

Degradation of Gasoline Oxygenates in the Subsurface

by

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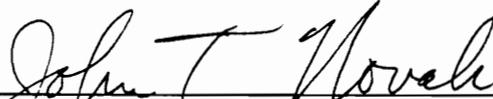
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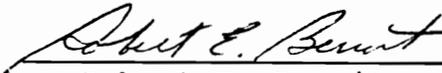
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## (ABSTRACT)

Tertiary butyl alcohol (TBA), methyl tertiary butyl ether (MTBE) and ethyl tertiary butyl ether (ETBE) are compounds with the potential for use as oxygenates in reformulated gasolines. Being relatively soluble in water, these organics, if accidentally discharged into the subsurface, may rapidly spread and pose threats to groundwater. The purpose of this work was to evaluate the biodegradation potential of these oxygenates in soils and to determine the influence of subsurface environments on their degradation.

Biodegradation was evaluated in static soil/water microcosms. Aquifer material was collected from various depths at three sites with different soil characteristics. Potential electron acceptors including  $O_2$  in the form of  $H_2O_2$ , nitrate or sulfate were added to induce the desired metabolism (aerobic respiration, denitrification, sulfate reduction, or methanogenesis). In each metabolic process, the influence of several subsurface environmental factors on biodegradation was investigated.

The data show that biodegradation potential of MTBE, ETBE

and TBA varied substantially with site and depth. TBA was the easiest compound to biodegrade, whereas MTBE was the most recalcitrant. Cleavage of the ether bond is the first and rate-limiting step in the degradation of ETBE and possibly MTBE.

Addition of  $H_2O_2$  caused chemical oxidation of MTBE and ETBE. The chemical oxidation was faster in the organically rich soils, but slower in the organic-poor soils. Soil microorganisms were able to catalyze the cleavage of the ether bond in ETBE but not MTBE. This biological reaction was not significant when chemical oxidation occurred. TBA, on the other hand, was aerobically biodegraded in all soils.

Under denitrifying and anaerobic conditions TBA degradation occurred in all soils but the degradation of ETBE and MTBE was only observed at one of three sites. TBA degradation was enhanced by nutrient addition in the nutrient-poor soil but hindered by the presence of other easily-degraded organic compounds. Degradation of MTBE and ETBE occurred only in soils containing low organic matter with a pH around 5.5. No degradation of MTBE and ETBE was observed in the organic-rich soils and in the organically poor soils, the addition of ethanol inhibited MTBE and ETBE degradation.

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## Chapter 1 Introduction

The Clean Air Act Amendments, which will become effective in 1992, require oxygenates to be added in reformulated gasolines to reduce carbon monoxide emissions. Methyl tertiary butyl ether (MTBE), ethyl tertiary butyl ether (ETBE) and tertiary butyl alcohol (TBA), three gasoline octane boosting chemicals, are likely candidates for use as gasoline oxygenates. Being highly soluble in water and relatively abundant in gasoline, these organics may spread through subsurface systems and pose an immediate threat to groundwater quality when an aquifer is contaminated by fuels.

Research has revealed that subsurface microorganisms are capable of degrading many organic compounds, including many gasoline components (Lee et al., 1988). While studies on some of the gasoline components are extensive, little is known about gasoline oxygenates.

Studies have shown that the degradability and degradation rates of organics in the subsurface varied with the characteristics of organic compounds, the existing microbial community, and subsurface environments (Alexander, 1978; Lee et al., 1988; Hickman et al., 1989). In addition, modification of the existing subsurface environments is often needed to increase the bioactivity and enhance the biodegradation. However, the appropriate strategies to induce microbial changes are unclear and often required

time-consuming laboratory studies. Thus the information concerning the degradability of the oxygenates and the subsurface factors that influence the degradation is essential in planning remediation efforts or protection strategies for groundwater systems.

The purpose of this work was to study the biodegradability of gasoline oxygenates, MTBE, ETBE and TBA, in subsurface microcosms and to determine the subsurface environmental factors that influence the biodegradation.

The specific objectives were to:

- (1) evaluate the biodegradation potential of MTBE, ETBE and TBA in previously uncontaminated subsurface systems;
- (2) study the effect of site variation on degradation; and
- (3) analyzed the influence of environmental factors on the biodegradation.

Data analysis and discussion were presented in different chapters. Chapter 4 presented soil characteristics at three sites. The degradation without amendments was discussed in chapter 5. Chapters 6 through 8 studied the degradation under aerobic, denitrifying and anaerobic conditions, respectively.

## Chapter 2 Literature Review

### 2.1 Introduction

Groundwater supplies drinking water for more than a half of the population in the United States. About 90-95 % of rural areas depend on groundwater as a sole drinking water resource. Because of inadequate management, groundwater has been threatening by contamination. Moreover, many incidents of groundwater contaminations occurred in heavily populated areas which rely on groundwater (EPA, 1987).

Leaking underground petroleum storage tanks are one of the major sources of soil and groundwater contamination. It is estimated that, in the U.S. alone, 10-30 % of the 3.5 million petroleum underground storage tanks may be leaking (Dowd, 1984). While most of the insoluble components of gasoline may be adsorbed and retained in soils, the more soluble components would spread through groundwater and could pose threats to drinking water resources.

In addition to the natural components of gasoline, the gasoline additives also raise concerns. Oxygenates are one of the major additives in unleaded gasolines. Beginning in late 1992, the Clean Air Act Amendments require oxygenates to be blended into reformulated gasolines to reduce carbon monoxide and ozone-forming hydrocarbon emissions. Table 2.1 lists the major chemicals used as oxygenates. They could be added into gasoline singularly or in mixtures. Among these compounds, MTBE is the most popular. The demand for MTBE is

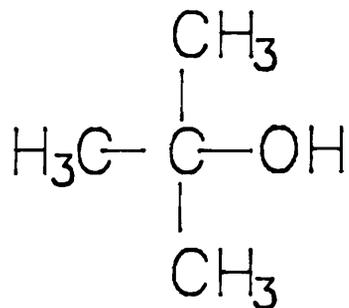
Table 2.1 Characteristics of unleaded gasoline (Black, 1991)

%	Premium	Regular
	Average (Range)	Average (Range)
<b>Components</b>		
Saturates	57.0 (37.1-88.0)	58.2 (35.0-72.9)
Aromatics	35.3 (14.7-53.6)	29.5 (15.8-41.5)
Olefins	6.8 (0.6-19.4)	12.4 (0.8-37.0)
<b>Oxygenates</b>		
Methanol	≤5.0	-
Ethanol	10.0	-
TBA	≤5.0	-
MTBE	2.0 (0.1-10.8)	0.5 (0.1-5.7)
ETBE	13.0	-

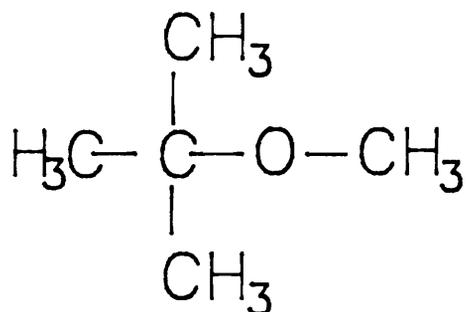
expected to increase 25 % each year (Alnsworth, 1991). ETBE is proposed to be an alternative to MTBE because it has lower volatility and is cheaper to produce (Anderson, 1988).

Concentrations of oxygenates in gasoline can range from 5 to 10 %, depending on blends and grades of gasoline (Table 2.1). Figure 2.1 and Table 2.2 show the structures and chemical properties of TBA, MTBE and ETBE. Being highly soluble in water and relatively abundant in gasoline, the oxygenates are likely to be found at high concentrations in groundwater when an aquifer is contaminated by oxygenated fuels. In an accidental leak in 1988, 8400 gallons of high-octane gasoline were released into an aquifer (Dey et al., 1991). Shortly after the leak, MTBE was detected in the groundwater at concentrations as high as 80 mg/l, while the combined concentrations of BTEX were only around 20 mg/l. MTBE was reported to still exist at detectable levels in a groundwater two years after a gasoline spill (Garrett, 1987; Garrett et al., 1987).

