). In this approach, an in situ biobarrier of MTBE degrading organisms is injected in the subsurface. Oxygen is also supplied through a series of injection wells. Results have indicated successful remediation of MTBE in both field and laboratory experiments. The limitations of bioaugmentation are that the microbes are slow to grow on MTBE in the subsurface, and the process can be very expensive. We are currently in the process of isolating the microorganisms that degrade MTBE. A mathematical model that can simulate intrinsic biodegradation of MTBE in a gasoline contaminated aquifer is also being developed.

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FIGURES AND TABLES

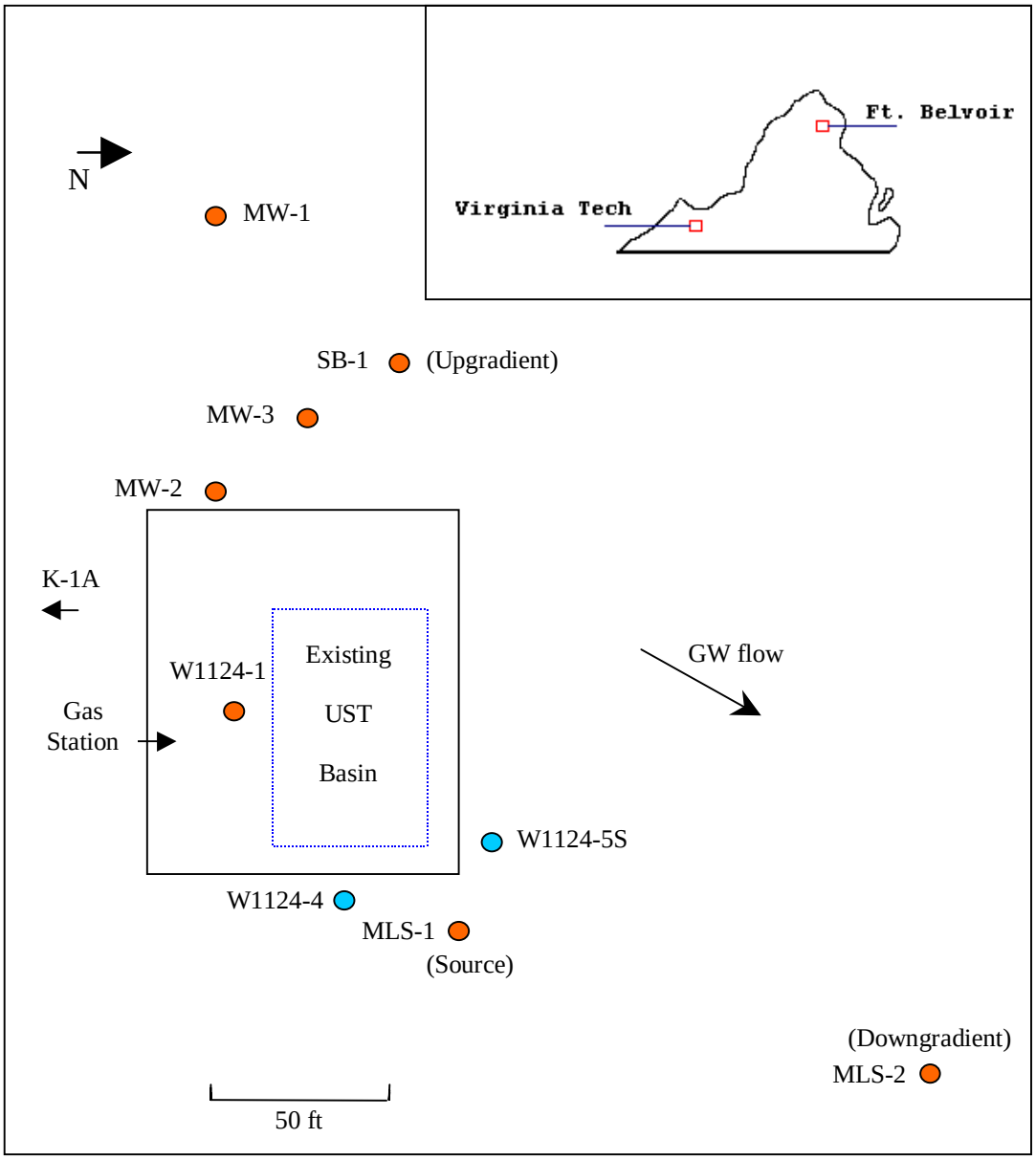


FIG. 1. Location of soil sampling. Upgradient, source area, and downgradient soil borings are labeled SB-1, MLS-1, and MLS-2 respectively.

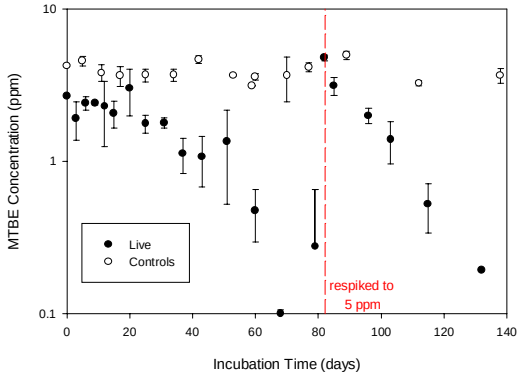


FIG. 2. MTBE concentration in source area (MLS-1) microcosms; only MTBE was added.

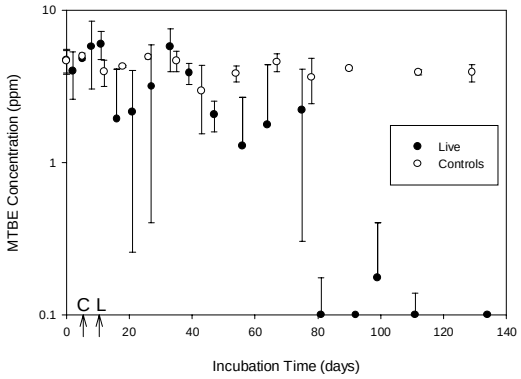


FIG. 3. MTBE concentration in source area (MLS-1) microcosms. Spiked with MTBE, toluene, and ethyl-benzene. (L) represents the disappearance of toluene and ethyl-benzene in live microcosms. (C) represents the disappearance of toluene and ethyl-benzene in control microcosms.

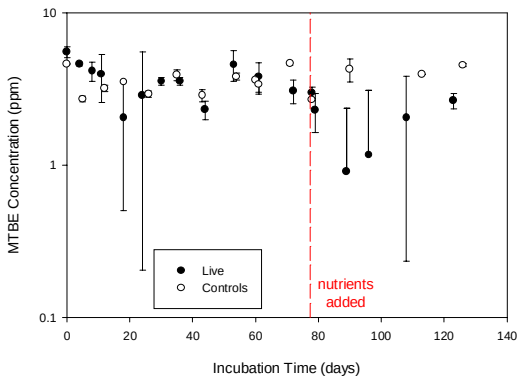


FIG. 4. MTBE concentration in downgradient (MLS-2) microcosms. Initially only MTBE was added; amended with nutrients at t= 78 days.

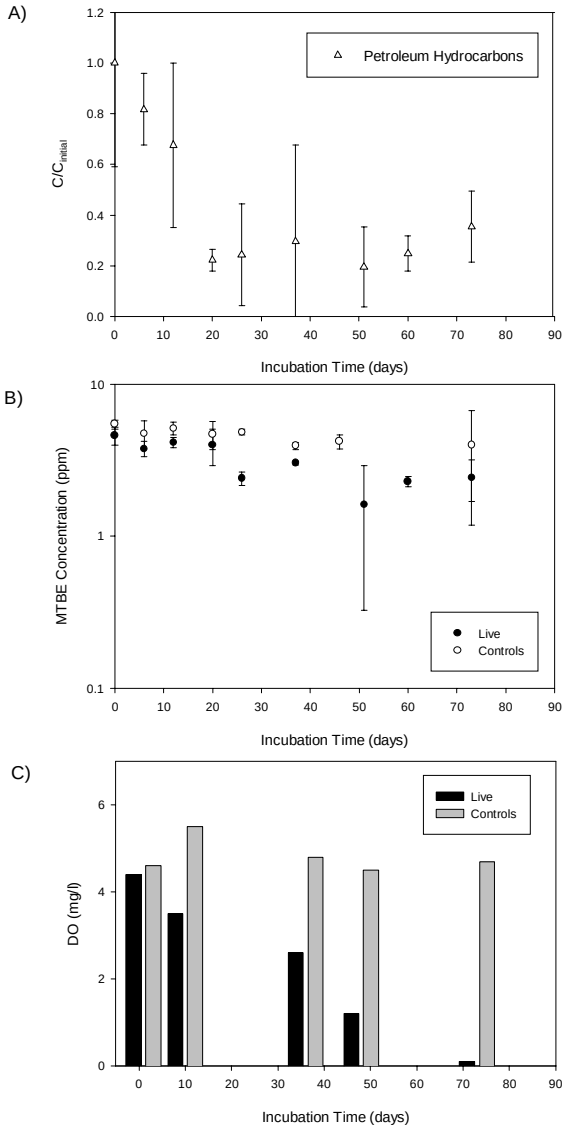


FIG 5. MLS-2 microcosms amaneded with nutrients. (A) MTBE concentration in live microcsms and controls, (B) Relative petroleum hydrocarbon concentration, (C) DO levels in live microcosms and controls

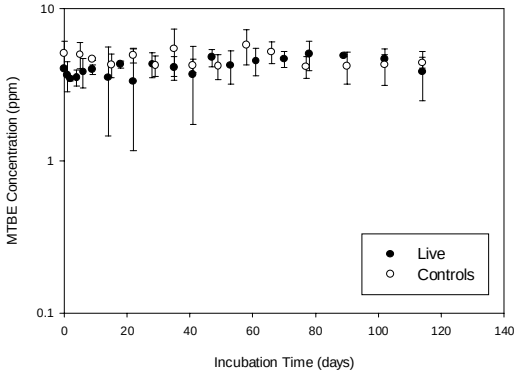


FIG. 6. MTBE concentration in upgradient (SB-1) microcosms; only MTBE was added.

