POTENTIAL FOR INTRINSIC BIODEGRADATION OF METHYL *TERT*-BUTYL ETHER (MTBE) IN THE UNCONFINED AQUIFER UNDERLYING THE DOVER NATIONAL TEST SITE, DOVER AIR FORCE BASE, DE

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Abstract

The potential for natural attenuation of methyl tert-butyl ether (MTBE) was investigated in laboratory microcosms constructed with aquifer sediment collected from contaminated and uncontaminated zones of the unconfined aquifer underlying the Dover National Test Site (DNTS), Dover Air Force Base, DE. Uncontaminated groundwater collected from an area outside of the MTBE plume was spiked with 2.5-3.5 mg/L neat MTBE and added to sediments. Microcosms were incubated under aerobic and anaerobic conditions while monitoring the disappearance of MTBE and the concentration of potential electron acceptors (O_2, NO_3^{2-}, SO_4^{2-}, and Fe^{2+}) over a 209-235 day incubation period. There was no measurable loss of MTBE detected under any of the incubation conditions tested. Despite evidence of denitrification and iron reduction in anaerobically-incubated microcosms, microbial activity was not associated with MTBE degradation. Phosphate and nitrogen amendments to microcosms were found not to stimulate MTBE degradation. Polymerase chain reaction (PCR) and denaturing gradient gel electrophoresis (DGGE) was also conducted in an attempt to characterize and compare microbial populations indigenous to MTBE-contaminated and MTBE-uncontaminated zones of the aquifer. Unfortunately the DNA extraction protocol used in this study was unsuccessful in isolating enough DNA from sediment. Microcosms results indicate that intrinsic MTBE biodegradation is limited and that natural attenuation may not be a good strategy for remediating this aquifer.

1 Introduction

Methyl *tert*-butyl ether (MTBE) was first introduced in the United States in the 1970's and by mid-1990 was being used extensively as a fuel additive to reduce atmospheric carbon monoxide levels (Sulfita and Mormile, 1999; Hunkeler et al., 2001). The widespread use of MTBE in the past 30 years has led to significant contamination of groundwater due in part to point sources such as the leakage of underground gasoline storage tanks. A recent survey conducted by Johnson et al. (2000) estimates that between 5-10% of potable water supplies in the United States have detectable levels of MTBE. While the potential toxicity of MTBE has not been fully investigated, it is currently considered a suspected human carcinogen (Squillance et al., 1997). Due to its pervasiveness in potable groundwater supplies the United States Environmental Protection Agency (USEPA) has recommended terminating its use and identifying remediation strategies for removing it from potable water supplies.

One approach to remediating contaminated aquifers is intrinsic bioremediation. Intrinsic bioremediation is a passive clean up technology that relies on microbial processes to degrade environmental contaminants. In combination with other processes such as dilution, dispersion, sorption,

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and volatilization, biodegradation can result in the natural attenuation of contaminants in the saturated subsurface. In order to determine whether intrinsic bioremediation is a viable option one must demonstrate that biodegradation is occurring. While intrinsic bioremediation has been demonstrated as a viable strategy for removal of the more soluble components of gasoline (benzene, tolulene, ethyl-benzene, and xylenes) from groundwater, intrinsic bioremediation of MTBE under ambient groundwater conditions is less clear. In early studies investigating the biodegradability of MTBE, MTBE was found to be resistant to microbial degradation (Sulfita and Mormile, 1999; Fiorenza and Rifai, 2003). MTBE is thought to be resistant to microbial degradation because of the tertiary carbon atom structure and presence of the ether bond (Fiorrenza and Rifai, 2003). Compounds containing ether bonds, for example lignin, are difficult to degrade because a large investment of energy is required by the microorganisms to break this linkage (White et al., 1996). More recently, studies investigating biodegradability of MTBE have reported varying degrees of degradation under both aerobic and anaerobic conditions (Salanitro et al., 1994; Borden et al., 1997).

In a study conducted by Bradley et al. (2001a), mineralization of [^{14}C]-MTBE to $^{14}CO_2$ (15%-66%) was observed within 50 days in aerobic surface-water sediments with previous exposure to MTBE and in sediment without previous exposure to MTBE. Similarly, in a study using mixed cultures, Pruden et al. (2001) demonstrated 99.9% removal of MTBE in aerobically maintained bioreactors with MTBE supplied as the sole carbon source and in aerobically maintained biorectors containing MTBE in combination with other common groundwater contaminants (diethyl ether, diisopropyl ether, ethanol, or benzene, tolulene, ethylbenzene, and xylenes). These data indicate that there is potential for intrinsic bioremediation of MTBE in aerobic environments.

Under anaerobic conditions the degree to which MTBE was degraded was dependent on available electron acceptors. Bradley et al. (2001b) demonstrated that MTBE was degraded under aerobic conditions and a range of anaerobic terminal electron-accepting conditions in microcosms containing surface-water sediments. The degree to which MTBE was mineralized was dependent on the available electron acceptor with mineralization of [^{14}C]-MTBE to $^{14}CO_2$ increasing in the order of SO_4^{2-} (9-28%), Fe(III) (14%), and Mn(IV) (6-11%) less than NO_3^{2-} (23-75%) less than O_2 (58-90%) (Bradley et al., 2001b). Finneran et al. (2001) monitored the potential for MTBE degradation in petroleum-contaminated sediments under anaerobic iron reducing conditions. No loss of MTBE (50mg/L) was observed after 275 days incubation in sediment amended with Fe(III) but when humic acids were added, MTBE was degraded below the detection limit (1 mg/L) within the 275 day incubation period. Humic acids are believed to stimulate MTBE degradation by acting as an electron shuttle between iron (Fe(III)) reducing microorganisms and insoluble iron oxides (Finneran et al., 2001). MTBE degradation has also been observed using surface-water sediments incubated under anaerobic denitrifying conditions (Bradley et al., 2001c). In this study 25% of [^{14}C]-MTBE was mineralized to $^{14}CO_2$ by 77days. No further degradation of MTBE was observed after 77 days.

While investigating the distribution of petroleum hydrocarbons in groundwater in the shallow, unconfined aquifer underlying the Dover National Test site (DNTS) at Dover Air Force Base, significant concentrations of MTBE (0.011-1.43 mg/L) were measured (Air Force Center for Environmental Excellence, 1999). MTBE was also detected in the vicinity of the DNTS during routine groundwater compliance sampling. The MTBE plume in the vicinity of the DNTS was later partially delineated and MTBE concentrations determined during a study conducted by the United States Geological Society (USGS) (Stewart et al., 2001). The objective of the present study was to evaluate the potential for intrinsic bioremediation of this MTBE plume. MTBE degradation was monitored in laboratory microcosms containing MTBE-contaminated aquifer sediment and MTBE-uncontaminated aquifer

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sediment prepared with groundwater containing MTBE. The microcosms were incubated under aerobic and anaerobic conditions while monitoring MTBE concentrations and changes in the levels of different electron acceptors (O_2, NO_3^{2-}, SO_4^{2-}, and Fe^{2+}). In addition, an attempt was made to characterize microbial populations indigenous to the unconfined aquifer using polymerase chain reaction (PCR) and denaturing gel electrophoresis (DGGE). In this molecular-based procedure, bacterial 16s rDNA is isolated from a sample and amplified using universal eubacterial and archaebacterial primers. The resulting PCR products are separated out on a denaturing gel producing a DNA profile. The DNA profile provides information about the number of species present (the number of visible DNA bands) and the abundance of each species (intensity of each DNA band) (Iwamoto et al., 2000). Because it does not rely on culturing microorganisms, this procedure provides more information about microbial community structure than traditional cultivation techniques do. It has been estimated that when traditional cultivation techniques are used less than 1% of the microorganisms in a community can be identified (Steffan et al., 1988).