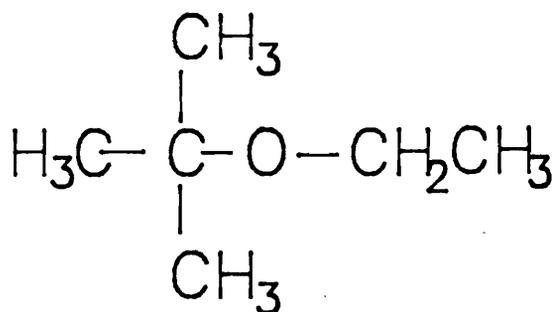
At present, there is little information on the health impact of oxygenates, especially MTBE and ETBE. The calculated Rfd (Reference Dose) of MTBE is 0.003 mg/kg/day, based on an inhalation study with mice and anaesthesia as an end point (Dietrich, 1988). The lethal concentration of MTBE for 50% of tested mice (LC<sub>50</sub>) is 150 ppm in the air (Budavari, 1988). TBA is considered to be relatively non-toxic, with an oral LC<sub>50</sub> of 3.5 g/kg tested mice



Tertiary Butyl Alcohol  
(TBA)



Methyl-t-Butyl Ether  
(MTBE)



Ethyl-t-Butyl-Ether  
(ETBE)

Figure 2.1 Chemical structures of TBA, MTBE and ETBE.

Table 2.2 Solubility of gasoline components and oxygenates

Aqueous solubility (25 °C, mg/l)	
<b>Components</b>	
Alkane	150
<b>Aromatics</b>	
Benzene	1780
Toluene	535
Xylene	200
Ethylbenzene	250
<b>Oxygenates</b>	
Methanol	100 %
Ethanol	100 %
TBA	100 %
MTBE	43000
ETBE	35000

(Budavari, 1989). Toxicity data for ETBE is not available. It was suggested that the health effects of continuous exposure to small concentrations of MTBE and ETBE will be similar to those of other ethers. The symptoms of exposure to ethers include loss of appetite, excessive thirst and fatigue (Sax et al., 1984).

## **2.2 Measurement of biodegradation potential**

For some years, indigenous soil microorganisms have been known to be metabolically active in degrading xenobiotic compounds, including many compounds in gasoline. Laboratory and field studies have shown that in situ bioremediation has great potential for restoring aquifers contaminated by fuels (Atlas, 1984; Lee et al., 1988). However, little effort has been focused on gasoline oxygenates.

In the past decade, studies were performed to gather information on the biological fate, degradation rates, and degradation kinetics of organics in subsurface systems. Such information is important in the risk assessment, evaluation of persistence and strategies for remediation (Alexander, 1988; Atlas, 1984). Furthermore, changes in subsurface environments are usually necessary in bioremediation efforts to stimulate microbial activity. Prior to in situ treatment, knowledge of the conditions and factors that affect the bioactivity are essential to optimize bioremediation efforts. Many methods are used to

evaluate biodegradation. They include (1) field studies, (2) laboratory studies using columns or microcosms containing aquifer material and simulating field environments, and (3) microbial studies using pure or mixed cultures (Schraa et al., 1985; Atlas, 1984). These methods are considered to be practical to evaluate biodegradation potential, to study the effects of environmental variables on degradation, and to compare the biodegradation of various compounds (Cooney, 1984). However, each method has its limitations and the results of biodegradation studies should be interpreted with caution.

Cooney (1984) stated that field measurements could provide direct information on in situ biodegradation. However, field measurements are often difficult and subject to many interferences. Laboratory studies, on the other hand, can control interferences but can not completely simulate subsurface environments. Therefore, the results of laboratory studies provides limited information. Moreover, because of the heterogeneity of subsurface and groundwater systems, results of biodegradation studies are often site-specific and are limited for general application (Cooney, 1984).

### **2.3. Subsurface microbiology**

#### **Methods for microbial characterization**

The distribution and density of microorganisms in the

subsurface have been found to vary with site and the method used to enumerate the microbial populations. Methods to estimate the types and population of subsurface microorganisms include Acridine Orange (AO) direct counts, plate counts, most probable number (MPN) analysis, biochemical (e.g. ATP) measurements, and electron microscopy. The microbial activity to degrade specific compounds can also be measured using selected media.

Each method has advantages and limitations. Direct microscopic counting (direct counts) is a common method for enumeration. One of its advantages is that this method avoid the selectivity of growth media and incubation conditions (Wollum, 1982). However, direct counts cannot easily distinguish viable and nonviable cells. Plate counts only enumerate those bacteria capable of growing on selected agar media and under specific incubation conditions. Therefore soil microbes not able to grow on agar media and under the designed incubation conditions are ignored. Studies have reported lower recoveries from viable counts than from AO direct counts (Balkwill & Ghiorse, 1985; Ghiorse and Balkwill, 1981; 1983; 1985).

The MPN method can provide simpler incubation environments for the enumeration of anaerobic microorganisms. Unlike plate count methods in which anaerobic microorganisms have to be incubated in a strictly anaerobic glove box, oxygen is excluded from well-sealed MPN

tubes; therefore offering an easier way for enumeration experiments. However, the MPN method cannot avoid the selectivity of media and incubation conditions.

### **Microbial population and distribution**

Soils are abundant in microorganisms because the soil surface attracts microbial substrates and nutrients and provides places for microbial attachment and growth (Bossert & Bartha, 1984). Viable counts in organically rich soils can reach  $10^8$  cells per gram of dry soil. Bacteria and fungi are the dominant groups.

Microbial populations and their distribution in the subsurface vary from site to site. In uncontaminated shallow aquifers in Oklahoma and Louisiana, the numbers of cells determined by AO direct count ranged from 0.1 to  $1 \times 10^7$  cells/g dry soil. In addition, the microbial distributions were consistent with depth (Balkwill and Ghiorse, 1985, Ghiorse and Balkwill, 1985). Novak et al. (1986) obtained similar results for soils from Pennsylvania, New York, and Virginia. On the other hand, a study of a landfill site indicated that the distribution of bacteria was concentrated in patches, probably a result of microzones of substrate distributions (Beloin et al., 1986). Bacterial counts were found to vary with geological layers, according to the study by Beloin et al. (1986). The microbial counts were higher on the top of the unsaturated zone of an artesian aquifer than those in the bedrock and confining layers.

The results of microbial enumerations must be applied with caution. The populations of specific microbial groups active in degradation are an important parameter in the estimation of biodegradation (Gunkel, 1968; Jones, 1969). However, most of the methods use specific substrates and the results therefore represent only fractions of the natural microflora in soils. In addition, the enumeration results represent the population of particular groups at a specific time in soil systems. But the microbial community is in a continual state of change, reflecting the physical, chemical and biological fluctuations in soils (Wollum, 1982). Changes in soil environments therefore can result in significant increases or decreases in microbial populations. For example, studies by Hickman (1988) and Gullic (1990) found that microbial populations were two orders of magnitude higher after incubation soil samples from the unsaturated zone in water for one week. The increased microbial populations probably were a result of new equilibrium between the soil environment and microbial populations during incubation.

Another limitation of enumeration results from the inability to estimate interactions among soil microbial groups. Microbial interactions such as proto cooperation, commensalism, competition, amensalism, and predation are common in soil ecosystems (Alexander, 1978). In soil systems, syntrophic interactions are often necessary for the

complete mineralization of organic compounds under anaerobic conditions. In these cases, organisms existing at relatively low populations may have significant influences in biodegradation processes (Wollum, 1982).

Several studies were attempted to develop rate-density relations in soil systems (Novak et al.; 1986; Hickman and Novak, 1989; Webster et al., 1985; Wilson et al., 1986). In activated sludge systems, substrate utilization rates are directly correlated to the active microbial density. Biodegradation rates of organic compounds therefore may be predicted from active microbial populations. However, the relation between degradation rates and microbial populations is more complex in soil systems. A positive relationship was observed between biodegradation rates of p-nitrophenol and p-nitrophenol-adapted organisms (Spain et al., 1984). The MPNs of p-nitrophenol degraders generally increased as the biodegradation rates increased. Hickman (1989), however, concluded that total counts are not a good indicator of biodegradation potential among sites. Studies have shown that soils with similar direct count results exhibited substantially different biodegradation rates (Webster et al., 1985; Wilson et al., 1986). The rate-density relation also depended on the type of organic compounds. Wilson et al. (1986) found a positive relation between toluene degradation rate and the ATP content of subsurface soils. However, a similar relation was not

observed for chlorobenzene degradation. The maximum rate of phenol mineralization was found to be positively correlated to total biomass in subsurface soils but the relationship was not strong (Dobbins et al., 1987).