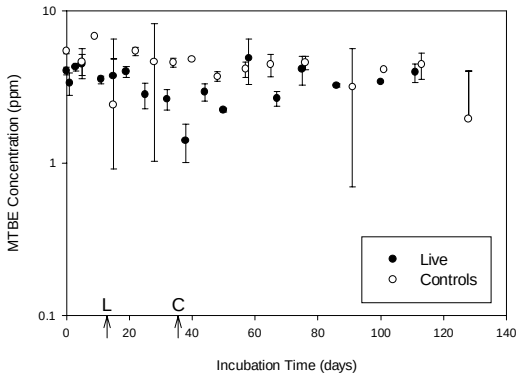


FIG. 7. MTBE concentration in upgradient (SB-1) microcosms; MTBE, toluene and ethyl-benzene added. (L) represents the disappearance of toluene and ethyl-benzene in live microcosms. (C) represents the disappearance of toluene and ethyl-benzene in control microcosms.

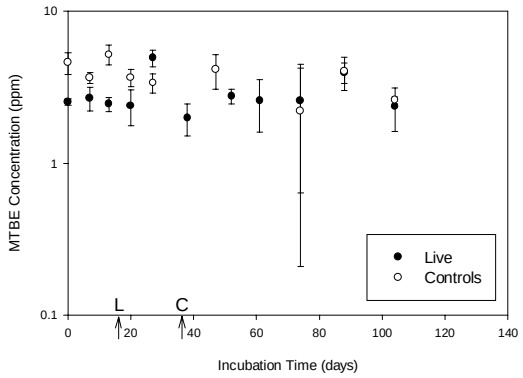


FIG. 8. MTBE concentration in upgradient (SB-1) microcosms; MTBE, toluene, and nutrients added. (L) represents toluene disappearance in live microcosms. (D) represents toluene disappearance in control microcosms.

TABLE 1. Chemical Characteristics of Groundwater

Well	Depth to GW (ft)	DO (mg/L)	SO_4 (mg/L)	NO_3 (mg/L)	Fe^{2+} (mg/L)	PH	MTBE (mg/L)	Toluene (mg/L)	Ethyl-benzene (mg/L)	Petroleum Hydrocarbons (mg/L)[a]
W1124-5S	20.4	BDL	12.92	0.17	NM	NM	1.588	0.044	0.271	22.536
MW-2	16.9	1.7	21.15	2.31	0.25	5.0	BDL	BDL	0.004	0.007
MW-3	15.0	4.2	20.03	0.39	0.20	5.5	0.012	BDL	BDL	BDL
K-1A	11.5	1.8	24.83	0.31	0.00	5.7	0.000	BDL	BDL	BDL

NM - Not Measured

[a] Petroleum hydrocarbon contamination was quantified by comparing the total gas chromatographic area to a standard curve created with unleaded gasoline

Table 2. Characteristics of Aquifer Sediments

Sample	Depth of sample (ft)	MTBE mg/kg	Moisture Content (%)	Absorbed Water (%)	Total Organic Carbon (%)	Grain Size Distribution	Petroleum Hydrocarbons (mg/kg)[a]
SB-1 (upgradient)	17-19	BDL	30.32	4.17	3.10	70% clay 30% silt	BDL
MLS-1 (source area)	19-21	1.06 - 56.89	13.91	0.46	3.47	>90% sand 50% fine 30% medium 20% coarse	0.526 - 27.373
MLS-2 (downgradient)	30-32	BDL - 1.01	17.59	0.71	3.04	>95% sand 25% fine 70% medium	BDL - 2.626

[a] Petroleum hydrocarbon contamination was quantified by comparing the total gas chromatographic area to a standard curve created with unleaded gasoline

TABLE 3. First-order Rate Constants for Source Area (MLS-1) Microcosms

Microcosm conditions	Rate Constant λ (days-1)	R^2 value	Points exluded from regression	Lag Time (days)
MTBE only added[a]				
(1st addition)	0.037	0.904	t= 68 days	20
(2nd addition)	0.063	0.991	none	0
MTBE, Toluene, and Ethyl-benzene added[b]	0.066	0.768	t= 92 days t= 134 days	20 - 40

[a] **see FIG. 2**
[b] **see FIG. 3**

APPENDIX

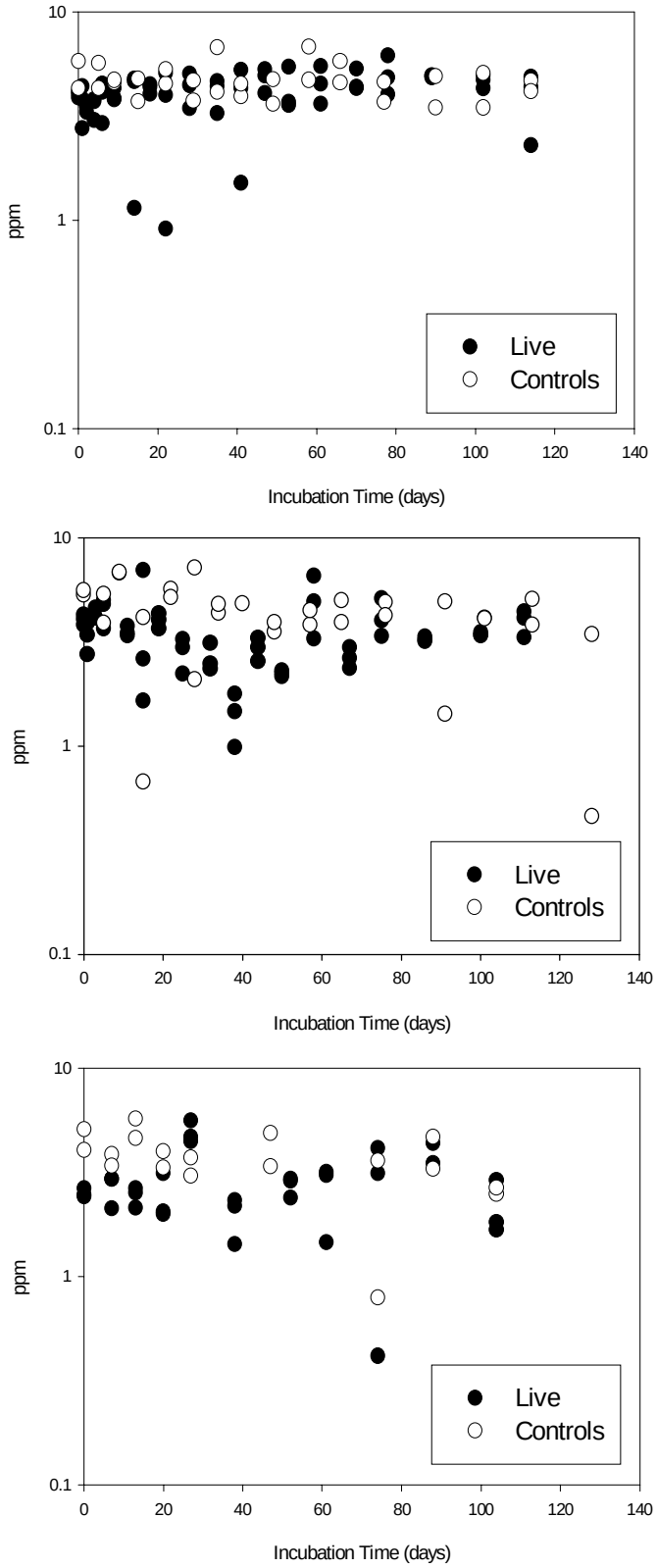


Figure A-1. Scatter plot of MTBE concentration in upgradient (SB-1) microcosms. (A) MTBE only added, (B) MTBE, toluene, and ethyl-benzene added, (C) MTBE, Toluene, and nutrients addded.

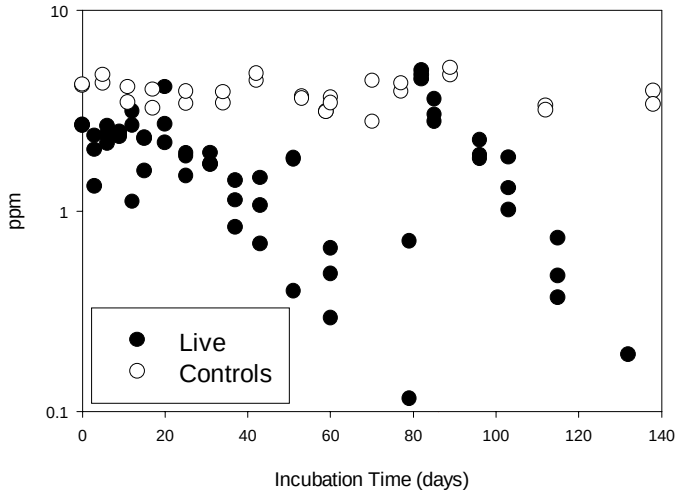


Figure A-2. Scatter plot of MTBE concentration in source area (MLS-1) micocosms; only MTBE was added. MTBE was respiked to 5 ppm at t=81 days.