2 Materials and Methods

2.1 Aquifer Sediment and Groundwater Collection

The potential for intrinsic biodegradation of MTBE was monitored in both MTBE-contaminated and MTBE-uncontaminated aquifer sediments. The uncontaminated sediment has no known prior exposure to MTBE or other petroleum hydrocarbons. Aquifer material was collected from two distinct locations (MTBE contaminated area and an area outside of the MTBE plume) in the unconfined aquifer using a Cone Penetrometer Drill Rig (CPT) and a Vertek soil sampler (Vertek, South Royalton, VT) (Figure 1). Aquifer material was collected over a depth of 6.0-8.0 m below ground surface in both locations for aerobic studies and over a depth of 8.0-10.0 m below ground surface in both locations for anaerobic studies. Sediment was aseptically removed from the core barrels, homogenized, and stored at 4 °C for no longer than 48 hr in sterile canning jars. Jars containing anaerobic sediment were deaerated by flushing with nitrogen gas before transferring sediment. All manipulations of anaerobic sediment were conducted in an anaerobic chamber (Coy Laboratories, Grass Lake, MI). All microcosms were prepared with MTBE-uncontaminated groundwater pumped from a depth of 10-11m from well ALP3 located outside the MTBE plume using a peristaltic pump (Figure 1).

2. 2 Laboratory Microcosms Studies

2.2.1 Microcosm Set Up

Aerobic microcosms were constructed in 120-ml serum bottles by aseptically dispensing into the bottles 40 g of MTBE-contaminated or MTBE-uncontaminated aquifer material and 60-ml of air-saturated groundwater containing approximately 3.5 mg/L neat MTBE (Sulpelco, Bellfonte, PA) with air in the headspace. Bottles were sealed with Teflon-lined septum and aluminum crimp top caps. Microcosms were incubated at room temperature in the dark and shaken periodically. A total of 24 aerobic microcosms were prepared for both contaminated and uncontaminated sediments. Nutrient amended aerobic microcosms were also prepared as previously described with the following exceptions. Sterile ammonia (0.1g/L as (NH4)2SO3) and phoshate (0.1g/L KH2PO4, and 0.1g/L K2HPO4) amendments were aseptically added to groundwater prior to dispensing into bottles. A total of 12 nutrient-amended aerobic microcosms were prepared for contaminated and uncontaminated sediments.

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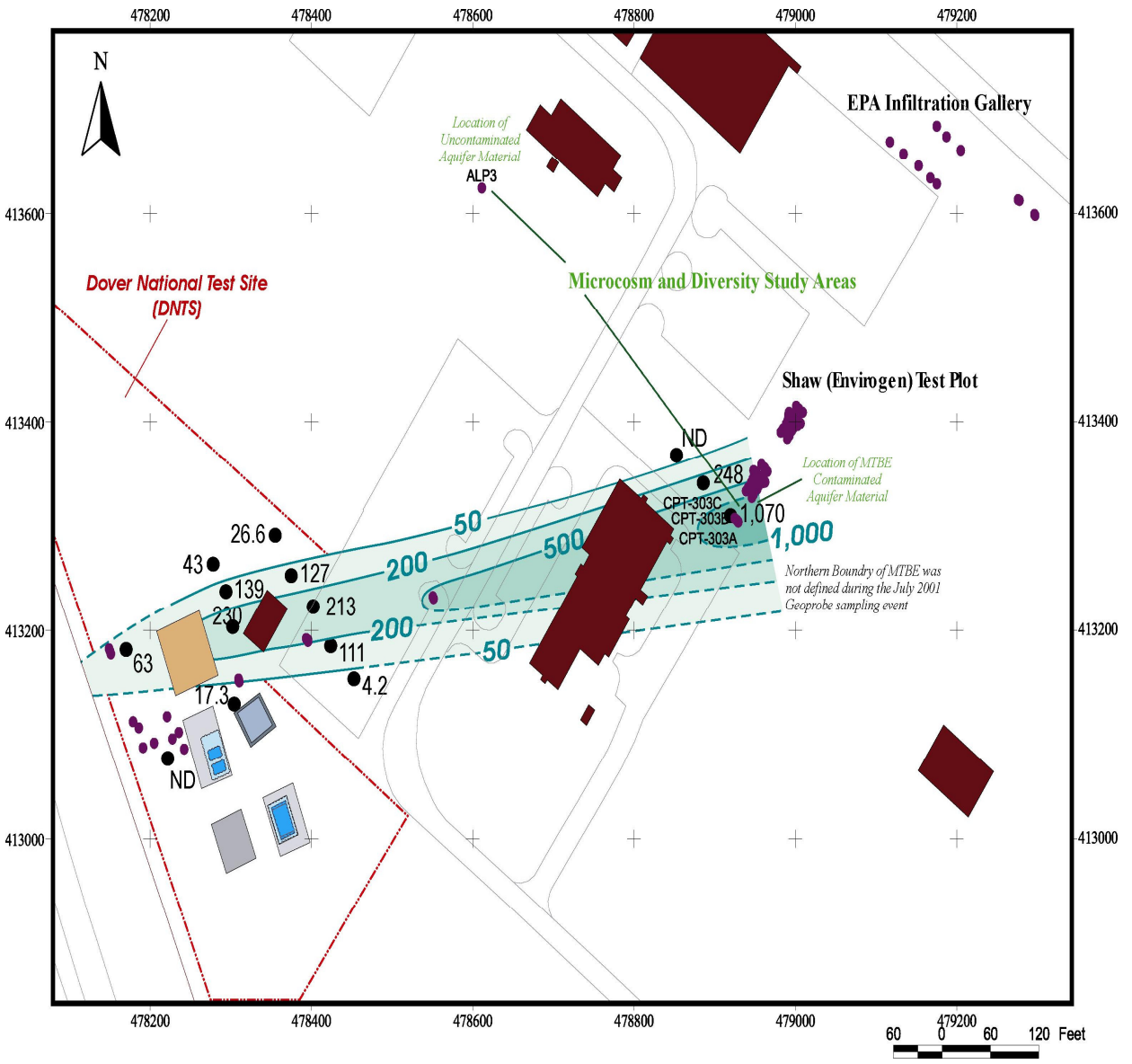

Figure 1: Site map showing locations of sediment collection and well from which MTBE-contaminated groundwater was collected

Anaerobic microcosms were constructed in 120-ml serum bottles by aseptically dispensing into the bottles 40g of MTBE-contaminated or MTBE-uncontaminated aquifer material and 120-ml nitrogen-saturated groundwater containing approximately 2.5 mg/L neat MTBE with nitrogen in the headspace. Bottles were sealed with Teflon-lined septum and aluminum crimp top caps. All manipulations and incubation were conducted at room temperature in an anaerobic chamber. A total of 24 anaerobic bottles were prepared for contaminated and uncontaminated sediments. As previously described for aerobic microcosms, nutrient-amended anaerobic treatments were also constructed. Sterile ammonia [0.1g/L as $(NH_4)_2SO_3$] and phosphate (0.1g/L KH_2PO_4 and 0.1g/L K_2HPO_4) solutions were aseptically added to deaerated groundwater prior to dispensing into bottles. A total of 24 nutrient-amended anaerobic microcosms were prepared for contaminated and uncontaminated sediments. Because background

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nitrate concentrations are low in the unconfined aquifer, additional anaerobic microcosms were prepared as previously describe but a sterile nitrate solution (0.1g/L KNO_3) was added to nitrogen-saturated groundwater before dispensing it into the bottles. A total of 12 nitrate-amended microcosms were prepared for contaminated and uncontaminated sediments.