### **Microorganisms in the subsurface**

Microorganisms use the energy and materials derived from the oxidation of organic compounds for maintenance and growth. The degradation pathways involve the oxidation of organic compounds (electron donors) and the transfer of electrons to an electron acceptor through a series of oxidation-reduction reactions. The terminal electron acceptors determine the types of catabolic pathways occurring in a system.

Oxygen is the most common electron acceptor in soil systems. When oxygen is depleted, organic compounds can be decomposed by anaerobic microorganisms through several anaerobic processes including iron reduction (iron reducers), denitrification (denitrifiers), fermentation (fermenters), sulfate reduction (sulfate reducers) and methanogenesis (methanogens).

#### **(a) Denitrifying bacteria**

Denitrification is an energy-efficient metabolic process. Denitrification involves three major steps: (1) the reduction of nitrate to nitrite; (2) the further reduction of nitrite to gaseous nitroxides (NO, N<sub>2</sub>O); and (3) final reduction to nitrogen gas (Skinner, 1975). Many denitrifiers

carry all the required enzymes for the complete reduction of nitrate to nitrogen gas. Some denitrifying bacteria however carry enzymes for only one step in the denitrification process. For example, species in the Pseudomadales, Eubacteriales, and Actinomycetales are only able to reduce nitrate to nitrite. These types of denitrifiers exist in large numbers in soils. The species that reduce nitrite are less diverse in soils (Tiedje, 1988).

Most denitrifying bacteria are facultative, using oxygen as a terminal electron acceptor when oxygen is available and using nitrogen oxides in anoxic or low-oxygen environments. In addition, denitrifiers are good at competing with other heterotrophs in aerobic environments, thus they are ubiquitous in soil systems (Tiedje, 1988). The basic requirements for denitrification to occur in subsurface systems include (1) anoxia or a limited supply of oxygen; (2) the presence of nitrate; (3) the presence of denitrifying bacteria; and (4) suitable electron donors (organic substrates).

Studies using pure or mixed cultures suggest that a wide range of organic compounds can be used as electron donors for denitrifying bacteria. Example compounds include aliphatics and aromatic hydrocarbons. A detailed list of compounds is shown in Table 2.3.

#### **(b) Sulfate-reducing bacteria**

When nitrate is depleted, sulfate serves as the next

Table 2.3 Organic compounds degradable under denitrifying conditions (after Hickman, 1988).

Compound	Reference
<i>p</i> -cresol	Bossert & Young, 1986; Bossert et al., 1986
<i>o</i> -phthalic acid	Astring, et al. 1981; Astring and Taylor, 1981
2-fluorobenzoate	Schennen et al., 1985
vanillic acid and other methoxyaromatics	Taylor, 1983
monofluorobenzoate and monochlorobenzoate	Taylor et al., 1979
benzoate	Williams & Evans, 1975
benzene	Taylor et al., 1970
mono-, di-, and trimethylamine	Meiberg & Harder, 1978
dibromochloropropane, dibromochloromethane, ethylene dibromide, 1,1,1-trichloroethane, bromodichloromethane, bromoform, carbon tetrachloride	Bouwer & McCarty, 1983b; Bouwer et al., 1986
protocatechuate, phenylalanine, <i>p</i> -hydroxybenzoate, benzoate, tyrosine, succinate	Oshima, 1965
benzoate, 2-aminobenzoate, 3-hydroxybenzoate, 4-hydroxybenzoate, 2-carboxylbenzoate (phthalate), 1-cyclohexenecarboxylate, adipate, pimelate, acetate, propionate, butyrate, caprolate, lactate, malate, fumarate, succinate, glucose, fructose, sucrose, maltose, acetoin, acetone	Braun & Gibson, 1984
benzene, toluene, xylene	Major et al., 1988
toluene, <i>m</i> -xylene, 3-ethyltoluene, <i>p</i> - and <i>m</i> -cresol, <i>p</i> -hydroxybenzoic acid, benzaldehyde, benzoate, <i>m</i> -toluylaldehyde, <i>m</i> -toluate, cyclohexanecarboxylic acid	Kuhn et al., 1988
naphthol, naphthalene, acenaphthene	Mihelcic & Luthy, 1988a,b
phenol, benzoate, 3-, and 4-hydroxybenzoate, 3,4-dihydroxybenzoate, <i>o</i> -, <i>m</i> -, and <i>p</i> -cresol	Bakker, 1977

alternative electron acceptor (Kaspar and Tiedje, 1982). In the sulfate respiration process, sulfate is reduced to hydrogen sulfide. The energy yield from sulfate reduction is far less than from aerobic respiration and denitrification.

Sulfate reduction requires obligate anaerobic environments (Eh between -150 and -300 mV), containing sulfate but no nitrate. Common sulfate reducing bacteria are species of *Desulfotomaculum*, *Desulfonema*, *Desulfococcus* and *Desulfosarcina*. Among these the genus of *Desulfovibria* is the predominant group (Thauer and Badziong, 1981).

Sulfate reducers are further broken into two groups "complete oxidizers" and "incomplete oxidizers", based on whether organic substrates are completely oxidized to carbon dioxide (Widdle, 1988). The incomplete oxidizers do not have enzymatic systems to oxidize acetate; therefore, the substrate oxidation stops at acetate. Incomplete oxidizers can grow faster than complete oxidizers; and might dominate in a system when conditions are favorable.

Sulfate reducers always use low-molecular-weight compounds from hydrolysis (fermentable products) or from fermentation (fermentation end products). In addition, they are able to use compounds such as propionate, butyrate, higher fatty acids or phenyl substituted organic acids that cannot be utilized by fermenters (Widdle, 1988). Electron donors used by sulfate reducing bacteria include organic acids and alcohols (Pfenning and Widdle, 1981; Postage,

1984). Aromatic compounds are also degradable via sulfate reduction (Gibson and Suflita, 1986; Suflita and Miller, 1985). A detailed list of compounds degraded by sulfate reducers is shown in Table 2.4.

### **(c) Methanogens**

Methanogens obtain energy through the oxidation of limited methanogenic substrates ( $H_2$  or a limited number of simple organics) with the reduction of  $CO_2$  to methane (Skinner, 1975). Methanogenic bacteria are all strict anaerobes and require the environments of very low redox potentials (Eh less than -200 mV). Because most of the organic matter entering soil systems is in the form of complex polymers, methanogens have to rely on other microorganisms for the supply of methanogenic substrates (Mah et al., 1977). Methanogenic degradation therefore involves two steps: (1) degradation of complex organics to simple acids, alcohols, hydrogen and carbon dioxide by fermenters; and (2) further oxidation of these simple organics to carbon dioxide by methanogens (Speece, 1983). In soils, the rate of methanogenesis is controlled by the rate and extent of conversion of complex organics and the diffusion rate of methanogenic substrates to methanogens.

Methanogenic substrates include acetate, formate, methanol, methylamine, dimethylamine, trimethylamine and some alcohols (Speece, 1983). Under syntrophic interactions, many compounds could be degraded under methanogenic

Table 2.4 Organic compounds degradable under sulfate reducing conditions (after Hickman, 1988).

Compound	Reference
C <sub>1</sub> -C <sub>18</sub> fatty acids C <sub>n</sub> H <sub>2n</sub> O <sub>2</sub> , benzoate, phenylacetate, 3-phenylpropionate	Pfennig & Widdel, 1981
H <sub>2</sub> /CO <sub>2</sub>	Badziong et al., 1978
cyclohexane, carboxylate, hydroxylbenzoate, hippurate	Peck, 1984
methanol, ethanol, propanol, butanol, lactate, pyruvate, malate, benzoate, succinate, oxamate, oxalate, fumarate, citrate, cysteine, choline, formate, acetate, glucose, glycerol	Postgate, 1984
benzoate	Balba & Evans, 1980
phenol, benzoate	Suflita & Miller, 1985 Gibson & Suflita, 1986
<i>o</i> -, <i>m</i> -, and <i>p</i> -cresol	Smolenski & Suflita, 1987
H <sub>2</sub> , formate, propionate, butyrate, valerate caproate, heptanoate, caprylate, pelargonate, methanol, ethanol, 1-propanol, 1-butanol, glycerol, lactate, pyruvate, succinate, fumarate, malate, oxaloacetate, choline	Nanninga & Gottschal, 1987

conditions (Table 2.5).