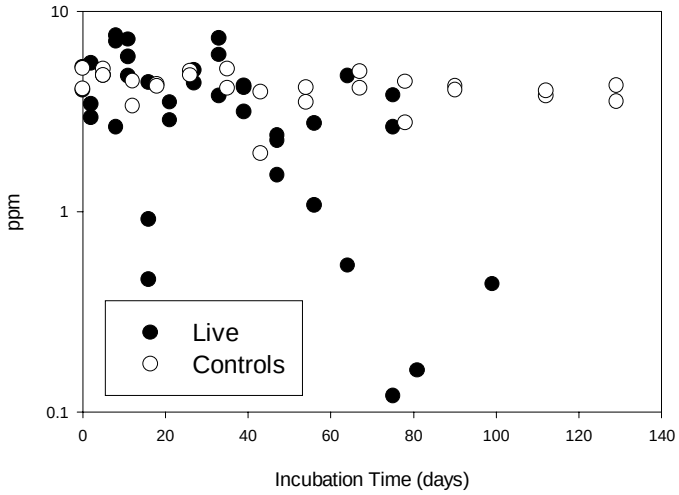


Figure A-3. Scatter plot of MTBE concentration in source area (MLS-1) microcosms. Spiked with MTBE, toluene, and ethyl-benzene.

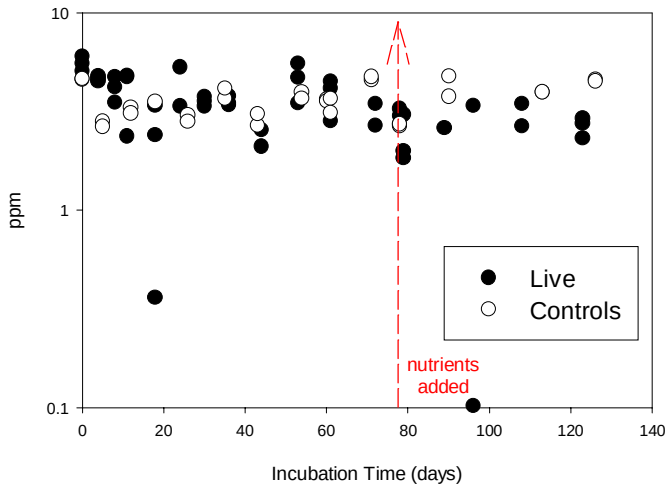


Figure A-4. Scatter plot of MTBE concentration in downgradient (MLS-2) microcosms. Initially only MTBE was added; amended with nutrients at t=78 days.

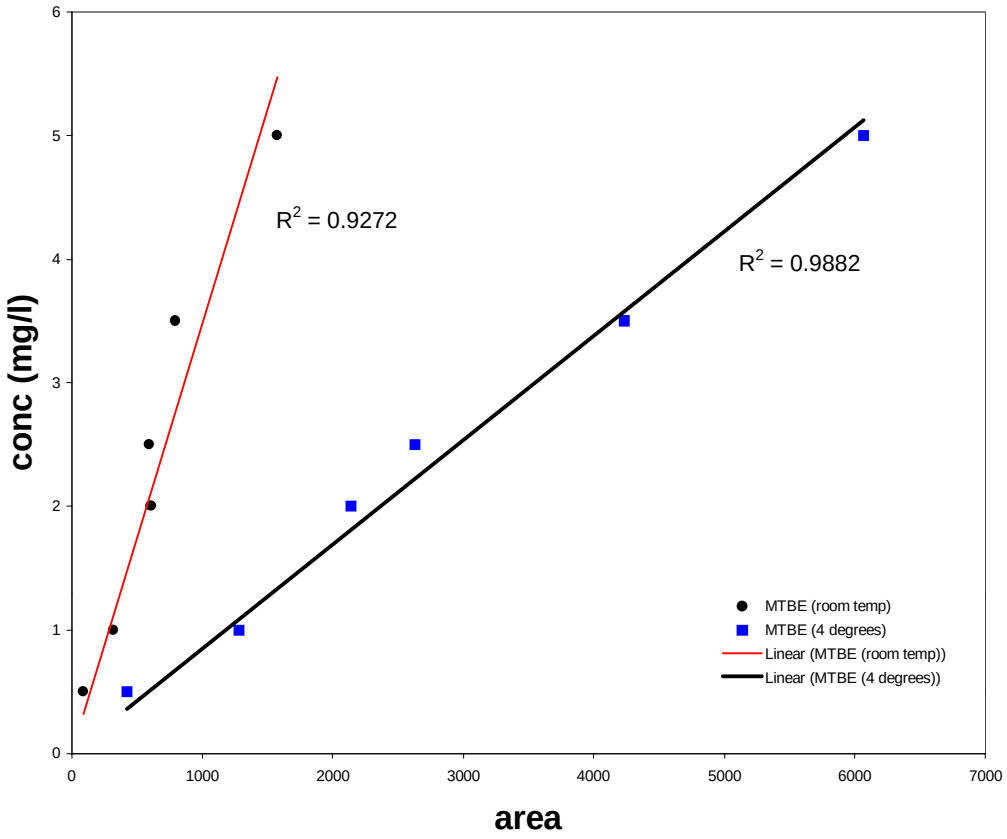


Figure A-5. MTBE standards prepared at 4 ^{o}C and 20 ^{o}C.

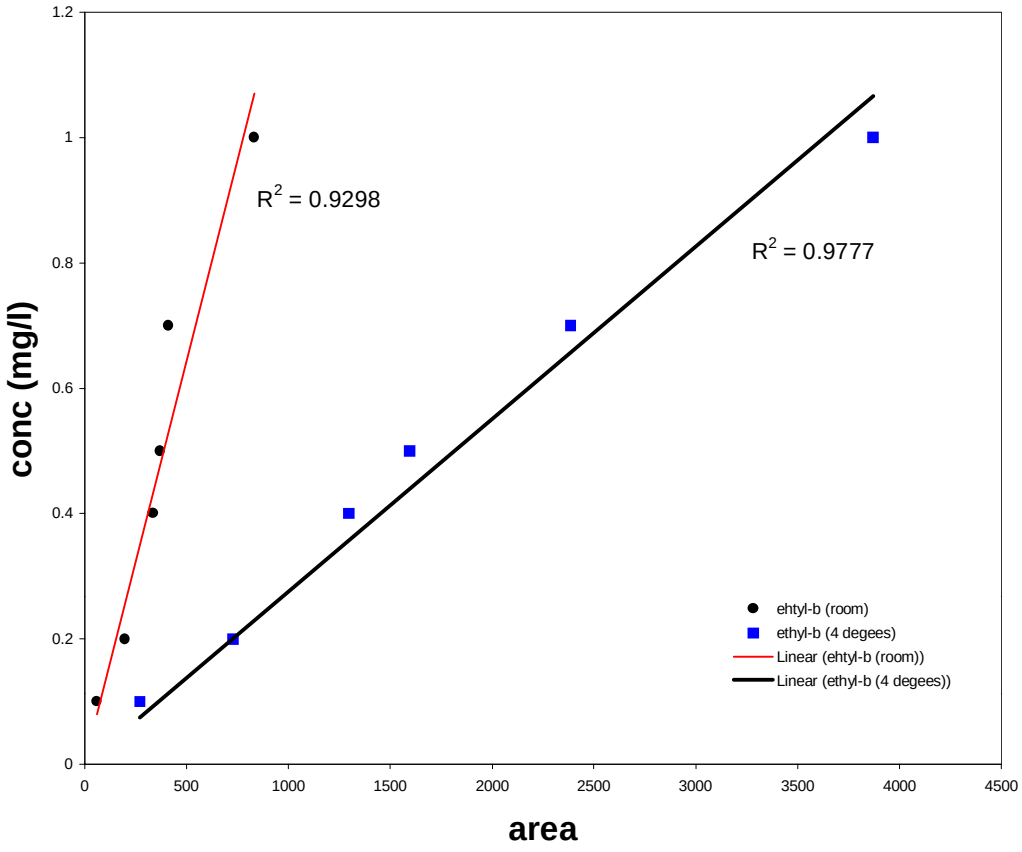
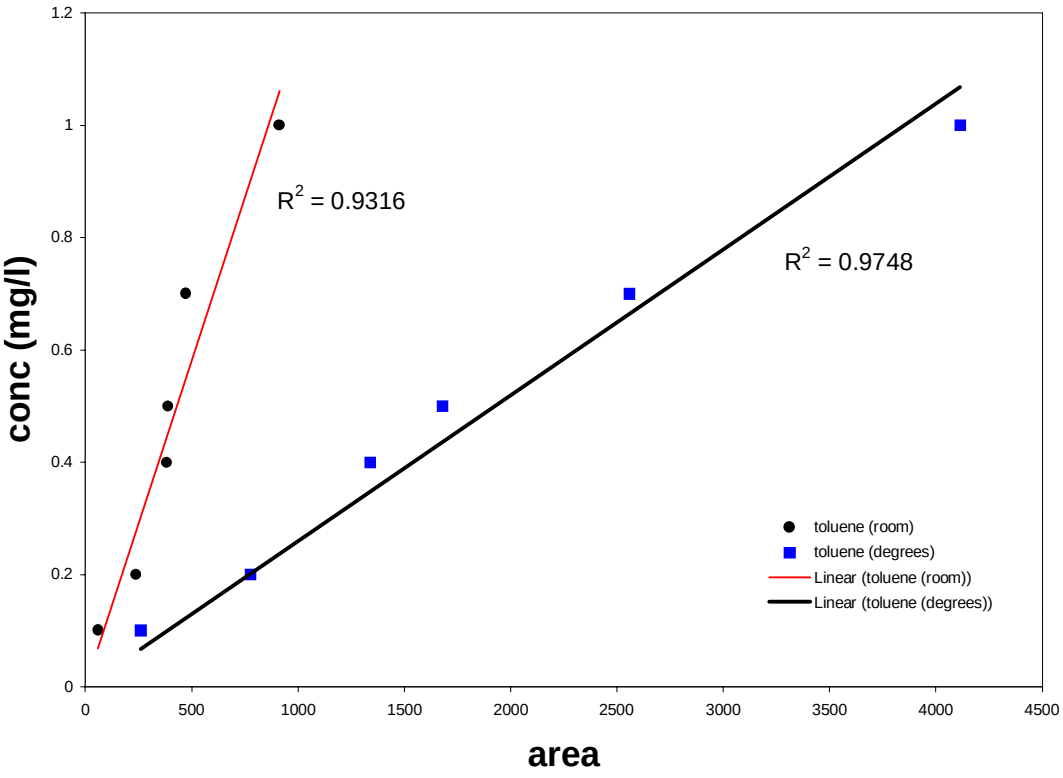


Figure A-6. Ethyl-benzene standards prepared at 4 ^{o}C and 20 ^{o}C.