Sterile control microcosms were prepared for all treatments by autoclaving aquifer sediment for 1 hr on three consecutive days, and adding sterile groundwater containing $HgCl_2$ (250 mg/L) and appropriate amendments. A total of 12 sterile control microcosms were prepared for all treatments.

At various times, triplicate treated and sterile control microcosms were sacrificed and analyzed. The total length of incubation was 209 days for aerobic microcosms and 235 days for anaerobic microcosms. All aerobic microcosms were analyzed for MTBE and dissolved oxygen (DO). Anaerobic microcosms without nitrate amendment were sacrificed and analyzed for DO, MTBE, SO_4^{2-}, and Fe^{2+}. Nitrate amended anaerobic microcosms were sacrificed and analyzed for MTBE, DO, and NO_3^{2-}. Dissolved oxygen, MTBE, NO_3^{2-}, SO_4^{2-}, and Fe^{2+} concentrations measured for experimental microcosms were compared to those measured in sterile control microcosms.

2.2.2 Analytical Methods

Groundwater was analyzed for MTBE (EPA 8021 VOC method) using a gas chromatograph (GC-HP 6890) (Hewlett-Packard, Palo Alto,CA) equipped with a flame ionization detector (FID) and a 60 m fused silica Supelco capillary column SPB-624 (Supelco, Bellefonte, PA). At every sampling event, 25 ml of groundwater were transferred to 25 ml glass teflon-lined screw cap vials containing two drops of HCL (50% v/v) to preserve samples until analysis. For MTBE analysis, 5ml were transferred to a 20 ml headspace vial. Vials were heated for 1 min (aerobic microcosms) or 5 min (anaerobic microcosms) prior to injecting 3ml of headspace onto the column. Detection limit for MTBE using this method is 0.5 µg/L.

Dissolved oxygen was monitored in both aerobic and anaerobic microcosms using an O_2 specific probe (Microelectrodes, Inc., Bedford, NH). After 25ml sample was removed for MTBE analysis, the O_2 probe was immersed in the remaining groundwater and left until a stable reading was obtained. Measurements were reported as % dissolved oxygen and later converted to mg/L using the following equations:

Solubility (moles/L) = (a/22.414)×(760-p)/760)×(r%/100)

where: a=absorption coefficient of gas at temperature
p=vapor pressure of water at temperature
r%=actual reading in percent oxygen

mg/L Oxygen = ((moles/L)×(32))/0.001

Nitrate, sulfate, and ferrous iron concentrations were monitored using a Hach photometer (Hach Company, Loveland, CO). After measuring dissolved oxygen, 25 ml aliquots were removed and analyzed for SO_4^{2-}, Fe^{2+}, or NO_3^{2-} as appropriate. The Cadmium Reduction method (detection range 0-30 mg/L) was used to measure NO_3^{2-}, the Sulfa Version 4 Method (detection range 0-70 mg/L) was used to measure SO_4^{2-}, and the Phenanthroline Method (detection range 0-3.0 mg/L) was used to measure Fe^{2+} (Methods adapted from the "Standard Methods for the Examination of Water and Wastewater", 1992).

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2.3 Diversity Studies

2.3.1 Nucleic Acid Extraction

Extraction of nucleic acids from a 100 g each of MTBE-contaminated and MTBE-uncontaminated aquifer sediment was attempted. Aquifer material was aseptically transferred to sterile 250 ml media bottles containing 75 ml sterile phosphate buffer (0.1M, pH 7.0) (Gerhardt et al., 1994) and 750 µl SDS. Bottles were shaken at 150 rpm for 1 hr. After shaking, large sediment particles were allowed to settle out prior to transferring the supernatant into sterile 50 ml centrifuge bottles (Fisher Scientific, Pittsburg, PA). To remove additional sediment, samples were centrifuged at 1000 rpm for 5.0 min. Resulting supernatant was pre-filtered through Whatman GFB glass fiber filters (Fischer Scientific, Pittsburg, PA) then filtered through 0.2 µm filters to trap suspended bacteria. Filters were transferred to sterile 1.5 ml microcentrifuge and DNA was extracted as described by Ausubel et al. (1988).

Bacterial 16s rRNA genes were amplified by the polymerase chain reaction (PCR) using eubacterial 16s primers Eub358f, Eub517r, and Eub907r (Murray et al., 1996) and Archaeal 16s primers Arch21f and Arch958r (DeLong, 1992). For amplification of eubacterial 16s rRNA genes PCR conditions were as follows: initial denature at 94°C for 3 min, denaturation, at 94°C for 30s, and touchdown primer annealing from 65 to 55°C (decrease 1°C each cycle for 10 cycles), primer annealing at 55°C for 30s for the next 20 cycles, primer extension at 72°C for 30s and a final extension for 5 min at 72°C. PCR conditions for archaebacterial primers were as follows: initial denature at 95°C for 3 min, denaturation at 95°C for 1.5 min, annealing at 55°C for 1.5 min, and extension at 72°C for 1.5 min for a total of 30 cycles. To confirm amplification of target DNA, PCR product was loaded onto 1% agarose gels, run at 90V for 2h then stained with ethidium bromide. A wide range DNA marker (Sigma, Location) was used to ensure that the appropriate size DNA had been amplified.

Nucleic acid extraction was also conducted on bacterial colonies previously isolated directly from sediment. This aquifer material was removed from the contaminated zone of the aquifer (as previously described) from a depth of 6 m below ground surface. Aquifer sediment (5g) was suspended in 45 ml sterile phosphate buffer (pH 7.0), shaken at 150 rpm for 30 min at room temperature, serially diluted then plated onto nutrient agar (NA) plates. After 5 days incubation at room temperature, different isolated colonies (based on gram stain and colony morphology) were restreaked onto to NA plates and used for control samples. Nucleic acids were extracted from the control cultures as previously described.

3 Results and Discussion

3.1 Laboratory Microcosm Studies

The potential for intrinsic biodegradation of MTBE in the unconfined aquifer was monitored in MTBE-contaminated and MTBE-uncontaminated aquifer material under aerobic and anaerobic incubation conditions. There was no evidence of MTBE degradation over 209 days incubation in aerobic microcosms containing either contaminated or uncontaminated aquifer material (Figure 2a and b). While aerobic degradation of MTBE has been observed by pure cultures (Hatzinger et al., 2001; Salanitro et al., 2001) as well as in mixed consortia from bioreactors (Salanitro et al., 1994) and enrichments from aquifer sediments (Hunkeler et al., 2001), the results obtained in the present study are consistent with earlier studies in which MTBE was found to be recalcitrant (Jensen and Arvin, 1990). In previous microcosm studies where degradation was observed under aerobic incubation conditions, the rate of