#### **2.4 Factors affecting degradation in the subsurface**

Soil microorganisms have to cope with variations in water content, availability and concentrations of electron acceptors, organic substrates, nutrients, redox potential, and pH in the subsurface. Understanding the chemical and biological dynamics in subsurface systems and how they affect the biodegradation are essential steps in bioremediation. The environmental characteristics are especially important because the distribution, proliferation and activity of specific microorganisms that are able to degrade certain compounds may be drastically affected by subsurface environments. The persistence of an organic compound in the subsurface may be due to (1) unfavorable growth environments such as absence of appropriate electron acceptors or growth factors; (2) the nature and concentration of organic compounds; and (3) inability of the specific microbial groups to proliferate from the microbial community due to competition (Schraa et al., 1986)

#### **Dynamics of redox potential in soil systems**

Many physico-chemical and biochemical reactions occurring in soils are regulated by the aeration status and redox potential (Bonneau, 1982). Aerobic degradation is the most common and the most important mechanism with respect to biodegradation of organics in the subsurface. Oxygen acts

Table 2.5 Organic compounds degradable under methanogenic conditions (after Hickman, 1988).

Compound	Reference
acetaldehyde, acetic anhydride, acetone, acrylic acid, adipic acid, aniline, 1-amino-2-propanol, 4-amino butyric acid, benzoic acid, butanol, butyraldehyde, butylene glycerol, catechol, cresol, crotonaldehyde, crotonic acid, diacetone gulusonic acid, dimethoxy benzoic acid, ethanol, ethyl acetate, ethyl acrylate, ferulic acid, formaldehyde, formic acid, fumaric acid, glutamic acid, glutaric acid, glycerol, hexanoic acid, hydroquinone, isobutyric acid, isopropanol, lactic acid, maleic acid, methanol, methyl acetate, methyl acrylate, methyl ethyl ketone, methyl formate, nitrobenzene, pentaerythritol, pentanol, phenol, phthalic acid, propanal, propanol, isopropyl alcohol, propionate, propylene glycol, protocatechuic acid, resorcinol, <i>s</i> -butanol, <i>s</i> -butylamine, sorbic acid, syringaldehyde, syringic acid, succinic acid, <i>t</i> -butanol, vanillic acid, vinyl acetate	Speece, 1983
tetrachloroethylene, chloroform, 1,1,2,2-tetrachloroethane, 1,1,1-trichloroethane, carbon tetrachloride, dibromochloropropane, bromodichloromethane, dibromochloromethane, bromoform, ethylene dibromide	Bouwer & McCarty, 1983a, 1985 Bouwer et al., 1986
trichloroethylene, 1,1-dichloroethylene, 1,2-dichloroethylene, ethylene dibromide, benzene, toluene, ethylbenzene, <i>o</i> -xylene	Wilson et al., 1986a
phenoxyacetate, 2,4-dichlorophenoxyacetate (2,4-D), 2,4,5-trichlorophenoxyacetate (2,4,5-T), 3-chlorobenzoate, 3,4-dichlorobenzoate, 3,5-dichlorobenzoate, 3-bromobenzoate, 3-iodobenzoate, 4-amino-3,5-dichlorobenzoate, phenol, 2-chlorophenol, 3-chlorophenol, 4-chlorophenol, 2,4-dichlorophenol, 2,5-dichlorophenol, 2,4,5-trichlorophenol	Horowitz et al., 1983 Sufliata & Miller, 1985 Gibson & Sufliata, 1986
1,1,1-trichloroethane	Vogel & McCarty, 1987
dimethylselenide	Oremland & Zehr, 1986

Table 2.5 (cont'd)

Compound	Reference
ethylene glycol, polyethylene glycol	Dwyer & Tiedje, 1983
<i>o</i> -, <i>m</i> -, and <i>p</i> -cresol	Smolenski & Suflita, 1987
vanillin, vanillic acid, ferulic acid, cinnamic acid, benzoic acid, catechol, protocatechuic acid, phenol, <i>p</i> -hydroxybenzoic acid, syringic acid, syringaldehyde	Healy & Young, 1979
phenol, phloroglucinol, hydroquinone, <i>p</i> -cresol	Young & Rivera, 1985
benzoate	Fina & Fiskin, 1966
syringic acid, 2,6-dimethoxyphenol, gallic acid, pyrogallol, vanillic acid, veratic acid	Kaiser & Hanselmann, 1982
2-, 3-, and 4-chlorophenol, <i>m</i> - and <i>p</i> -cresol, <i>o</i> -, <i>m</i> -, and <i>p</i> -nitrophenol, 2,3-, 2,4-, 2,5-, 2,6-, 3,4-, and 3,5-dichlorophenol, phenol	Boyd et al., 1983 Boyd & Shelton, 1984
toluene, benzene	Grbic-Galic & Vogel, 1987

not only as an electron acceptor, but also an active reactant in many degradation pathways. For example, the degradation of alkane requires the insertion of an oxygen atom into the carbon chain. As a result, oxygen is often one of the limiting factors in the degradation of petroleum components in the subsurface (Bossert & Bartha, 1984).

The rates of anaerobic biodegradations are usually slower than those under aerobic conditions (Lee et al., 1988). In addition, organic compounds may not be completely degraded to CO<sub>2</sub> under anaerobic conditions even after long periods of time. However, some advantages of anaerobic biodegradation have been stated. Biodegradation of DDT and dehalogenation of many halogenated organics occur more rapidly under anaerobic conditions (Kaspar and Tiedje, 1982). Furthermore, anaerobic degradation requires less inorganic nutrients and produces less biomass (Lee et al., 1988). On the other hand, the rapid growth of aerobic microorganisms might lead to accumulation of biomass in the subsurface and increase the potential of plugging soil voids. In addition, the supply of oxygen may be limited in low permeability aquifers.

The aeration status of a soil is controlled by a number of environmental factors, including the total amount of air-filled pore space, the size of the pores and the rate of oxygen consumption (Bossert & Bartha, 1984; Bonneau, 1982). Oxygen may be depleted very fast in heavy clay, water-logged or organic-rich soils. Bonneau (1982) defined the succession

of redox potential in three phases. In the first phase, dissolved oxygen is present. The Eh is controlled by the concentrations of oxygen and aerobic respirations are the dominant activity. As oxygen decreases, Eh decreases but still remains at positive values. Iron remains in the ferric state. When dissolved oxygen disappears from small pores, a partial anaerobic conditions is developed (the second phase). In the second phase, portions of ferric iron are reduced to more mobile Fe(+2). When conditions reach complete anaerobic conditions (the third phase), all iron is reduced and Eh becomes negative.

#### **Nutrient availability**

Microorganisms require carbon, nitrogen, phosphate and other trace nutrients for growth. Among these required nutrients, nitrogen is considered as a major limiting factor in soils (Bossert & Bartha, 1984; Stevenson, 1982). In a soil system, nitrogen fixation,  $\text{NH}_4^+$  and  $\text{NO}_3^-$  from precipitation and the input of nitrogen-containing organics are major nitrogen sources in the soil N cycle (Stevenson, 1982). Nitrogen is subject to losses from soils by volatilization of  $\text{NH}_3$ , bacterial denitrification and leaching of nitrate. Ammonium fixation on clay material can strongly reduce the nitrogen availability in soils (Young & Aldag, 1982; Nommik and Vahtras, 1982). Soils rich in clay minerals of the three-layer or 2:1 type are able to fix the ammonium ion and make it unavailable to

microorganisms.