Figure A-7. Toluene standards prepared at 4 °C and 20 °C.



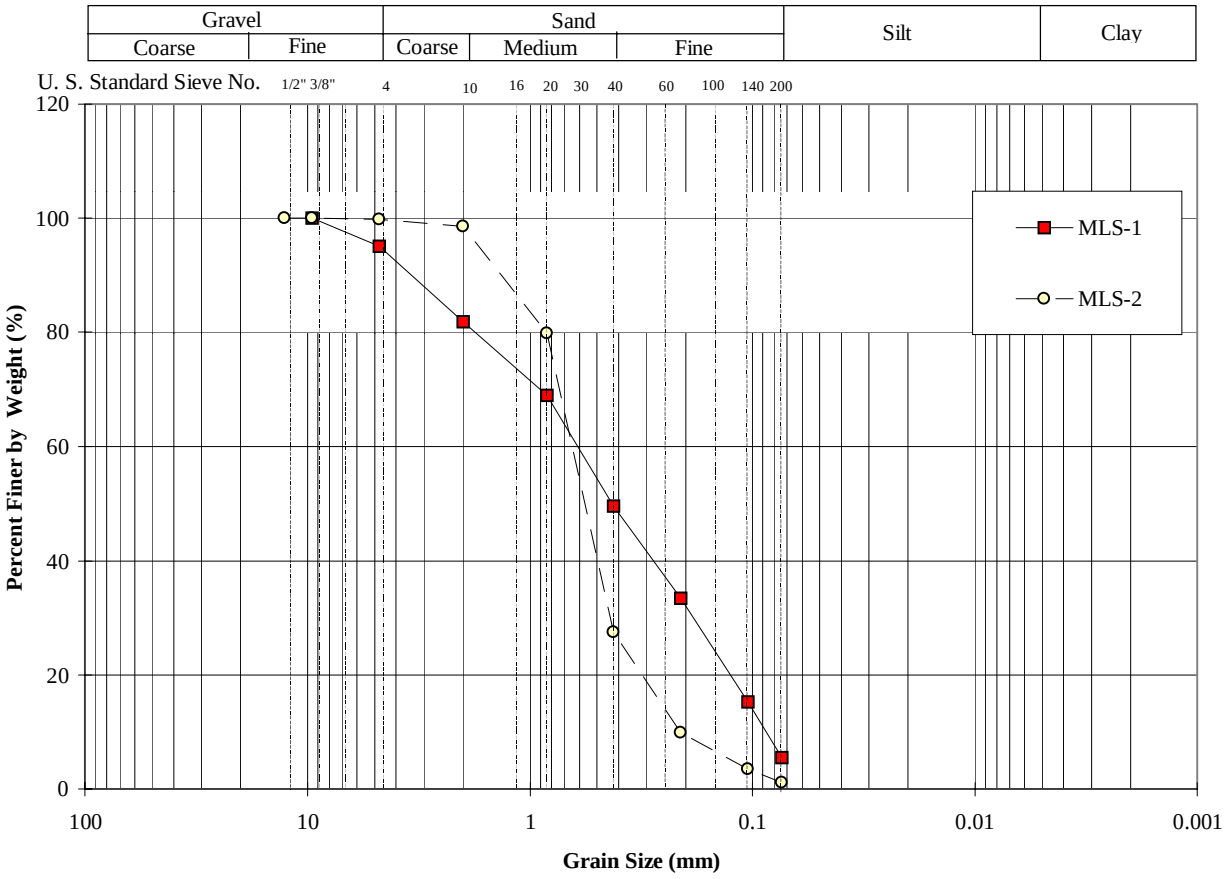


Figure A-8. Grain size distribution for aquifer samples MLS-1 and MLS-2.

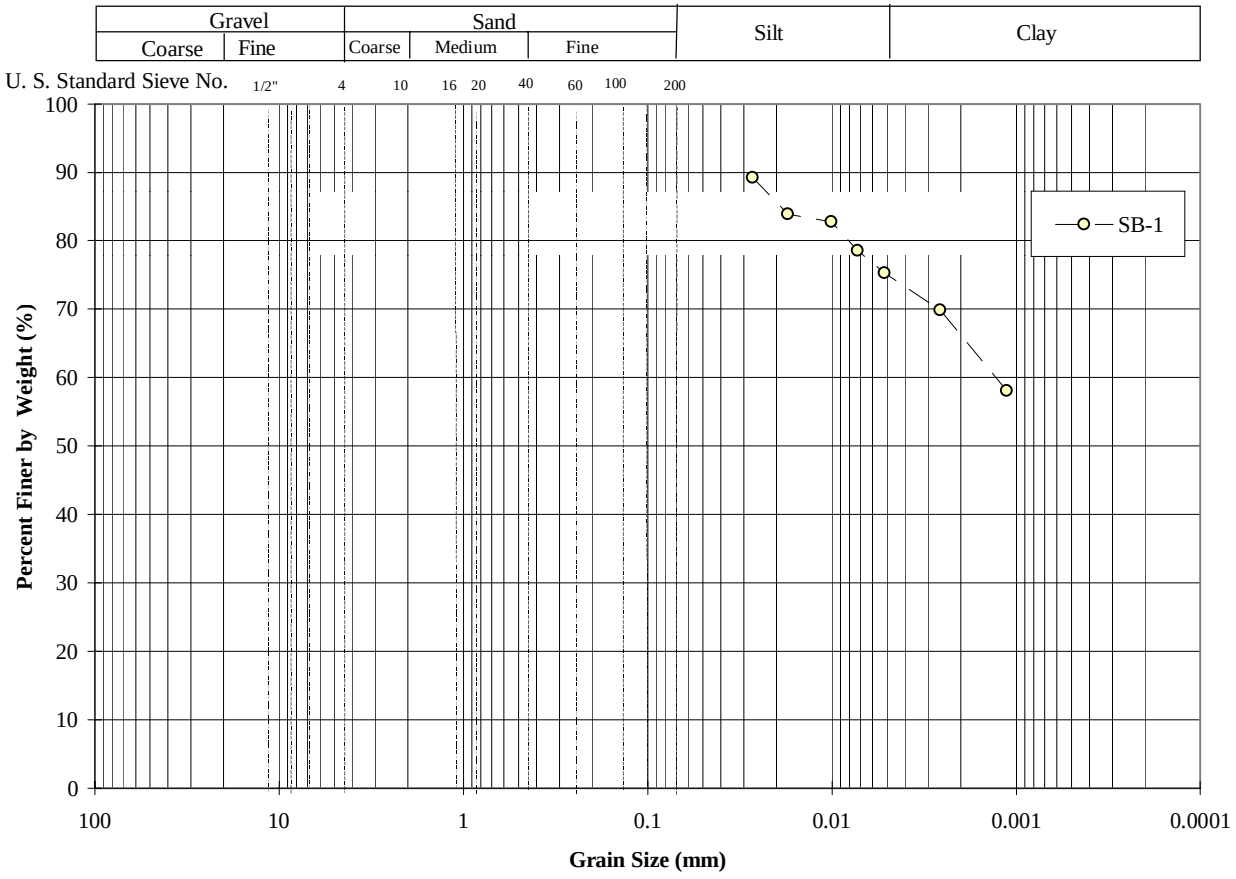


Figure A-9. Hydrometer analysis for aquifer sample SB-1.

Table A-1. GC Method 1 (used for microcosms constructed in the fall of 1998).

Color	Soil	Condition	Initial Concentration (mg/l)		
			MTBE	Toluene	Ethyl-B
Red	SB-1	Uncontaminated	5	1	1
Green	SB-1	Uncontaminated	5	1	1
Orange	MLS-1	Mostly MTBE	5	1	1
Purple	MLS-1	Mostly MTBE	5	1	1
Yellow	MLS-2	Petroleum	5	1	1

Column tubing:	glass
Coating material :	5% SP-1200/1.75% Bentone
Support :	100/120 Supelcoport

Paramater	Setting
Detector	FID
Carrier gas	Nitrogen
Carrier gas flow rate (ml/min)	30
Air pressure	38
Air Flowrate (ml/min)	300
Hydrogen Pressure	36
Hydrogen Flowrate	30
Temperature:	
Injector 2 (C)	130
Detector 2 (C)	250
Column Temperature Program:	
Initial Temperature (C)	50
Initial Time (min)	3
Program Rate (C/min)	5
Final temperature (C)	80
Final Time (min)	2
GC integrator and chart recorder :	
Attenuation	2▲6
Threshold	-4
Peak width	0.32
Signal	C
Chart Speed (cm/min)	1

Table A-2. GC Method 2 (used for microcosms constucted in January of 1999).