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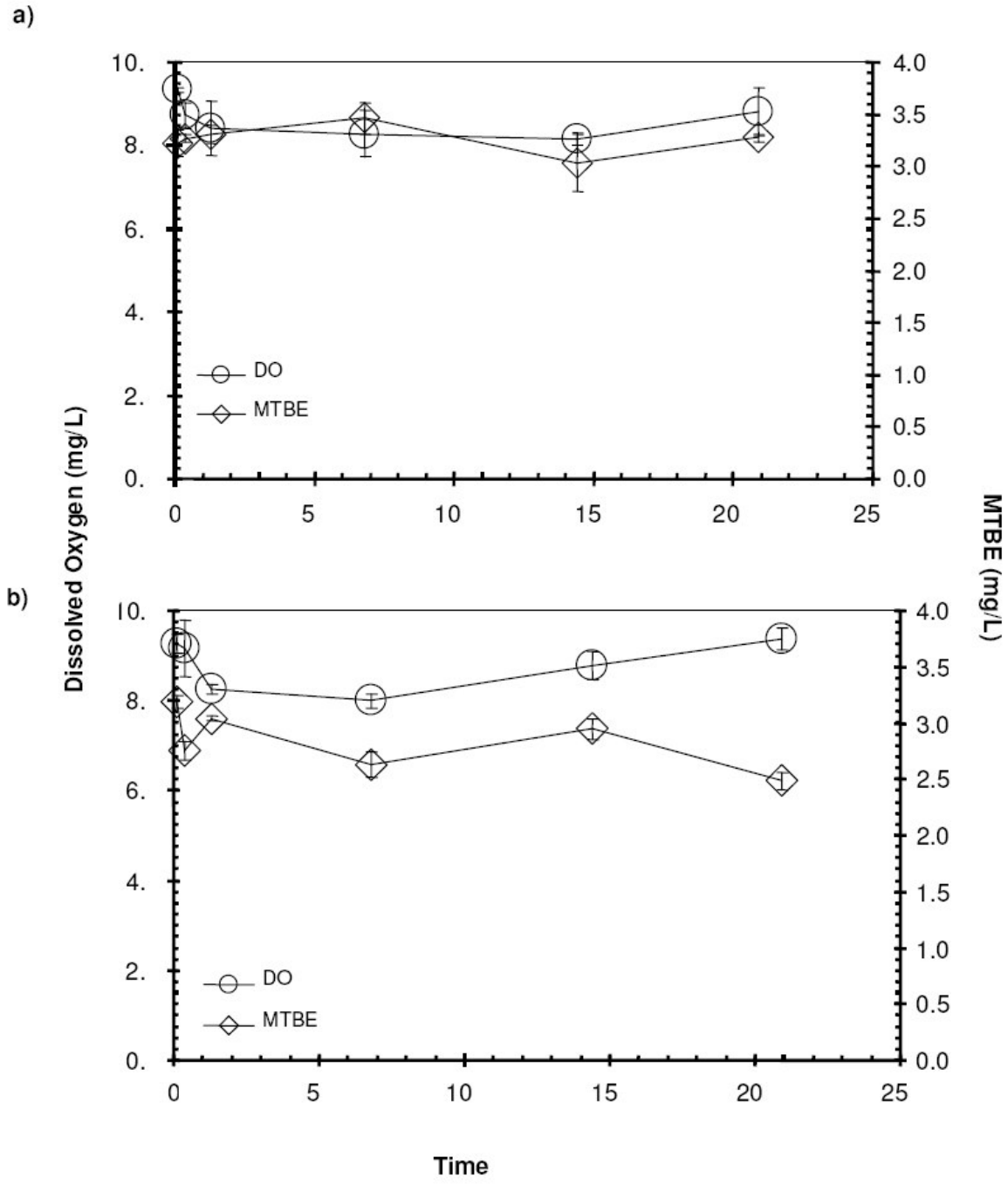

Figure 2: Aerobic, unamended microcosms using a) contaminated aquifer material and b) uncontaminated aquifer material

degradation was most rapid in the absence of other metabolizable carbon sources (Schirmer et al., 2003). In this study, the persistance of MTBE is not likely due to competing substrates; but is more likely the result of a lack of bacteria capable of degrading MTBE. This finding is supported by O_2 data. Dissolved oxygen concentrations remained stable at 9.0 mg/L during the incubation period indicating a lack of

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aerobic microbial activity in both contaminated and uncontaminated sediments incubated under aerobic conditions. The lack of DO uptake may indicate that an easily degradable carbon source is not present in the uncontaminated sediment. It is unclear why DO was not utilized in the contaminated sediment because it is likely that alternative degradable carbon sources are present in this material. Because the aquifer in the vicinity of the DNTS is anaerobic, it is possible that aerobic microorganisms required more time than was provided in this study (longer than 209 days) to become metabolically active.

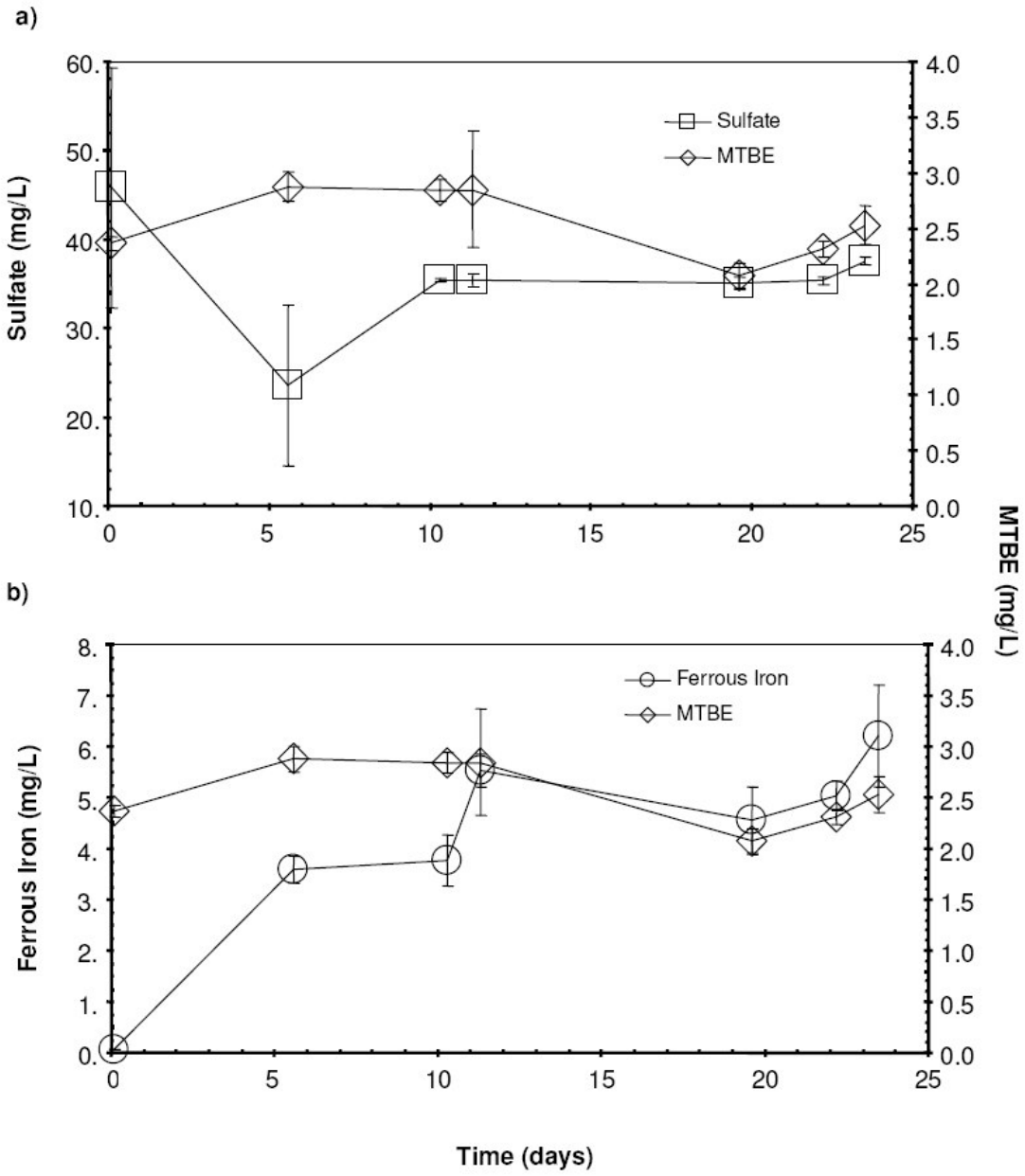

Figure 3: Anaerobic, unamended microcosms using contaminated aquifer material showing a) sulfate concentrations and b) ferrous iron concentrations during the incubation period