Many xenobiotic compounds entering subsurface systems do not contain nitrogen or phosphorus. Thus, the activity of microorganisms may be limited by the amount of nitrogen or phosphorus available in the subsurface heavily contaminated by organic compounds (Bossert & Bartha, 1984). For example, gasoline components from oil spills provide large quantities of readily available organic carbon, and nitrogen and other mineral nutrients may be depleted very quickly in a subsurface system.

Large quantities of inorganic nutrients have been applied to enhance bioactivity in the contaminated aquifers (Lee et al., 1988). It was reported that enhancement of biodegradation was achieved only through the addition of nitrogen and phosphate together. Adding nitrogen or phosphate alone did not result in significant effects. Jamison et al. (1975) observed that the addition of nitrogen and phosphorus increased bioactivity and enhanced aerobic degradation of gasoline in contaminated aquifers. Others also reported success in enhancing biodegradation in soil systems by adding nitrogen and phosphate nutrients (Kincannon, 1972; Veratraete et al., 1976; Dibble and Bartha, 1979; Lee and Ward, 1985; Raymond, 1974; Raymond et al., 1976; Jamison et al., 1975).

The methods and the quantities of nutrients applied influenced the extent of enhancement (Bossert and Bartha,

1984). Hunt et al. (1973) reported that the soil respiration activity, measured in term of CO<sub>2</sub> evolution rates, corresponded to the increases in nitrogen application rates. When the nitrogen concentration was over 100 ppm, however, soil respiration was inhibited, probably due to ammonium or nitrite toxicity.

In spite of the general success in enhancing gasoline degradation by nutrient additions, the effectiveness of nutrient addition varied. While many studies observed immediate and significant increases in oil degradation by nutrient additions, several also reported slow or no response after long terms of incubation. Jobson et al. (1974) reported the stimulation of biodegradation by nutrients did not occur until 66 days of incubation. Odu (1978) found that significant enhancement by nutrient additions happened after 12 weeks of incubation. It is suggested that the various responses of soil systems to the nutrient amendments might be due to other unfavorable environmental factors such as nitrogen reserves of the soil, nitrogen fixation, temperature, oxygen, water or pH (Bossert & Bartha, 1984).

#### **Nature and concentration of organics**

The biodegradability of organics is dependent on the existence of microbial enzymes that are capable of catalyzing the degradation reactions. One example of the influence of the nature of organics on their degradability

is the biodegradation of halogenated organic compounds. Culture studies on denitrifiers indicated that denitrifiers are capable of degrading fluoro- and bromo-hydrocarbons but not the chlorinated compounds. Methanogenic bacteria, on the other hand, only degrade chlorinated compounds but not fluoro-substituted organics (Suflita et al., 1982; Schenneu et al., 1985; Tiedje, 1988).

In addition to the nature of organics, many organic compounds can become recalcitrant at trace concentrations. The aspect of minimum concentration ( $S_{\min}$ ) was proposed by Rittmann et al. (1980) and McCarty et al., (1981). They proposed that below minimum concentrations, microorganisms can not obtain enough energy for growth and maintenance and no biodegradation will occur.

The types of organics, electron acceptors and microorganisms influence the values of  $S_{\min}$ . For example, the  $S_{\min}$  value for the degradation of acetate and methanol ranged from 0.1 to 1 mg/l under denitrification, while under methanogenesis,  $S_{\min}$  was 350 mg/l (McCarty et al., 1981).

However, a compound at concentrations below  $S_{\min}$  can still be biodegraded if a primary substrate is added to support the microbial growth (second substrate utilization). The application of secondary substrate utilization was studied using an isolated strain adapted to degrade methylene chloride (LaPat-Polasko et al., 1984). Addition of acetate increased the degradation of methylene chloride

at trace concentrations. However, the enhancement of the degradation of the secondary substrate diminished when the concentrations of the primary substrate reached certain levels.

The effectiveness of applying a primary substrate to enhance the biodegradation of organics in the subsurface might not be as significant as those seen in the culture study. Dibble and Bartha (1979) found that amendment with yeast or sludge actually inhibited oil biodegradation. They suggested the organic amendment might deplete oxygen or inhibit the synthesis of hydrocarbon-degrading enzymes.

### **Microbial interactions**

The successful decomposition of organic matter in a soil system often is a result of interactions of different metabolic groups. Interactions among soil microorganisms include commensalism, competition, and cometabolism (Alexander, 1978). Microbial interactions may play important roles with respect to the complete biodegradation of organic compounds in the subsurface. The following sections review the interactions between sulfate reducers and methanogenic bacteria and their effects on anaerobic degradation of organics in soils.

#### **(a) Interspecies hydrogen transfer**

Sulfate reducers and methanogens are the terminal microorganisms in the anaerobic microbial food chain. They degrade the metabolic products produced by fermenters to

carbon dioxide. Figure 2.2 summarizes the possible substrate and electron flows when sulfate reducers and methanogens serve at the terminal stage (Zehnder and Colberg, 1986). The pathways in which the substrates flow through anaerobic systems are highly dependent on the chemical nature of substrates, electron acceptors, and the biological activity of microorganisms.

The interactions between sulfate reducers and methanogens have profound effects on the decomposition of xenobiotic organic compounds entering soil systems. For example, the biodegradation of various cresol isomers was reported to occur at sites that were actively reducing sulfate but not at methanogenic sites. Inhibition of sulfate reduction decreased biodegradation of cresols; whereas, stimulation of sulfate reducing bacteria favored cresol mineralization (Smolenski and Suflita, 1985). Methanogenic activity, on the other hand, determined the degradation potential of halogenated compounds. The reductive dehalogenation processes of halogenated organics by methanogens could be slowed or inhibited by sulfate reducing bacteria. Chlorophenolic compounds, for example, were only degraded at methanogenic sites. Therefore, the chlorophenols were persistent in the soils where sulfate reduction was the main activity (Suflita and Miller, 1985). The addition of sulfate to the methanogenic sites activated sulfate reducing bacteria and inhibited the dehalogenation process. As soon

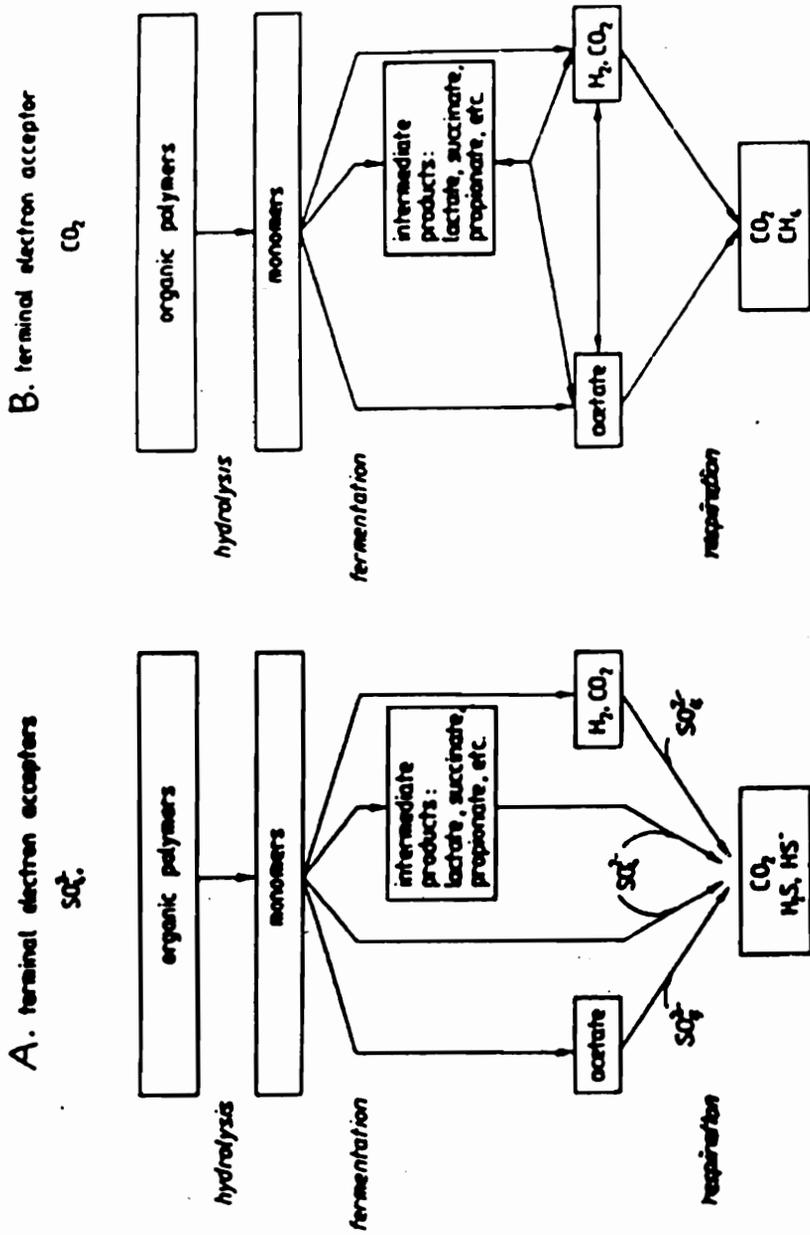


Figure 2.2 Carbon flows during methanogenesis and sulfate reductions (Zehnder and Colberly, 1986).

as sulfate was depleted by acetate, degradation of chlorinated aromatics was recovered (Gibson and Suflita, 1986).