Color	Soil	Condition	Initial Concentration (mg/l)			Nutrients (mg/l)	
			MTBE	Toluene	Ethyl-B	N-NH4	P-PO4
green	SB-1	Uncontaminated	5	10	0	50	10
black	MLS-2	Petroleum	5	10	0	50	10
Purple	MLS-1	Mostly MTBE	>5	10	0	0	10

Column tubing:	glass
Coating material :	5% SP-1200/1.75% Bentone
Support :	100/120 Supelcoport

Paramater	Setting
Detector	FID
Carrier gas	Nitrogen
Carrier gas flow rate (ml/min)	30
Air pressure	38
Air Flowrate (ml/min)	300
Hydrogen Pressure	36
Hydrogen Flowrate	30
Temperature:	
Injector 2 (C)	130
Detector 2 (C)	250
Column Temperature Program:	
Initial Temperature (C)	60
Initial Time (min)	2
Program Rate (C/min)	7
Final temperature (C)	123
Final Time (min)	4
GC integrator and chart recorder :	
Attenuation	2▲9
Threshold	-4
Peak width	0.32
Signal	C
Chart Speed (cm/min)	1

Table A-3. Headspace Method (Used with both GC method 1 & 2)

Parameter	Setting
Headspace method	1
Equilibration time	0
Bath temperature (C)	65
Valve/loop temp. (C)	70
Injections per vial	1
Last vial #	varies
sampling interval	21 min
method sequence	1
Carrier Gas	Nitrogen
Carrier Gas Flowrate	25 ml/min
Carrier gas pressure (bars)	1.3-1.7
Aux pressure (bars)	0-0.2
Servo air (bars)	2.0-3.0

Table A-4. Depth of aquifer samples, and description of use

Sample	Depth (ft)	Description of use
SB-1	17-19	MTBE only (live)
MLS-2	30-32	MTBE only (controls)
MLS-2	30-32	Soil characterization
MLS-2	32-34	MTBE only (controls)
MLS-2	30-32	MTBE only (live)
SB-1	19-21	MTBE + 10 ppm toluene (live)
SB-1	19-21	Soil characterization
SB-1	15-17	MTBE + 1 ppm toluene + 1ppm ethyl-benzene (live)
MLS-1	18-20	MTBE + nutrients (live)
MLS-1	18-20	MTBE + nutrients (live)
MLS-1	18-20	MTBE + nutrients (controls)
SB-1	17-19	MTBE only (controls)
MLS-2	30-32	MTBE + nutrients @ t=0 (live)
MLS-2	28-30	MTBE + nutrients @ t=0 (live)
MLS-2	28-30	MTBE + nutrients @ t=0 (controls)
SB-1	19-21	MTBE + 10 ppm toluene (controls)
MLS-2	32-34	MTBE only (live)
MLS-2	32-34	Soil characterization
SB-1	15-17	MTBE + 1 ppm toluene + 1ppm ethyl-benzene (controls)
MLS-1	18-20	MTBE only (live)
MLS-1	18-20	MTBE only (controls)
SB-1	15-17	MTBE + 1 ppm toluene + 1ppm ethyl-benzene (live)
MLS-1	16-18	MTBE + 1 ppm toluene + 1ppm ethyl-benzene (live)
MLS-1	18-20	MTBE only (live)
MLS-1	16-18	MTBE + 1 ppm toluene + 1ppm ethyl-benzene (controls)
SB-1	19-21	Soil characterization
SB-1	17-19	MTBE only (live)
MLS-1	18-20	Soil characterization
SB-1	17-19	MTBE only (controls)
MLS-1	15-17	MTBE + 1 ppm toluene + 1ppm ethyl-benzene (live)

Table A-5. MTBE concentration in SB-1live microcosms; only MTBE added.

Vial #	date	Time (days)	MTBE (mg/L)	AVG	STDEV
1	10/19/98	0	4.132		
2	10/19/98	0	3.868		
3	10/19/98	0	4.040	4.014	0.134
4	10/20/98	1	2.752		
5	10/20/98	1	3.815		
6	10/20/98	1	4.388	3.652	0.830
7	10/21/98	2	3.307		
8	10/21/98	2	3.520		
9	10/21/98	2	3.538	3.455	0.129
10	10/23/98	4	3.015		
11	10/23/98	4	3.708		
12	10/23/98	4	3.791	3.505	0.426
13	10/25/98	6	2.905		
14	10/25/98	6	4.111		
15	10/25/98	6	4.504	3.840	0.833
16	10/28/98	9	3.836		
17	10/28/98	9	4.288		
18	10/28/98	9	3.785	3.970	0.277
19	11/2/98	14	1.143		
20	11/2/98	14	4.644		
21	11/2/98	14	4.759	3.515	2.055
22	11/6/98	18	4.478		
23	11/6/98	18	4.029		
24	11/6/98	18	4.348	4.285	0.231
25	11/10/98	22	0.907		
26	11/10/98	22	5.073		
27	11/10/98	22	3.977	3.319	2.159
28	11/16/98	28	3.433		
29	11/16/98	28	4.434		
30	11/16/98	28	5.052	4.307	0.817
31	11/23/98	35	3.259		
32	11/23/98	35	4.639		
33	11/23/98	35	4.384	4.094	0.734
34	11/29/98	41	4.281		
35	11/29/98	41	5.256		
36	11/29/98	41	1.505	3.681	1.946
37	12/5/98	47	4.076		
38	12/5/98	47	4.924		
39	12/5/98	47	5.294	4.765	0.624
41	12/11/98	53	5.425		
42	12/11/98	53	3.561		
43	12/11/98	53	3.688	4.225	1.041
44	12/19/98	61	4.507		
45	12/19/98	61	5.458		
46	12/19/98	61	3.603	4.523	0.928
47	12/28/98	70	5.322		
48	12/28/98	70	4.305		
49	12/28/98	70	4.343	4.657	0.577
51	1/5/99	78	6.18		
52	1/5/99	78	3.994		
53	1/5/99	78	4.835	5.003	1.103
54	1/16/99	89	4.927		
55	1/16/99	89	4.920		
56	1/16/99	89	4.827	4.891	0.056
57	1/29/99	102	4.950		
58	1/29/99	102	4.704		
59	1/29/99	102	4.278	4.644	0.340
61	2/10/99	114	2.284		
62	2/10/99	114	4.874		
63	2/10/99	114	4.355	3.838	1.370

Table A-6. MTBE concentration in SB-1control microcosms; only MTBE added.

Vial #	date	Time (days)	MTBE (mg/L)	AVG	STDEV
101	11/1/98	0	4.293		
102	11/1/98	0	5.791	5.042	1.059
104	11/6/98	5	5.659		
105	11/6/98	5	4.296	4.978	0.964
106	11/10/98	9	4.581		
107	11/10/98	9	4.687	4.634	0.075
108	11/16/98	15	4.781		
109	11/16/98	15	3.727	4.254	0.745
110	11/23/98	22	4.528		
111	11/23/98	22	5.272	4.900	0.526
112	11/29/98	29	3.749		
113	11/29/98	29	4.669	4.209	0.651
114	12/5/98	35	4.108		
115	12/5/98	35	6.751	5.430	1.869
117	12/11/98	41	3.923		
118	12/11/98	41	4.513	4.218	0.417
119	12/19/98	49	3.621		
120	12/19/98	49	4.728	4.175	0.783
121	12/28/98	58	6.791		
122	12/28/98	58	4.682	5.737	1.491
124	1/5/99	66	4.582		
125	1/5/99	66	5.803	5.193	0.863
126	1/16/99	77	3.679		
127	1/16/99	77	4.621	4.150	0.666
128	1/29/99	90	3.471		
129	1/29/99	90	4.888	4.180	1.002
130	2/10/99	102	5.083		
131	2/10/99	102	3.451	4.267	1.154
132	2/22/99	114	4.652		
133	2/22/99	114	4.142	4.397	0.361

TABLE A-7. MTBE, toluene, and ethyl-benzene concentration in SB-1 live microcosms.