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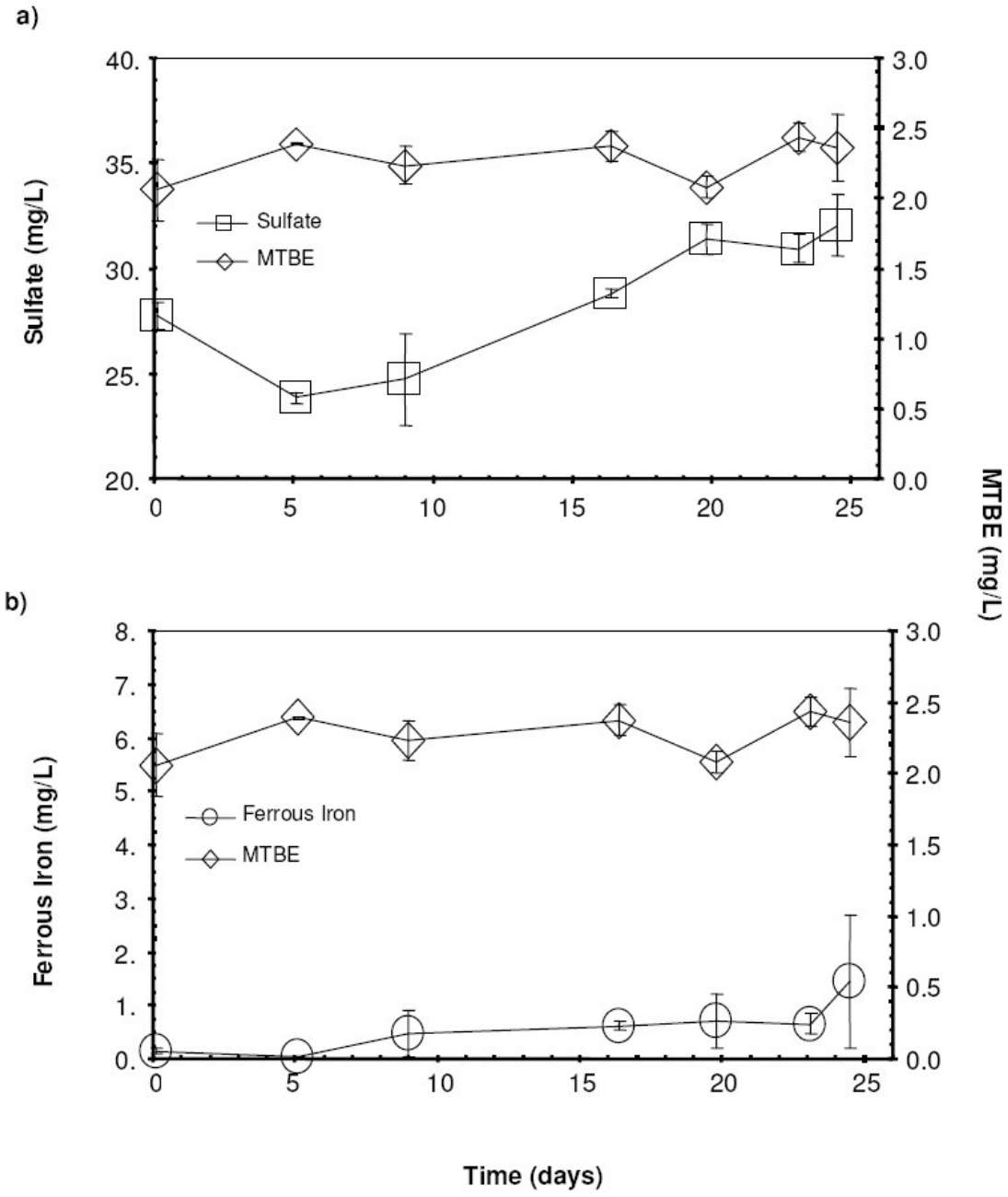

Figure 4: Anaerobic, unamended microcosms using uncontaminated aquifer material showing a) sulfate concentrations and b) ferrous iron concentrations during the incubation period

Similar results were found for aquifer sediments incubated under anaerobic conditions. There was no loss of MTBE over 229 days of incubation for microcosms prepared with contaminated aquifer material (Figure 3a and b). Sulfate concentrations remained stable during the incubation period (mean sulfate concentration = 35.0 mg/l) (Figure 3a), but an increase in Fe^{2+} production was observed (0.6 at day 1 up to 6.20 mg/L at day 235) (Figure 3b). Ferrous iron production is indicative of ferric iron (Fe^{3+}) reduction due to the activity of iron-reducing bacteria; however, in this experiment it was not associated

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with MTBE degradation. These data suggest that there are alternative carbon sources available in the contaminated sediment for iron-reducing bacteria to metabolize. The source of alternative carbon sources may be components of jet fuel from an earlier release (Parsons Engineering Science Inc., 1999).

There was no detectable loss of MTBE after 231 days incubation for anaerobic microcosms prepared with uncontaminated aquifer material (Figures 4a and 4b). Similar to anaerobic microcosms prepared with contaminated aquifer material, SO_4^{2-} concentrations remained steady (mean sulfate concentration = 28.5 mg/L) during the incubation period (Fig. 4a). While a similar increasing trend over the incubation period was observed for Fe^{2+} concentrations, the concentrations were much lower than those observed for microcosms containing contaminated aquifer material (0.15-0.7 mg/L) (Fig. 4b).