Competition between sulfate reducers and methanogens involves the process of interspecies hydrogen transfer in which more oxidized end products and hydrogen are generated. In the interspecies hydrogen transfer process, hydrogen produced by fermenters is immediately consumed by methanogens or sulfate reducers. The products of such a syntrophic association are acetate, carbon dioxide, and hydrogen sulfide or methane (Zehnder and Colberg, 1986). By interspecies  $H_2$  transfer, the theoretical energy yield for fermenters is increased per mole of substrate converted.

The interspecies  $H_2$  transfer has significant advantages for the mineralization of organics and the ecology in the anaerobic soil system. As stated by Zeikus (1977) and Mah et al. (1977), these advantages include:

1. displacement of unfavorable reaction equilibria, providing an energy-efficient mechanism for electron disposal;
2. alternation of electron flow, resulting in different proportions of reduced end products;
3. use of otherwise unfermentable substrates as carbon and energy sources;
4. more complete oxidation of substrates by fermentative microorganisms;
5. generation of  $H_2$  from complex substrates, which can be

- used by sulfate reducers and methanogens;
6. increased substrate utilization; and
  7. increased growth of fermentative microorganisms, sulfate reducers and/or methanogens.

**(b) Competition for acetate and hydrogen**

The major substrates involved in competition between methanogens and sulfate reducers are acetate and hydrogen. In anaerobic natural systems where sulfate is present, sulfate reducers can outcompete methanogens. The competition can be explained from both thermodynamic and kinetic aspects.

**. Thermodynamic aspects**

Competition between sulfate reducers and methanogens for acetate and hydrogen has been well studied. Table 2.6 presents the theoretical free energy change for sulfate reduction and methanogenesis, using acetate and hydrogen as electron donors. The free energy changes derived from the oxidation of H<sub>2</sub> and acetate are more negative through sulfate reduction than through carbon dioxide reduction.

**. Kinetic aspects**

The success of sulfate reducers in competition for H<sub>2</sub> and acetate can be explained not only by thermodynamics but also by sulfate reducers' higher affinity for substrates. Sulfate reducers can use substrates more efficiently than methanogens. Two models were proposed to describe the partitioning of hydrogen and acetate between sulfate

**Table 2.6 Theoretical free energy change for the oxidation of H<sub>2</sub> and acetone under sulfate reducing and methanogenic conditions (after Hickman, 1988).**

Electron Donor	Bacterial Group	Reaction	$\Delta G^\circ$ , kcal/mole of Electron Donor
H <sub>2</sub>	SRB	$4\text{H}_2 + \text{SO}_4^{2-} + \text{H}^+ \rightarrow \text{HS}^- + 4\text{H}_2\text{O}$	-36.3
H <sub>2</sub>	MPB	$4\text{H}_2 + \text{HCO}_3^- + \text{H}^+ \rightarrow \text{CH}_4 + 3\text{H}_2\text{O}$	-31.3
acetate	SRB	$\text{CH}_3\text{COO}^- + \text{SO}_4^{2-} \rightarrow \text{HS}^- + 2\text{HCO}_3^-$	-11.2
acetate	MPB	$\text{CH}_3\text{COO}^- + \text{H}^+ \rightarrow \text{CH}_4 + \text{CO}_2$	-8.6

SRB = sulfate-reducing bacteria

MPB = methane-producing bacteria

Source: Frea, 1984

reducers and methanogens (Lovley, 1985).

The first one is the Michaelis-Menten model. As described by Healy (1980), the nutrient uptake and growth rates of microorganisms are a function of external nutrient concentrations. These relationships can be empirically described by the equation:

$$V = V_m * S / K_m + S$$

where V = rate of nutrient uptake or growth, V<sub>m</sub> = maximum rate of nutrient uptake or growth, S = substrate concentration and K<sub>m</sub> = half-saturation value, or substrate concentration supporting half the maximum rate of nutrient uptake or growth.

The ratio V<sub>m</sub>/K<sub>m</sub> is useful in comparisons between different strains or species of microorganisms during uptake or growth under the same conditions. While competition for the limited nutrients occurs, the species with the highest V<sub>m</sub>/K<sub>m</sub> have the advantages. A modified model in which cell mass (growth yield) was taken into account was derived by Widdle (1988). The ratio of substrate consumption rates between two species can be expressed as:

$$\frac{R_1}{R_2} = \frac{V_{m1}(K_{m2} + S)M_1}{V_{m2}(K_{m1} + S)M_2}$$

V<sub>m1</sub> and V<sub>m2</sub> are the corresponding specific maximum consumption rates, K<sub>m1</sub> and K<sub>m2</sub> are the substrate concentrations at which the consumption rates are half of the maximum, and M<sub>1</sub> and M<sub>2</sub> are the cell masses of species 1

and species 2, respectively.

At very low substrate concentrations ( $S \ll K_m$ ) such as those present in soils, the following approximation was derived:

$$\frac{R_1}{R_2} = \frac{(V_{m1}/K_{m1})M_1}{(V_{m2}/K_{m2})M_2}$$

The data on Table 2.7 and Table 2.8 show that sulfate reducers tend to have higher  $V_m/K_m$  for  $H_2$  and acetate (Widdle, 1988). It was noted by Widdle (1988) that when no growth occurs, the maximum substrate consumption rates (growth rates) of sulfate reducers and methanogens are quite similar. However, sulfate reducers have higher growth yields than methanogens (Table 2.9), so when growth takes place, sulfate reducers appear to be the dominant species after a certain time of growth in enriched cultures. Thus, sulfate reducers can completely outcompete methanogens after some generations of growth on hydrogen or acetate.

An alternative model for  $H_2$  and acetate competition, based on field studies on natural habitats, is the threshold model (Lovley et al., 1982; Lovley, 1985). According to this model, competition is independent of maximum growth rates ( $V_m$ ). Table 2.10 shows the  $K_m$  for sulfate reducers and methanogens when using  $H_2$  or acetate as the electron donor. When sulfate is not limiting, the lower half-saturation constant ( $K_m$ ) of sulfate reducers allows more effective consumption of substrates; thus sulfate reducers inhibit

Table 2.7 Kinetic parameters of hydrogen utilization by sulfate reducers and methanogens (adapted from Widdle, 1988).

Species	Vmax ( $\mu\text{mol/g/h}$ )	Km ( $\mu\text{mol/l}$ )	Vm/Km ( $\text{l/g/h}$ )
<b>Sulfate reducers</b>			
desulfuricans	5280	1.8	2930
sp. (G11)	3300	1.1	3000
sp. (PS1)	3300	1.7	4710
<b>Methanogens</b>			
methanobrevibacter	8510	6.6	1290
methanospirillum			
hungati (JF-1)	4200	5.0	840
sp. (PM1)	5400	2.5	2160

Table 2.8 Kinetic parameters of acetate utilization by sulfate reducers and methanogens (adapted from Widdle, 1988).