Vial#	DATE	TIME (days)	MTBE mg/L	Toluene mg/L	Ethyl-B mg/L	AVG MTBE	AVG TOL	AVG Ethyl-B	STDEV MTBE	STDEV TOL	STDEV Ethyl-B
1	10/22/98	0	3.813	0.679	0.779						
2	10/22/98	0	4.268	0.774	0.939						
3	10/22/98	0	4.047	0.650	0.745	4.043	0.701	0.821	0.228	0.065	0.104
4	10/23/98	1	3.421	1.068	1.032						
5	10/23/98	1	3.892	1.262	1.032						
6	10/23/98	1	2.758	1.030	0.892	3.357	1.120	0.985	0.569	0.124	0.081
7	10/25/98	3	4.117	0.000	0.282						
8	10/25/98	3	4.152	0.000	0.252						
9	10/25/98	3	4.590	0.000	0.316	4.286	0.000	0.283	0.263	0.000	0.032
10	10/27/98	5	4.796	0.000	0.584						
11	10/27/98	5	3.655	0.000	0.605						
12	10/27/98	5	4.997	0.000	0.651	4.483	0.000	0.614	0.724	0.000	0.034
13	11/2/98	11	3.383	0.000	0.053						
14	11/2/98	11	3.484	0.000	0.000						
15	11/2/98	11	3.771	0.000	0.025	3.546	0.000	0.026	0.201	0.000	0.026
16	11/6/98	15	6.975	0.000	0.001						
17	11/6/98	15	1.647	0.000	0.013						
18	11/6/98	15	2.629	0.000	0.000	3.750	0.000	0.005	2.836	0.000	0.007
19	11/2/98	19	3.653	0.000	0.000						
20	11/2/98	19	4.018	0.000	0.000						
21	11/10/98	19	4.317	0.000	0.000	3.996	0.000	0.000	0.333	0.000	0.000
22	11/10/98	25	3.248	0.000	0.000						
23	11/10/98	25	2.214	0.000	0.016						
24	11/6/98	25	2.979	0.000	0.022	2.814	0.000	0.013	0.536	0.000	0.011
25	11/16/98	32	3.121	0.000	0.000						
26	11/16/98	32	2.347	0.000	0.000						
27	11/16/98	32	2.475	0.000	0.000	2.648	0.000	0.000	0.415	0.000	0.000
28	11/23/98	38	0.987	0.000	0.000						
29	11/23/98	38	1.462	0.000	0.000						
30	11/23/98	38	1.777	0.000	0.000	1.409	0.000	0.000	0.398	0.000	0.000
31	11/29/98	44	2.543	0.000	0.177						
32	11/29/98	44	2.976	0.000	0.113						
33	11/29/98	44	3.298	0.333	0.000	2.939	0.111	0.097	0.379	0.192	0.090
35	12/5/98	50	2.295	0.000	0.000						
36	12/5/98	50	2.230	0.000	0.000						
37	12/5/98	50	2.166	0.000	0.000	2.230	0.000	0.000	0.064	0.000	0.000
38	12/11/98	58	4.926	0.000	0.000						
39	12/11/98	58	6.542	0.000	0.000						
40	12/11/98	58	3.276	0.000	0.000	4.915	0.000	0.000	1.633	0.000	0.000
41	12/19/98	67	2.970	0.000	0.000						
42	12/19/98	67	2.643	0.000	0.000						
43	12/19/98	67	2.370	0.000	0.000	2.661	0.000	0.000	0.300	0.000	0.000
45	12/28/98	75	3.369	0.000	0.000						
46	12/28/98	75	3.998	0.000	0.000						
47	12/28/98	75	5.111	0.000	0.000	4.159	0.000	0.000	0.882	0.000	0.000
48	1/5/99	86	3.342	0.000	0.000						
49	1/5/99	86	3.176	0.000	0.000						
50	1/5/99	86	3.225	0.000	0.000	3.248	0.000	0.000	0.085	0.000	0.000
51	1/16/99	100	3.414	0.000	0.000						
52	1/16/99	100	3.391	0.000	0.000						
53	1/16/99	100	3.513	0.000	0.000	3.439	0.000	0.000	0.065	0.000	0.000
54	1/30/99	111	3.321	0.000	0.000						
55	1/30/99	111	4.412	0.000	0.000						
56	1/30/99	111	4.106	0.000	0.000	3.946	0.000	0.000	0.563	0.000	0.000

Table A-8. MTBE, toluene, and ethyl-benzene concentration in SB-1 control microcosms.

Vial#	Date	TIME (days)	MTBE mg/L	Toluene mg/L	Ethyl-B mg/L	AVG MTBE	AVG TOL	AVG Ethyl-B	STDEV MTBE	STDEV TOL	STDEV Ethyl-B
101	11/1/98	0	*5.319*	*0.008*	*0.165*						
102	11/1/98	0	*5.582*	*0.004*	*0.133*	5.451	0.006	0.149	0.186	0.003	0.023
104	11/6/98	5	*3.887*	*0.000*	*0.291*						
105	11/6/98	5	*5.349*	*0.000*	*0.182*	4.618	0.000	0.237	1.034	0.000	0.077
106	11/10/98	9	*6.799*	*0.000*	*0.020*						
107	11/10/98	9	*6.835*	*0.000*	*0.055*	6.817	0.000	0.038	0.025	0.000	0.025
108	11/16/98	15	*4.151*	*0.725*	*1.980*						
109	11/16/98	15	*0.674*	*0.396*	*1.894*	2.413	0.561	1.937	2.459	0.233	0.061
110	11/23/98	22	*5.665*	*0.000*	*0.888*						
111	11/23/98	22	*5.180*	*0.000*	*0.000*	5.423	0.000	0.444	0.343	0.000	0.628
112	11/29/98	28	*2.086*	*0.000*	*0.000*						
113	11/29/98	28	*7.182*	*0.000*	*0.000*	4.634	0.000	0.000	3.603	0.000	0.000
114	12/5/98	34	*4.352*	*0.641*	*0.000*						
115	12/5/98	34	*4.794*	*0.153*	*0.029*	4.573	0.397	0.015	0.313	0.345	0.021
117	12/11/98	40	*4.819*	*0.000*	*0.000*						
118	12/11/98	40	*NA*	*NA*	*NA*	4.819	0.000	0.000	0.000	0.000	0.000
119	12/19/98	48	*3.525*	*0.000*	*0.000*						
120	12/19/98	48	*3.913*	*0.000*	*0.000*	3.719	0.000	0.000	0.274	0.000	0.000
121	12/28/98	57	*3.810*	*0.000*	*0.000*						
122	12/28/98	57	*4.485*	*0.000*	*0.000*	4.148	0.000	0.000	0.477	0.000	0.000
124	1/5/99	65	*3.917*	*0.000*	*0.000*						
125	1/5/99	65	*4.990*	*0.000*	*0.000*	4.454	0.000	0.000	0.759	0.000	0.000
126	1/16/99	76	*4.891*	*0.000*	*0.000*						
127	1/16/99	76	*4.234*	*0.000*	*0.000*	4.563	0.000	0.000	0.465	0.000	0.000
128	1/31/99	91	*1.425*	*0.000*	*0.000*						
129	1/31/99	91	*4.942*	*0.000*	*0.000*	3.184	0.000	0.000	2.487	0.000	0.000
130	2/10/99	101	*4.122*	*0.000*	*0.000*						
131	2/10/99	101	*4.086*	*0.000*	*0.000*	4.104	0.000	0.000	0.025	0.000	0.000
132	2/22/99	113	*3.819*	*0.000*	*0.000*						
133	2/22/99	113	*5.051*	*0.000*	*0.000*	4.435	0.000	0.000	0.871	0.000	0.000
134	3/8/99	128	*0.460*	*0.000*	*0.000*						
135	3/8/99	128	*3.431*	*0.000*	*0.000*	1.946	0.000	0.000	2.101	0.000	0.000

Table A-9. MTBE and toluene concentration in live SB-1 microcosms; MTBE and toluene added.

Vial#	Date	Time (days)	MTBE (mg/L)	Toluene (mg/L)	AVG MTBE	STDEV MTBE	AVG Toluene	STDEV Toluene
1	1/29/99	0	2.475	8.637				
2	1/29/99	0	2.424	9.917				
3	1/29/99	0	2.660	10.377	2.520	0.124	9.644	0.902
4	2/5/99	7	2.944	0.008				
5	2/5/99	7	2.127	1.865				
6	2/5/99	7	2.942	0.003	2.671	0.471	0.625	1.074
7	2/11/99	13	2.136	0.005				
8	2/11/99	13	2.651	0.001				
9	2/11/99	13	2.524	0.000	2.437	0.268	0.002	0.002
13	2/18/99	20	2.047	0.000				
14	2/18/99	20	3.126	0.000				
15	2/18/99	20	2.000	0.000	2.391	0.637	0.000	0.000
10	2/25/99	27	4.673	0.004				
11	2/25/99	27	4.464	0.000				
12	2/25/99	27	5.624	0.000	4.920	0.618	0.001	0.002
16	3/7/99	38	2.321	0.000				
17	3/7/99	38	1.432	0.000				
18	3/7/99	38	2.178	0.000	1.977	0.477	0.000	0.000
19	3/21/99	52	2.948	0.000				
20	3/21/99	52	2.880	0.000				
21	3/21/99	52	2.396	0.000	2.742	0.301	0.000	0.000
22	3/30/99	61	3.185	0.000				
23	3/30/99	61	1.455	0.000				
24	3/30/99	61	3.071	0.000	2.570	0.968	0.000	0.000
25	4/12/99	74	4.125	0.000				
26	4/12/99	74	0.414	0.000				
27	4/12/99	74	3.139	0.000	2.559	1.922	0.000	0.000
31	4/26/99	88	3.501	0.000				
32	4/26/99	88	4.365	0.000				
33	4/26/99	88	NM	0.000	3.933	0.611	0.000	0.000
28	5/12/99	104	2.896	0.000				
29	5/12/99	104	1.829	0.000				
30	5/12/99	104	1.673	0.000	2.362	0.754	0.000	0.000

Table A-10. MTBE and toluene concentration in control SB-1 microcosms; MTBE and toluene added.

Vial#	Date	Time (days)	MTBE (mg/L)	Toluene (mg/L)	AVG MTBE	STDEV MTBE	AVG Toluene	STDEV Toluene
101	1/29/99	0	5.093	5.597				
102	1/29/99	0	4.042	5.166	4.567	0.743	5.382	0.305
103	2/5/99	7	3.840	0.063				
104	2/5/99	7	3.409	0.398	3.624	0.304	0.231	0.236
105	2/11/99	13	5.714	0.007				
106	2/11/99	13	4.624	0.003	5.169	0.771	0.005	0.003
107	2/18/99	20	3.319	0.009				
108	2/18/99	20	3.996	0.000	3.657	0.479	0.005	0.007
109	2/25/99	27	3.038	0.006				
110	2/25/99	27	3.725	0.000	3.382	0.486	0.003	0.004
111	3/16/99	47	3.376	0.009				
112	3/16/99	47	4.866	0.000	4.121	1.054	0.004	0.006
113	4/12/99	74	0.793	0.000				
114	4/12/99	74	3.607	0.000	2.200	1.990	0.000	0.000
117	4/26/99	88	3.286	0.000				
118	4/26/99	88	4.694	0.000	3.990	0.995	0.000	0.000
120	5/12/99	104	2.487	0.000				
121	5/12/99	104	2.675	0.000	2.581	0.133	0.000	0.000

Table A-11. MTBE concentration in MLS-1 live microcosms; MTBE only added.