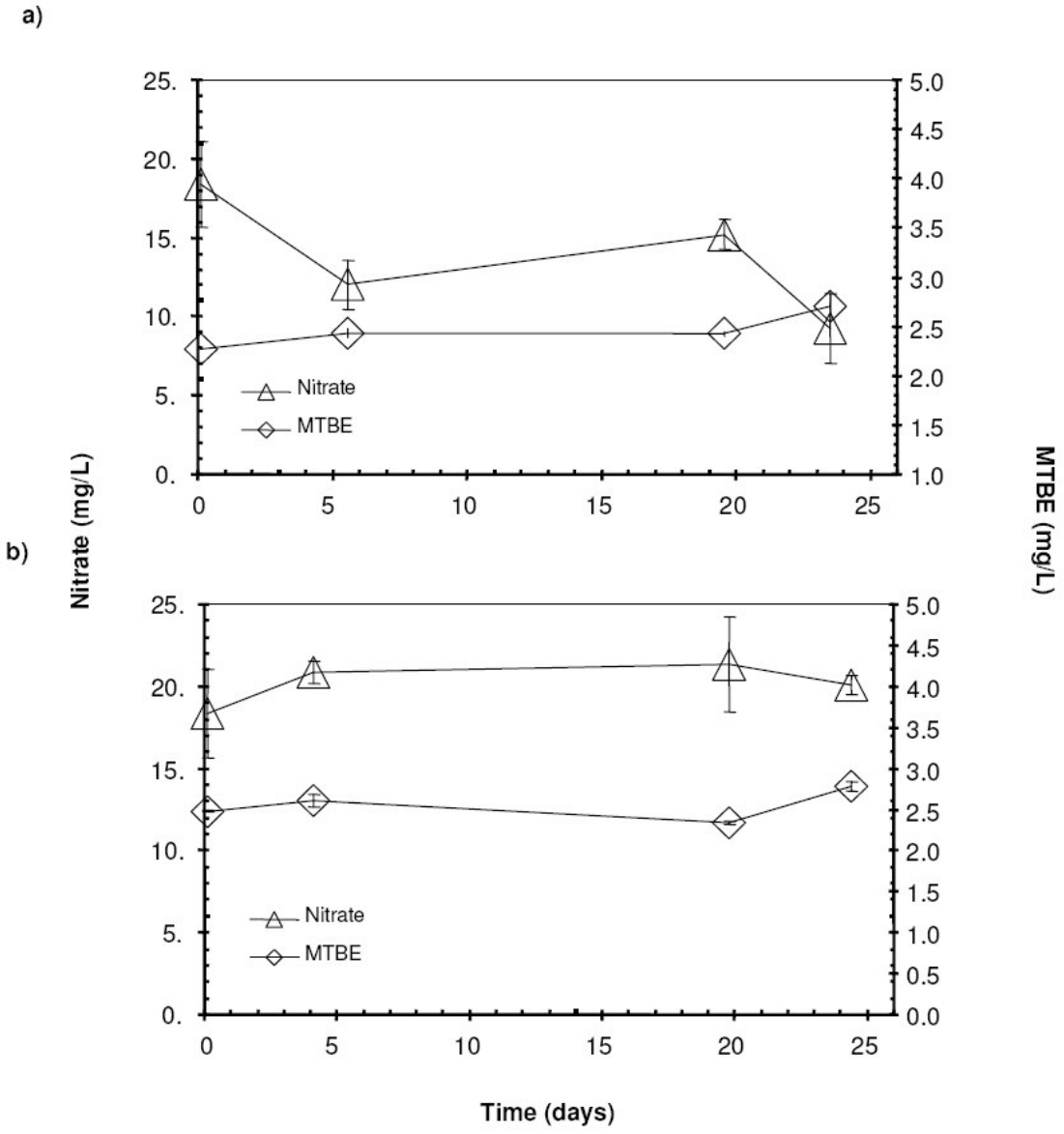

Figure 5: Anaerobic, nitrate-amended microcosms using a) contaminated aquifer material and b) uncontaminated aquifer material

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Ferrous iron concentrations are lower in these sediments because they were retrieved from an area of the aquifer that has had no known previous exposure to jet fuel, and therefore apparently does not contain utilizable carbon sources. Similarly, while there was no detectable loss of MTBE during the 235-day incubation period in nitrate-amended microcosms (Figure 5a and b), in microcosms constructed with contaminated aquifer material, NO_3^{2-} concentrations decreased over time (18 mg/L at day 1 down to 9.0 mg/L at day 235), indicating denitrifying activity. These data support the findings that there is a degradable source of carbon in the contaminated zone for denitrifying and iron reducing bacteria.

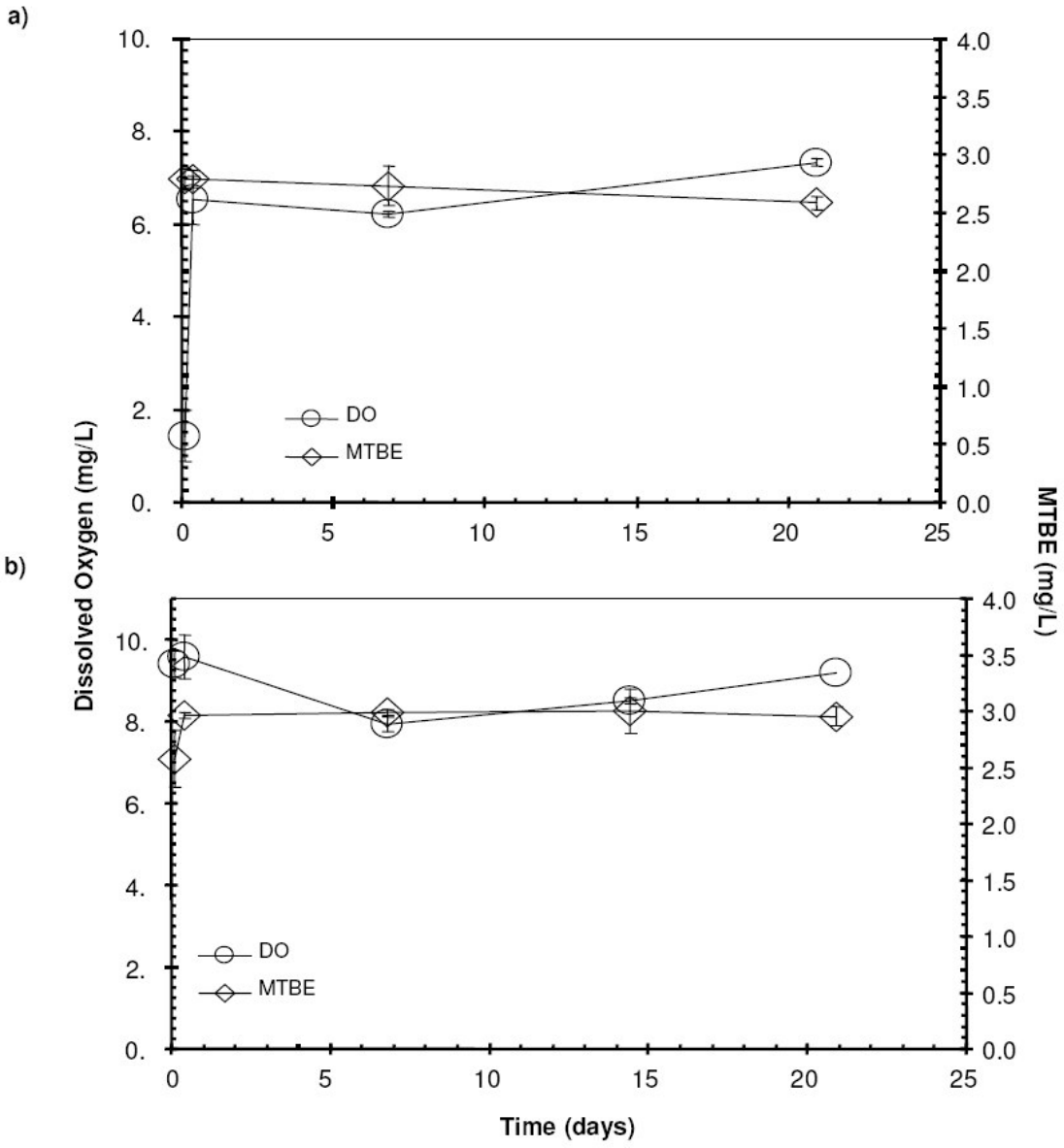

Figure 6: Aerobic, nutrient-amended microcosms using a) contaminated aquifer material, and b) uncontaminated aquifer material