Species	Vmax ( $\mu\text{mol/g/h}$ )	Km ( $\mu\text{mol/l}$ )	Vm/Km ( $1/\text{g/h}$ )
<b>Sulfate reducers</b>			
desulfobacter	830	230	3.6
postgatei(ac9)	3100	64	48
<b>Methanogens</b>			
methanosarcina			
barkeri (Fusaro)	2240	3000	0.75
barkeri (227)	6800	nd	nd

Table 2.9 Yield coefficient Y of sulfate reducers and methanogens on utilization of H<sub>2</sub> and acetate (after Widdle, 1988).

Species (strain)	Energy Substrates	Y (g cell dry mass per mol H <sub>2</sub> or acetate dissimilated)
<i>Desulfovibrio</i>	H <sub>2</sub> + SO <sub>4</sub> <sup>2-</sup>	2.1; 2.9 <sup>b</sup>
<i>vulgaris</i> (Marburg)	H <sub>2</sub> + S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	4.2
<i>Desulfotomaculum</i>	H <sub>2</sub> + SO <sub>4</sub> <sup>2-</sup>	1.9; 3.1 <sup>b</sup>
<i>orientis</i> (Singapore I)	H <sub>2</sub> + SO <sub>3</sub> <sup>2-</sup>	4.0 <sup>b</sup>
	H <sub>2</sub> + S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	4.5 <sup>b</sup>
<i>Methanosarcina</i>	H <sub>2</sub> + CO <sub>2</sub>	0.7-2.2
<i>barkeri</i> strains		
Other H <sub>2</sub> -utilizing methanogens	H <sub>2</sub> + CO <sub>2</sub>	0.5-1.0
<i>Desulfobacter</i>	Acetate + SO <sub>4</sub> <sup>2-</sup>	4.8
<i>postgatei</i> (2ac9)	Acetate + SO <sub>3</sub> <sup>2-</sup>	11.2
	Acetate + S <sub>2</sub> O <sub>3</sub> <sup>2-</sup>	10.4
<i>Desulfotomaculum</i>	Acetate + SO <sub>4</sub> <sup>2-</sup>	3.4-4.8
<i>acetoxidans</i> (5575)		
<i>Desulfovibrio</i>	Acetate + SO <sub>4</sub> <sup>2-</sup>	0.7-1.4
<i>baarsii</i> (2st14)		
<i>Desulfuromonas</i>	Acetate + S	4.2
<i>acetoxidans</i> (11070)	Acetate + malate	16.5
<i>Methanosarcina</i>	Acetate	1.6-3.2
<i>barkeri</i> strains		
<i>Methanotherix</i>	Acetate	1.4
<i>soehngenii</i> (Opfikon)		

<sup>a</sup> Only real Y values are listed, but no extrapolated Y<sub>max</sub> values (μ = ∞).

<sup>b</sup> From chemostat culture.

Table 2.10 Half-saturation ( $K_m$ ) values of sulfate reducers and methanogens on utilization of  $H_2$  and acetate

(from Hickman, 1988)

Electron Donor	Bacterial Group	$K_m$	Reference
$H_2$	SRB	141 Pa	Lovley et al. 1982
$H_2$	MPB	597 Pa	
$H_2$	SRB	$1\mu M$	Kristjansson et al. 1982
$H_2$	MPB	$6\mu M$	
acetate	SRB	0.2mM	Schönheit et al. 1982
acctate	MPB	3mM	

methanogens by lowering hydrogen or acetate concentrations below the levels where methanogenesis is energetically unfavorable.

**(c) Competition for fermentable substrates**

Sulfate reducers also oxidize fermentable compounds such as lactate, ethanol, or amino acids. Therefore, in the utilization of degradation products that can be fermented further, sulfate reducers have the ability to compete with fermentative microorganisms (Widdle, 1988). At growth-limiting concentrations in chemostat enrichments, both lactate and ethanol were directly used by sulfate reducers rather than by fermenters (Laanbroek et al., 1982). Similar to the observation in pure culture studies, field studies (Widdle, 1988) showed that lactate, ethanol and amino acids at low concentrations were utilized directly by sulfate reducers. Methanogens are not able to use fermentative substrates, thus they do not compete with sulfate reducers for fermentative substrates. However, methanogens depend on fermenters to oxidize fermentative compounds to methanogenic substrates. Therefore, sulfate reducers can indirectly inhibit the activity of methanogens by channeling substrate flows to sulfate reduction and blocking the substrates for fermentation and methanogenesis.

**(d) Variations in the degree of competition**

The degree of competition between sulfate reducers and methanogens varies in different environments. According to

the study by Mountfort et al. (1980), methanogens competed poorly in an intertidal sediment when sulfate was in excess. Methane production could not be stimulated by addition of acetate or H<sub>2</sub>. In a study on a salt-marsh sediment (Abram and Nedwell, 1978), H<sub>2</sub> did stimulate methanogenesis during active sulfate reduction, indicating that methanogens seemed to be slightly more competitive in sulfate-limited sediments.

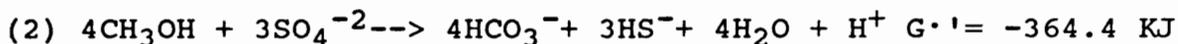
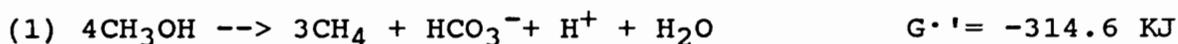
The basis for differences in the degree of competition may be due to the supply of sulfate and the extent of organic matter loading which can be converted to acetate and H<sub>2</sub> (Ward and Winfery, 1985). Since sulfate reducers require sulfate as an external electron acceptor, the successful competition of sulfate-reducing bacteria depends on an abundant supply of sulfate. In environments such as soils where sulfate supply is limited or fluctuated, increasing the organic loading could cause a sharp depletion of sulfate and thus decrease the competitive ability of sulfate reducers. Winfery and Zeikus (1977) showed that the competition could be uncoupled if electron donors were provided in excess.

#### **(e) Non-competitive substrates**

Although sulfate reduction clearly dominates methanogenic activity in the environments where sulfate is non-limiting, methanogenesis could still occur during active sulfate reduction. This was due to the presence of methyl compounds

which can not be utilized by sulfate reducers (Ward and Winfrey, 1985). Naturally occurring methyl compounds include methanol derived from pectin degradation, trimethylamine from the decomposition of choline and methoxyl groups of the aromatic lignin monomers (Widdle, 1988). These methyl compounds are poor substrates for sulfate reducers. Very few types of sulfate reducers are known to use methoxyl groups from aromatic compounds.

Although methanol oxidation through sulfate reduction is thermodynamically more favorable than through methanogenesis (equation 1 and 2), sulfate reducers grow more slowly on methanol than methanogens. This is due to the relatively low affinity to methanol of sulfate reducers (Widdle, 1988).



Sulfate reducers also degrade fermentable substrates by different catabolic mechanisms to sulfate reduction when sulfate is lacking. Without sulfate, sulfate reducers can grow on lactate, ethanol or pyruvate in a manner similar to hydrogen-producing microorganisms (Widdle, 1988). Under this circumstance, a syntrophic relationship, instead of competition, between sulfate reducers (acting as hydrogen-producing bacteria) and methanogens (hydrogen-consuming bacteria) is developed.

**(f) Effects of competition on degradation of halogenated organic compounds**

Sulfate reducers inhibit methanogenesis by altering normal carbon and electron flows during anaerobic mineralization of organic matter. As little as 0.2 mM sulfate was reported to completely inhibit methanogenesis for 10 hours. Higher concentrations of sulfate can inhibit methanogenesis for longer periods of time (Winfery and Zeikus, 1977). The outcome of competition could have important implications in the decomposition of xenobiotic organic compounds that occur only under methanogenic conditions. One of the examples is the degradation of halogenated organic compounds, which were found to occur only under methanogenic conditions and were inhibited by sulfate reduction due to the competition.

Halogenated organics entering the subsurface include pesticides, plasticizers, plastics, and solvents. The key process for the mineralization of halogenated compounds is the removal of halogenated substituents from the organic compounds, so-called reductive dehalogenation (Reineke and Knackmuss, 1988).