Vial #	Date	Time (days)	MTBE (mg/L)	AVG	STDEV
1	10/28/98	0	NA		
2	10/28/98	0	2.686		
3	10/28/98	0	2.658	2.672	0.020
4	11/1/98	3	1.324		
5	11/1/98	3	2.380		
6	11/1/98	3	2.025	1.909	0.537
7	11/4/98	6	2.170		
8	11/4/98	6	2.648		
9	11/4/98	6	2.409	2.409	0.239
10	11/7/98	9	2.480		
11	11/7/98	9	2.405		
12	11/7/98	9	2.341	2.409	0.070
13	11/10/98	12	1.116		
14	11/10/98	12	2.665		
15	11/10/98	12	3.126	2.303	1.053
16	11/13/98	15	2.316		
17	11/13/98	15	1.580		
18	11/13/98	15	2.287	2.061	0.416
19	11/18/98	20	2.707		
20	11/18/98	20	4.164		
21	11/18/98	20	2.182	3.018	1.027
22	11/23/98	25	1.489		
23	11/23/98	25	1.945		
24	11/23/98	25	1.870	1.768	0.244
25	11/29/98	31	1.697		
26	11/29/98	31	1.945		
27	11/29/98	31	1.713	1.785	0.139
28	12/5/98	37	0.830		
29	12/5/98	37	1.415		
30	12/5/98	37	1.129	1.125	0.293
32	12/11/98	43	1.064		
33	12/11/98	43	1.463		
34	12/11/98	43	0.682	1.070	0.391
35	12/19/98	51	1.800		
36	12/11/98	51	0.396		
37	12/19/98	51	1.835	1.344	0.821
38	12/28/98	60	0.291		
39	12/28/98	60	0.483		
40	12/28/98	60	0.649	0.474	0.179
42	1/5/99	68	0.004		
43	1/5/99	68	0.016		
44	1/5/99	68	0.007	0.009	0.006
45	1/16/99	79	0.705		
46	1/16/99	79	0.007		
47	1/16/99	79	0.115	0.276	0.376
**	1/19/99	82	5.002		
**	1/19/99	82	4.753		
**	1/19/99	82	4.546	4.767	0.228
48	1/22/99	85	3.032		
49	1/22/99	85	3.596		
50	1/22/99	85	2.783	3.137	0.417
51	2/3/99	96	2.252		
52	2/3/99	96	1.908		
53	2/3/99	96	1.820	1.993	0.228
54	2/10/99	103	1.300		
55	2/10/99	103	1.007		
56	2/10/99	103	1.849	1.385	0.427
57	2/22/99	115	0.473		
58	2/22/99	115	0.368		
59	2/22/99	115	0.732	0.524	0.187
60	3/16/99	132	0.192		
61	3/16/99	132	0.192	0.192	0.000

Table A-12. MTBE concentration in MLS-1 control microcosms; MTBE only added.

Vial #	Date	Time (days)	MTBE (mg/L)	AVG	STDEV
101	11/24/98	0	4.219		
102	11/24/98	0	4.277	4.248	0.041
103	11/29/98	5	4.321		
104	11/29/98	5	4.779	4.550	0.324
105	12/5/98	11	3.475		
106	12/5/98	11	4.161	3.818	0.485
108	12/11/98	17	4.037		
109	12/11/98	17	3.251	3.644	0.556
110	12/19/98	25	3.433		
111	12/19/98	25	3.927	3.680	0.349
112	12/28/98	34	3.450		
113	12/28/98	34	3.919	3.685	0.332
115	1/5/99	42	4.471		
116	1/5/99	42	4.836	4.654	0.258
117	1/16/99	53	3.709		
118	1/16/99	53	3.622	3.666	0.062
119	1/22/99	59	3.123		
120	1/22/99	59	3.120	3.122	0.002
**	1/25/99	60	3.698		
**	1/25/99	60	3.454	3.576	0.173
126	2/3/99	70	2.798		
127	2/3/99	70	4.481	3.640	1.190
124	2/10/99	77	3.943		
125	2/10/99	77	4.343	4.143	0.283
121	2/22/99	89	4.762		
122	2/22/99	89	5.177	4.970	0.293
123	3/16/99	112	3.353		
128	3/16/99	112	3.179	3.266	0.123
131	4/12/99	138	3.958		
132	4/12/99	138	3.387	3.673	0.404

TABLE A-13. MTBE, toluene, and ethyl-benzene concentration in MLS-1 live microcosms.

Vial#	DATE	TIME (days)	MTBE mg/L	Toluene mg/L	Ethyl-B mg/L	AVG MTBE	AVG TOL	AVG Ethyl-B	STDEV MTBE	STDEV TOL	STDEV Ethyl-B
1	11/2/98	0	NA	NA	NA						
2	11/2/98	0	4.049	0.861	0.876						
3	11/2/98	0	5.297	1.159	1.158	4.673	1.010	1.017	0.883	0.210	0.200
4	11/4/98	2	3.437	0.879	0.627						
5	11/4/98	2	2.951	0.536	0.497						
6	11/4/98	2	5.509	1.062	0.982	3.966	0.826	0.702	1.359	0.267	0.251
7	11/7/98	5	NA	NA	NA						
8	11/7/98	5	4.790	0.000	0.488						
9	11/7/98	5	4.829	0.000	0.215	4.810	0.000	0.352	0.028	0.000	0.193
10	11/10/98	8	7.113	0.024	0.050						
11	11/10/98	8	2.634	0.000	0.080						
12	11/10/98	8	7.562	0.000	0.100	5.770	0.008	0.077	2.725	0.014	0.025
13	11/13/98	11	7.256	0.000	0.000						
14	11/13/98	11	4.757	0.000	0.147						
15	11/13/98	11	5.930	0.000	0.000	5.981	0.000	0.049	1.250	0.000	0.085
16	11/18/98	16	0.915	0.000	0.000						
17	11/18/98	16	4.411	0.000	0.000						
18	11/18/98	16	0.459	0.000	0.000	1.928	0.000	0.000	2.162	0.000	0.000
19	11/23/98	21	3.532	0.000	0.000						
20	11/23/98	21	0.000	0.000	0.000						
21	11/23/98	21	2.871	0.000	0.000	2.134	0.000	0.000	1.878	0.000	0.000
22	11/29/98	27	0.000	0.000	0.000						
23	11/29/98	27	5.081	0.000	0.000						
24	11/29/98	27	4.398	0.000	0.000	3.160	0.000	0.000	2.758	0.000	0.000
25	12/5/98	33	3.785	0.000	0.000						
26	12/5/98	33	7.354	0.000	0.000						
27	12/5/98	33	6.082	0.000	0.000	5.740	0.000	0.000	1.809	0.000	0.000
29	12/11/98	39	3.149	0.000	0.000						
30	12/11/98	39	4.174	0.000	0.000						
31	12/11/98	39	4.276	0.000	0.000	3.866	0.000	0.000	0.623	0.000	0.000
32	12/19/98	47	1.524	0.000	0.000						
33	12/19/98	47	2.262	0.000	0.000						
34	12/19/98	47	2.399	0.000	0.000	2.062	0.000	0.000	0.471	0.000	0.000
35	12/28/98	56	0.011	0.000	0.000						
36	12/28/98	56	1.077	0.000	0.000						
37	12/28/98	56	2.763	0.000	0.000	1.284	0.000	0.000	1.388	0.000	0.000
39	1/5/99	64	4.759	0.000	0.000						
40	1/5/99	64	0.542	0.000	0.000						
41	1/5/99	64	0.000	0.000	0.000	1.767	0.000	0.000	2.605	0.000	0.000
42	1/16/99	75	0.120	0.000	0.000						
43	1/16/99	75	3.837	0.000	0.000						
44	1/16/99	75	2.645	0.000	0.000	2.201	0.000	0.000	1.898	0.000	0.000
46	1/22/99	81	0.046	0.000	0.000						
47	1/22/99	81	0.023	0.000	0.000						
48	1/22/99	81	0.162	0.000	0.000	0.077	0.000	0.000	0.075	0.000	0.000
49	2/3/99	92	0.000	0.000	0.000						
50	2/3/99	92	0.000	0.000	0.000						
51	2/3/99	92	0.001	0.000	0.000	0.000	0.000	0.000	0.001	0.000	0.000
51	2/10/99	99	0.437	0.000	0.000						
52	2/10/99	99	0.019	0.000	0.000						
53	2/10/99	99	0.065	0.000	0.000	0.174	0.000	0.000	0.229	0.000	0.000
54	2/22/99	111	0.000	0.000	0.000						
55	2/22/99	111	0.067	0.000	0.000						
56	2/22/99	111	0.000	0.000	0.000	0.022	0.000	0.000	0.039	0.000	0.000
57	3/16/99	134	0.000	0.000	0.000						
58	3/16/99	134	0.000	0.000	0.000						
59	3/16/99	134	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

TABLE A-14. MTBE, toluene, and ethyl-benzene concentration in MLS-1 control microcosms.