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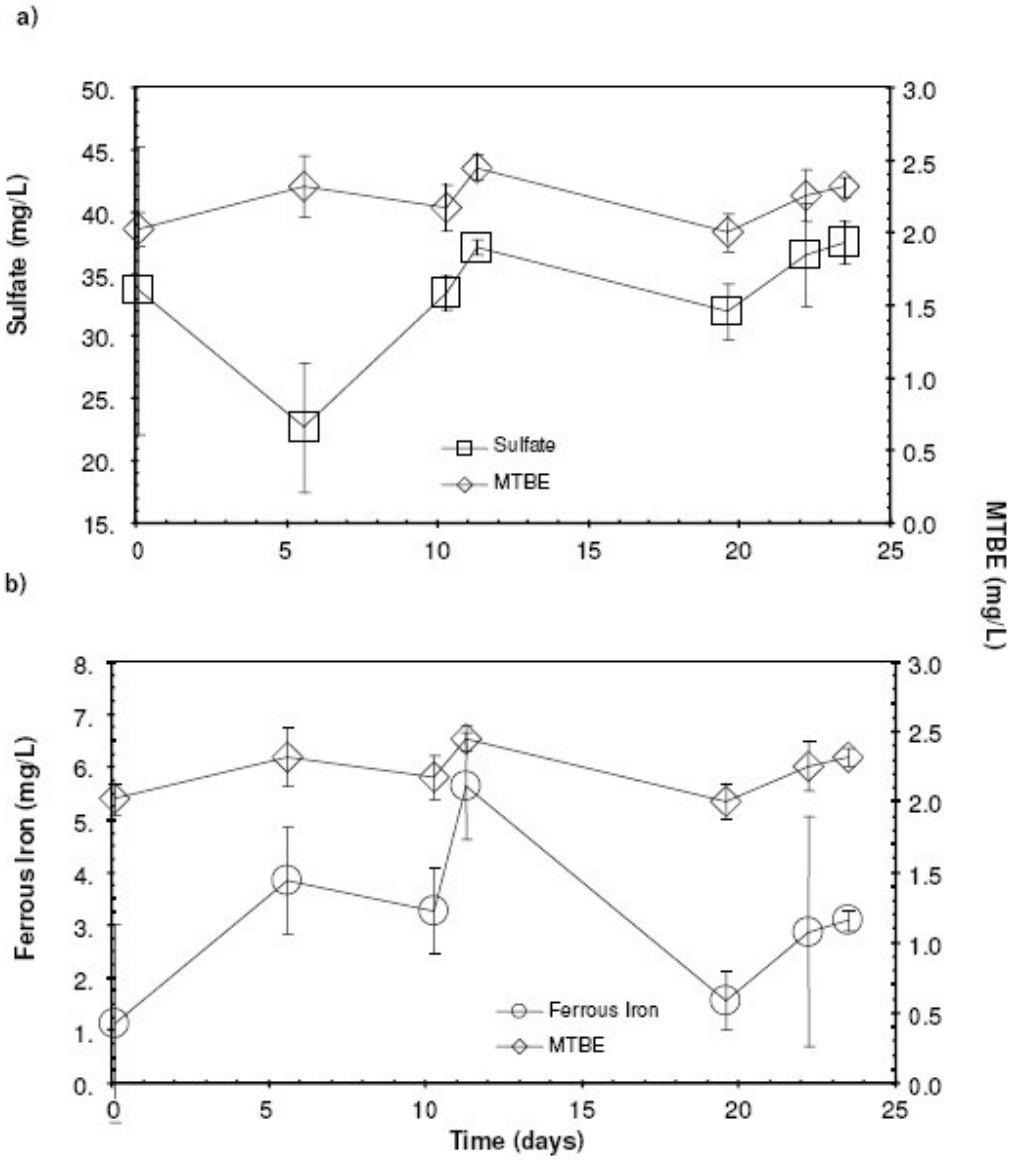

Figure 7: Anaerobic, nutrient-amended microcosms using contaminated aquifer material showing a) sulfate concentrations and b) ferrous iron concentrations during the incubation period

Often saturated subsurface environments do not contain sufficient amounts of essential nutrients required for microbial growth, thereby limiting bacterial numbers and extent of biodegradation (Barbaro et al., 1994). To ensure that the indigenous microbial populations were not nitrogen or phosphate limited, these nutrients were included in the construction of additional microcosms. It was hypothesized that nutrient addition would increase the numbers of bacteria and the amount of MTBE degradation. However, the addition of nitrogen and phosphate amendments to microcosms did not stimulate degradation of MTBE in the aerobic (Figure 6) or anaerobic microcosms (Figures 7 and 8). These data indicate that nitrogen and phosphate limitation is not responsible for the lack of MTBE degradation in this aquifer.

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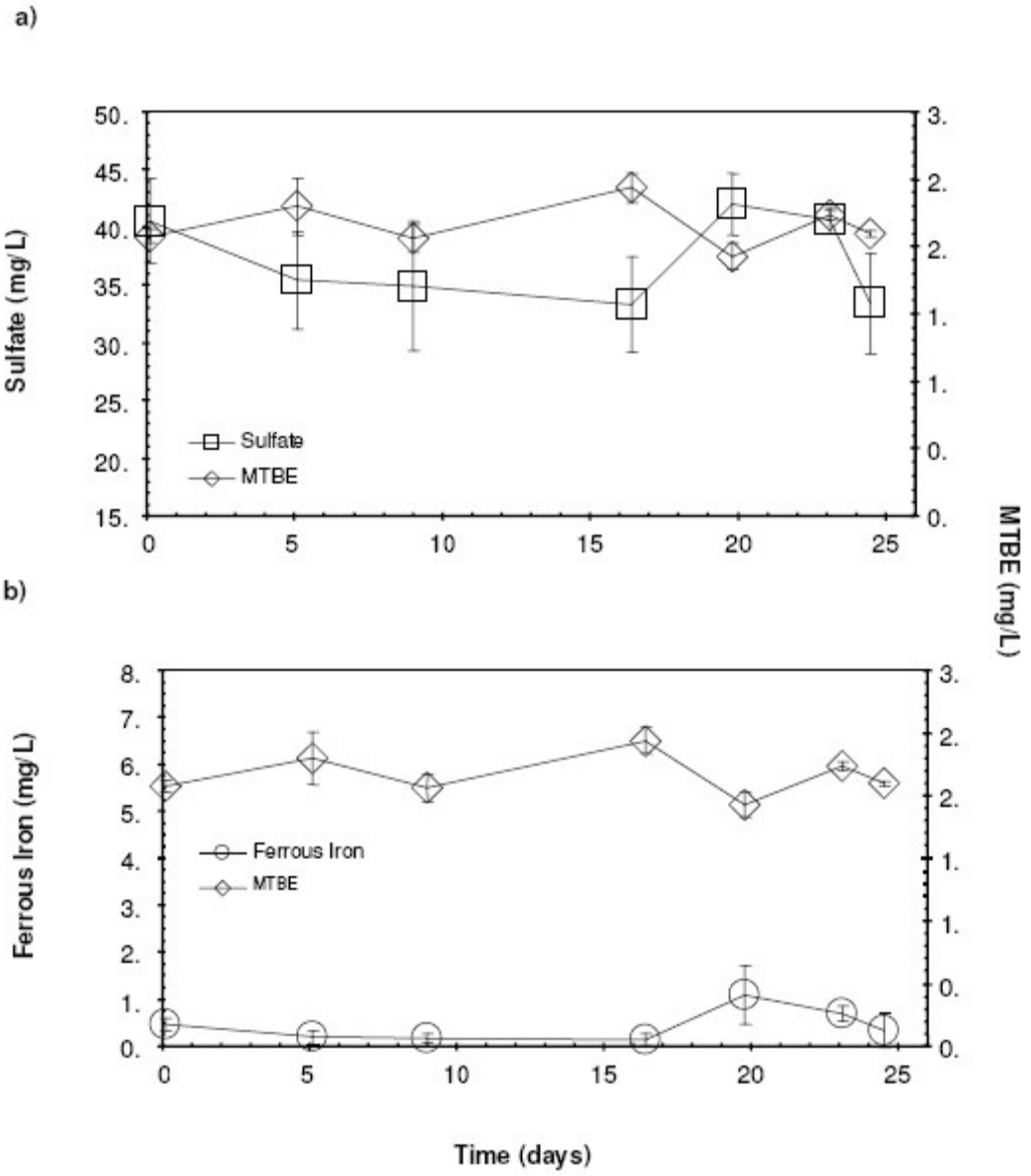