Vial#	DATE	TIME (days)	MTBE mg/L	Toluene mg/L	Ethyl-B mg/L	AVG MTBE	AVG TOL	AVG Ethyl-B	STDEV MTBE	STDEV TOL	STDEV Ethyl-B
101	11/24/98	0	4.108	0.857	0.064						
102	11/24/98	0	5.190	1.021	0.000	4.649	0.939	0.032	0.765	0.116	0.045
103	11/29/98	5	5.157	0.000	0.000						
104	11/29/98	5	4.781	0.000	0.000	4.969	0.000	0.000	0.266	0.000	0.000
105	12/5/98	12	4.482	0.000	0.000						
106	12/5/98	12	3.371	0.000	0.000	3.927	0.000	0.000	0.786	0.000	0.000
108	12/11/88	18	4.312	0.000	0.000						
109	12/11/98	18	4.224	0.000	0.000	4.268	0.000	0.000	0.062	0.000	0.000
110	12/19/98	26	5.066	0.000	0.000						
111	12/19/98	26	4.815	0.000	0.000	4.941	0.000	0.000	0.177	0.000	0.000
112	12/28/98	35	5.163	0.000	0.000						
113	12/28/98	35	4.139	0.000	0.000	4.651	0.000	0.000	0.724	0.000	0.000
115	1/5/99	43	3.950	0.000	0.000						
116	1/5/99	43	1.954	0.000	0.000	2.952	0.000	0.000	1.411	0.000	0.000
117	1/16/99	54	4.164	0.000	0.000						
118	1/16/99	54	3.519	0.000	0.000	3.842	0.000	0.000	0.456	0.000	0.000
119	1/29/99	67	4.138	0.000	0.000						
120	1/29/99	67	5.002	0.000	0.000	4.570	0.000	0.000	0.611	0.000	0.000
123	2/10/99	78	2.780	0.000	0.000						
124	2/10/99	78	4.472	0.000	0.000	3.626	0.000	0.000	1.196	0.000	0.000
121	2/22/99	90	4.237	0.000	0.000						
122	2/22/99	90	4.050	0.000	0.000	4.144	0.000	0.000	0.132	0.000	0.000
127	3/16/99	112	3.786	0.000	0.000						
128	3/16/99	112	4.024	0.000	0.000	3.905	0.000	0.000	0.168	0.000	0.000
125	4/3/99	129	3.536	0.000	0.000						
126	4/3/99	129	4.256	0.000	0.000	3.896	0.000	0.000	0.509	0.000	0.000

Table A-15. MTBE concentration in MLS-2 live microcosms; only MTBE added.

Vial #	date	Time (days)	MTBE (mg/L)	AVG	STDEV	
1	11/5/98	0	6.013			
2	11/5/98	0	5.563			
3	11/5/98	0	5.084	5.553	0.464	
4	11/9/98	4	4.501			
5	11/9/98	4	4.761			
6	11/9/98	4	4.631	4.631	0.130	
7	11/13/98	8	3.526			
8	11/13/98	8	4.210			
9	11/13/98	8	4.738	4.158	0.608	
10	11/16/98	11	4.737			
11	11/16/98	11	2.359			
12	11/16/98	11	4.813	3.970	1.395	
13	11/23/98	18	2.398			
14	11/23/98	18	3.386			
15	11/23/98	18	0.360	2.048	1.543	
16	11/29/98	24	0.000			
17	11/29/98	24	5.289			
18	11/29/98	24	3.353	2.881	2.676	
19	12/5/98	30	3.572			
20	12/5/98	30	3.772			
21	12/5/98	30	3.359	3.568	0.207	
23	12/11/98	36	3.409			
24	12/11/98	36	3.486			
25	12/11/98	36	3.804	3.566	0.209	
26	12/19/98	44	2.554			
27	12/19/98	44	2.096			
28	12/19/98	44	NA	2.325	0.324	
29	12/28/98	53	3.489			
30	12/28/98	53	4.725			
31	12/28/98	53	5.568	4.594	1.046	
33	1/5/99	61	2.829			
34	1/5/99	61	4.497			
35	1/5/99	61	4.132	3.819	0.877	
36	1/16/99	72	NA			
37	1/16/99	72	3.473			
38	1/16/99	72	2.690	3.082	0.554	
39	1/22/99	78	2.736			
40	1/22/99	78	3.252			
41	1/22/99	78	3.013	3.000	0.258	
**	1/23/99	79	3.043			nutrients added
**	1/23/99	79	1.996			
**	1/23/99	79	1.842	2.294	0.653	
44	2/3/99	89	2.608			
45	2/3/99	89	0.073			
46	2/3/99	89	0.041	0.907	1.473	
48	2/10/99	96	0.031			
49	2/10/99	96	0.102			
50	2/10/99	96	3.387	1.173	1.917	
52	2/22/99	108	3.457			
53	2/22/99	108	2.672			
54	2/22/99	108	0.004	2.044	1.810	
50	3/8/99	123	2.314			
51	3/8/99	123	2.908			
52	3/8/99	123	2.749	2.657	0.308	
55	3/30/99	145	2.986			
56	3/30/99	145	2.956			
57	3/30/99	145	2.591	2.844	0.220	

Table A-16. MTBE concentration in MLS-2 control microcosms; only MTBE added.

Vial #	date	Time (days)	MTBE (mg/L)	AVG	STDEV
101	11/24/98	0	4.589		
102	11/24/98	0	4.636	4.613	0.033
103	11/29/98	5	2.802		
104	11/29/98	5	2.640	2.721	0.115
105	12/5/98	12	3.308		
106	12/5/98	12	3.089	3.199	0.155
108	12/11/98	18	3.529		
109	12/11/98	18	NA	3.529	0.000
110	12/19/98	26	3.025		
111	12/19/98	26	2.821	2.923	0.144
112	12/28/98	35	3.673		
113	12/28/98	35	4.139	3.906	0.330
115	1/5/99	43	2.684		
116	1/5/99	43	3.071	2.878	0.274
117	1/16/99	54	3.966		
118	1/16/99	54	3.689	3.828	0.196
119	1/22/99	60	3.659		
120	1/22/99	60	3.566	3.613	0.066
**	1/23/99	61	3.686		
**	1/23/99	61	3.121	3.404	0.400
121	2/3/99	71	4.581		
122	2/3/99	71	4.733	4.657	0.107
123	2/10/99	78	2.673		
124	2/10/99	78	2.725	2.699	0.037
125	2/22/99	90	4.777		
126	2/22/99	90	3.747	4.262	0.728
127	3/16/99	113	3.961		
128	3/16/99	113	3.952	3.957	0.006
130		126	4.622		
131		126	4.490	4.556	0.093

Table A-17. MTBE and relative petroleum hydrocarbon concentration in MLS-2 live microcosms microcosms; MTBE and nutrients added.

Vial #	date	Time (days)	MTBE (mg/L)	AVG	STDEV	Relative PH	STDEV
1	1/30/99	0	4.986				
3	1/30/99	0	4.903				
4	1/30/99	0	3.888	4.593	0.611	1.000	0.408
5	2/5/99	6	3.949				
6	2/5/99	6	4.054				
7	2/5/99	6	3.268	3.757	0.427	0.817	0.141
8	2/11/99	12	3.803				
9	2/11/99	12	4.442				
10	2/11/99	12	4.197	4.147	0.322	0.676	0.324
11	2/19/99	20	3.217				
12	2/19/99	20	5.201				
13	2/19/99	20	3.517	3.978	1.069	0.223	0.043
14	2/25/99	26	NA				
15	2/25/99	26	2.568				
16	2/25/99	26	2.225	2.397	0.242	0.244	0.200
17	3/7/99	37	3.043				
18	3/7/99	37	3.098				
19	3/7/99	37	2.943	3.028	0.079	0.296	0.380
20	3/21/99	51	0.190				
21	3/21/99	51	1.955				
22	3/21/99	51	2.709	1.618	1.292	0.195	0.158
23	3/30/99	60	2.466				
24	3/30/99	60	2.270				
25	3/30/99	60	2.126	2.287	0.171	0.248	0.069
26	4/12/99	73	2.282				
27	4/12/99	73	3.232				
28	4/12/99	73	1.776	2.430	0.739	0.355	0.140
34	4/26/99	87	3.036				
35	4/26/99	87	2.969				
36	4/26/99	87	3.072	3.026	0.052	NA	NA
29	5/12/99	103	2.706				
30	5/12/99	103	3.078				
31	5/12/99	103	2.557	2.780	0.268	NA	NA

Table A-18. MTBE concentration in MLS-2 control microcosms; MTBE and nutrients added.

Vial #	date	Time (days)	MTBE (mg/L)	AVG	STDEV
101	1/30/99	0	5.178		
102	1/30/99	0	5.700	5.439	0.369
103	2/5/99	6	4.029		
104	2/5/99	6	5.450	4.739	1.004
105	2/11/99	12	4.775		
106	2/11/99	12	5.471	5.123	0.492
107	2/19/99	20	3.983		
108	2/19/99	20	5.387	4.685	0.993
115	2/25/99	26	4.701		
111	2/25/99	26	4.986	4.843	0.201
111	3/7/99	37	4.050		
112	3/7/99	37	3.784	3.917	0.189
113	3/16/99	46	3.871		
114	3/16/99	46	4.515	4.193	0.455
116	4/12/99	73	5.909		
118	4/12/99	73	1.999	3.954	2.765
119	4/26/99	87	3.165		
120	4/26/99	87	4.326	3.745	0.821
**	5/12/99	103	2.403		
**	5/12/99	103	4.518	3.460	1.495

VITA

Jeff Radcliffe Zoeckler was born on August 25, 1974 in Richmond, Virginia. He graduated from Monacan High School in June of 1992. In August of 1992, he enrolled at Radford University where he studied computer science. In August of 1993, he transferred to Virginia Polytechnic Institute and State University, where he studied geology. In December of 1996, he received a B.S. in Geological Sciences from Virginia Polytechnic Institute and State University. In August of 1997, he began work on his Master's degree in Environmental Engineering. In August of 1999, he will begin work as an engineer with Malcom Pirnie in Tucson, Arizona.