Figure 8: Anaerobic, nutrient-amended microcosms using uncontaminated aquifer material showing a) sulfate concentrations and b) ferrous iron concentrations during the incubation period

3.2 Diversity Studies

PCR and DGGE were conducted to characterize the aquifer microbial community within MTBE-contaminated and MTBE-uncontaminated zones of the unconfined aquifer. The effectiveness of PCR-based techniques for analyzing community structure is dependent on the efficient recovery of DNA from the environmental sample (Steffan et al., 1988). It was anticipated that if MTBE degradation was observed in aquifer sediments, DNA profiles of sediments in which degradation was not observed could be compared and contrasted to DNA profiles of aquifer sediment in which MTBE degradation did occur so that microbial populations important for MTBE degradation could be identified. Even though MTBE

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degradation was not observed, this technique was still conducted as it can provide information for better understanding the microbial ecology of the unconfined aquifer.

Based on gram stain and colony morphology, 11 aerobic bacteria were isolated from contaminated aquifer sediment (Table 1). DNTS isolates 2, 4, 6, 9 and 11 were arbitrarily picked to be controls for nucleic acid extraction, PCR, and DGGE. To evaluate the effectiveness of the nucleic acid extraction procedure PCR-amplified products for control strains and MTBE-contaminated and MTBE-uncontaminated aquifer sediment were loaded on to agarose gels then stained to visual DNA product. The universal eubacterial primer sets EUB358f and EUB 517r, and EUB358f and EUB907r used in PCR produced sufficient product of the appropriate size (159 bp and 549 bp respectively) for the bacterial controls tested (Figure 9). No product was seen for aquifer sediment samples indicating that the nucleic acid procedure used in this study was not successful in extracting enough DNA from aquifer material. Similar results were obtained using archael primers ARCH21f and ARCH958r (data not shown). Because the nucleic acid extraction was unsuccessful, DGGE was not performed.

Table 1: Gram reaction and colony morphology of aerobic bacterial strains isolated on nutrient agar plates

Strain Designation	Gram Reaction	Colony Morphology
DNTS 1	-ve Coccus	Pink
DNTS 2	-ve Diplococcus	Yellow Opaque
DNTS 3	+ve Diplococcus	Orange Opaque
DNTS 4	+ve Rods (long with pointed ends)	Beige Opaque
DNTS 5	-ve Rods (short plump rods)	Beige Opaque
DNTS 6	-ve Rods (in pairs)	Beige Opaque
DNTS 7	-ve Rods (short plump rods)	Orange
DNTS 8	-ve Rods (long with rounded ends)	Beige
DNTS 9	+ve Rod	Beige
DNTS 10	+ve Cocci (in tetrads)	Yellow Opaque
DNTS 11	Variable (in chains)	Beige Opaque

Note: Gram stains were conducted on 5 day old cultures.

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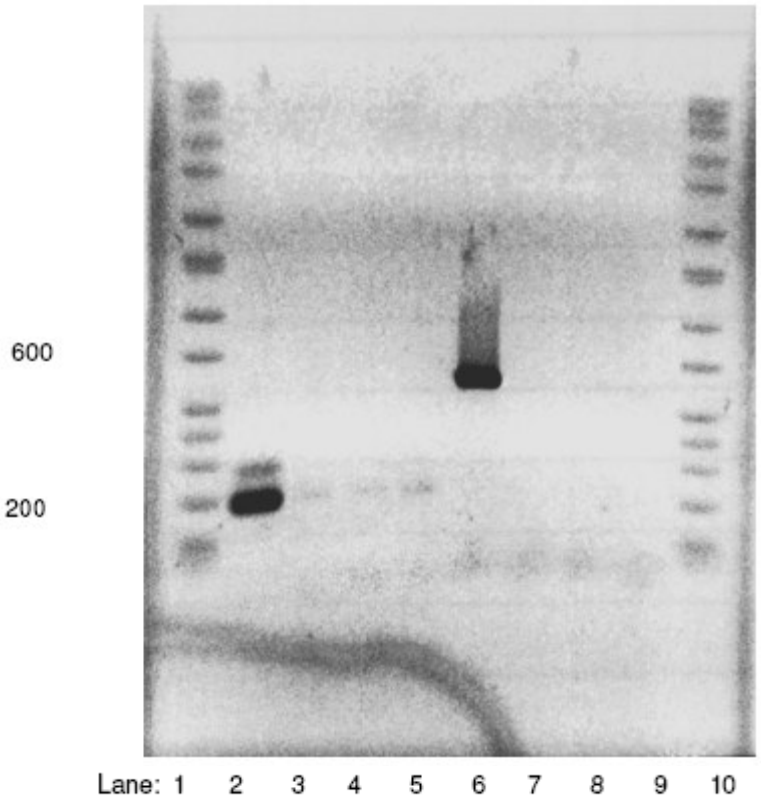

Figure 9: Polymerase chain reaction analysis and gel electrophoresis using eubacterial primers Eub358f and Eub517r, and Eub358f and Eub907r to amplify 16s ribosomal DNA in control bacterial strains, contaminated, and uncontaminated aquifer sediment. Lane 1: molecular marker; Lanes 2-4: PCR with Eub 358f and Eub 517r; Lane 2: DNTS 9; Lane 3: uncontaminated aquifer sediment; Lane 4: contaminated aquifer sediment. Lane 5: negative control. Lanes 6-8: PCR with Eub 358f and Eub 907r; Lane 6: DNTS 9; Lane 7: uncontaminated aquifer sediment; Lane 8: contaminated aquifer sediment; Lane 9: negative control; Lane 10: molecular marker.

Summary

In order to evaluate potential for intrinsic bioremediation of MTBE in the unconfined aquifer underlying the DNTS at Dover Air Force Base, MTBE degradation was monitored in aerobic and anaerobic microcosms prepared with aquifer material collected from MTBE-contaminated and MTBE-uncontaminated zones of the aquifer. Despite evidence of iron and nitrate reduction in anaerobic microcosms, microbial activity was not associated with MTBE degradation. MTBE concentrations remained steady for the duration of the study, suggesting that anaerobic bacteria are not capable of degrading MTBE and that alternate sources of carbon were used as substrates for iron reducing and denitrifying microorganisms. Similarly, MTBE was not degraded under aerobic incubation conditions. Surprisingly, as indicated by steady dissolved oxygen concentrations during the incubation period, there was no evidence of microbial activity in the aerobic microcosms. A longer acclimation period may be necessary for aerobic bacteria to become metabolically active. Addition of nitrogen and phosphate amendments did not stimulate microbial activity or MTBE degradation in any of the experimental

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microcosms suggesting that microbial activity is not inhibited by limited nitrogen or phosphate nutrients. Overall results indicate that the aquifer does not support microbial populations capable of degrading MTBE. There may be additional unknown factors limiting (concentration effects) or inhibiting (metabolic degradation products) MTBE degradation that require further study. At present, the data collected in this study suggest that intrinsic bioremediation would not be a successful MTBE remediation strategy for this aquifer.

Lastly, information regarding microbial community structure in the Dover aquifer was not obtained. Efficient DNA extraction is critical for the success of PCR based methodologies, but the DNA extraction protocol used in this study was unsuccessful. Because information about microbial community structure would be useful for further studies designed to evaluate the potential for MTBE bioremediation in this aquifer, this work will continue.

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