Reductive dehalogenation involves the removal of halogen atoms by oxidation-reduction reactions. As illustrated in Figure 2.3, two possible pathways are involved. In essence, a primary organic compound is required. The electrons generated from the oxidation of organic compounds (primary substrates) are transferred to halogenated compounds via a mediator. The mediator could be microorganisms, nonliving

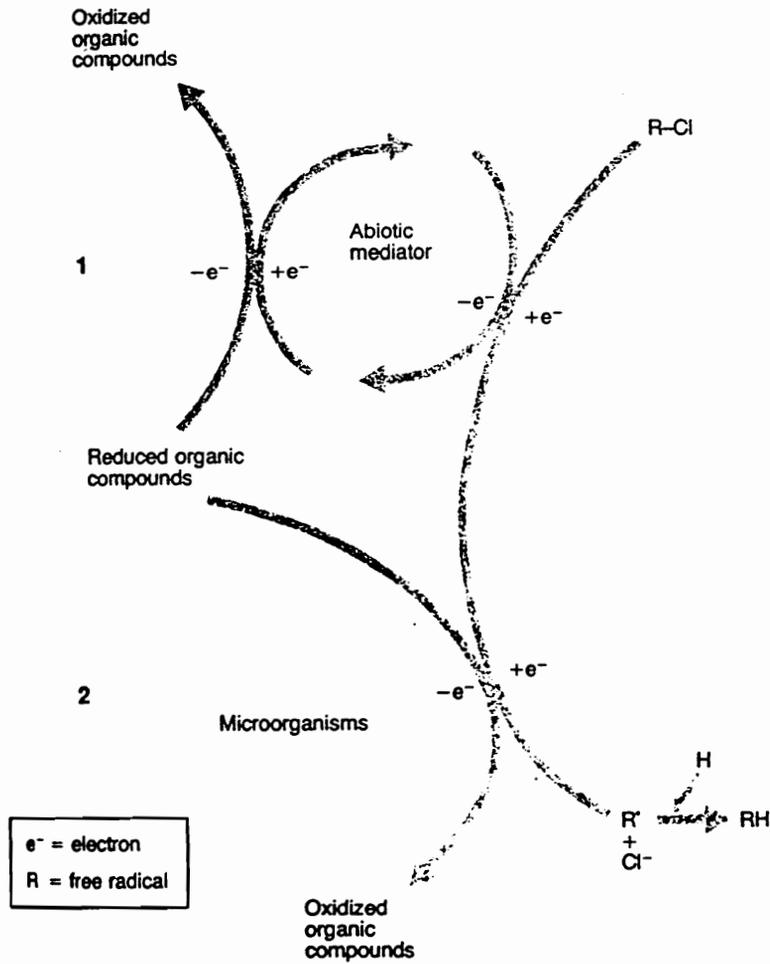


Figure 2.3 Two possible routes for reductive dechlorination (Kobayashi and Rittmann, 1982).

compounds (e.g.,  $\text{Fe}^{+3}$ ), or biological products (e.g., NAD(P), flavin). The major requirements for the reductive dehalogenation process are the availability of free electrons (the reducing power) from a primary substrate and the direct contact between the electron donors, mediators and electron acceptors (halogenated compounds) (Kobayashi and Rittmann, 1982). The products after reductive dehalogenation could be further utilized by microorganisms or have no benefits to microorganisms.

In an earlier investigation, Suflita et al. (1982) found that the chlorine on a number of meta-substituted chlorinated benzoates was replaced with hydrogen using an anaerobic microbial consortium isolated from sewage sludge. After all the chlorine atoms were successively replaced, the aromatic ring was broken and degraded to  $\text{CO}_2$  and  $\text{CH}_4$ .

A food chain for degradation of 3-chlorobenzoates was proposed by Shelton and Tiedje (1984) in which three key organisms were involved: the dechlorinating organism (DCB-1), the benzoate degrader (BZ-1) and methanogens (Fig 2.4). The reducing power ( $\text{H}_2$ ) required for reductive dechlorination is obtained from the oxidation of benzoate. One third of the hydrogen generated was consumed via the reductive dechlorination, while two thirds was left to the methanogens. The interspecies hydrogen transfer between benzoate degraders and methanogens allowed the transformation of benzoate to  $\text{H}_2$  to proceed and hence the



reductive dechlorination was enhanced.

Many non-aromatic halogenated hydrocarbons such as perchloroethylene (PCE) are also subjected to reductive dehalogenation under methanogenic conditions. According to the study by Fathepure and Boyd (1988), the reductive dechlorination of PCE to TCE occurred only during methanogenesis, and no dechlorination proceeded when methane production stopped. Methanogens directly acted as the dehalogenating microorganisms. Again, a source of reducing power is required (Fig. 2.5). The hydrogen from primary substrates was transferred to PCE through an electron transfer agent inside the cells of methanogenic bacteria. The data in Table 2.11 show that the reductive dechlorination of PCE was directly proportional to the concentrations of the primary carbon substrates. The dechlorinating ability under different methanogenic substrates suggested that dechlorination could take place at any one or all of the potential methanogenic sites (Fathepure and Boyd, 1988).

**(g) Effects of sulfate reducers on reductive dehalogenation**

Reductive dehalogenation, the critical process in biodegradation of halogenated compounds, is not found at active sulfate-reducing sites. Furthermore, the addition of sulfate to stimulate sulfate reduction at active methanogenic sites inhibited the reductive dehalogenation process. The microbial metabolism of chlorophenolic

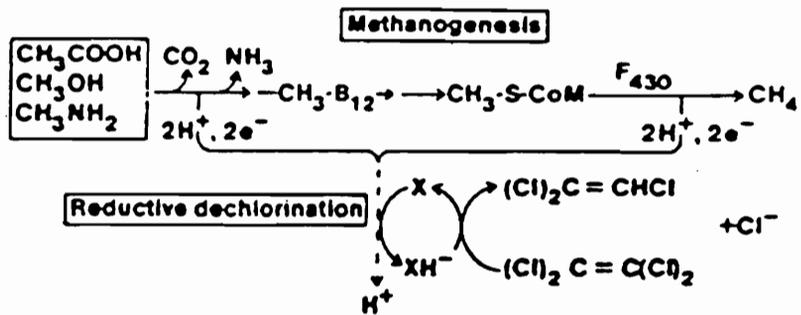


Figure 2.5 Hypothetical scheme for the transfer of electrons to PCE during methanogenesis (Fathepure and Boyd, 1988).

Table 2.11 Effect of methanol concentration on PCE dechlorination by *Methanosarcina* sp. strain DCM (Fathepure and Boyd, 1988).

Methanol (mmol)	TCE formed (nmol)	CH <sub>4</sub> formed (mmol)	CH <sub>4</sub> expected (mmol)	TCE formed per mmol of CH <sub>4</sub> (nmol)
0	9.6	0.15	0	64.0
0.25	12.9	0.25	0.188	51.6
0.50	21.1	0.44	0.38	48.0
1.0	34.9	0.79	0.75	44.2
1.5	57.8	1.19	1.125	48.6
2.0	84.9	1.70	1.5	49.9
2.5	91.0	1.76	1.875	51.7
5.0	154.0	2.80	3.75	55.0

\* *Methanosarcina* sp. strain DCM was first grown in 50 ml of growth medium containing 1.25 mmol of methanol for one growth period. At the end of the first growth cycle, different amounts of methanol and 150 µg (3 ppm) of PCE were added to the bottles, which were then incubated for 2 weeks.

compounds in an aquifer contaminated by municipal landfill leachate was studied by Suflita and Miller (1985). Two types of sites were found in the aquifer: active sulfate-reducing sites where sulfate concentrations were high and sulfate reducers were the dominant group; and active methanogenic sites where sulfate concentrations were low and methanogenesis was the major process. Their results indicated that microorganisms present in the soils at actively methanogenic sites were able to metabolize chlorophenols by replacing the chlorine with hydrogen atoms, whereas the chlorophenols tended to persist in the soils at active sulfate-reducing sites.

Another evidence of the inhibition of reductive dehalogenation by sulfate reducers was given by Gibson and Suflita (1986). Soil microorganisms at a sulfate-reducing site were unable to carry out reductive dehalogenation. The sulfate concentration appeared to be a critical factor in inhibiting the dehalogenation process. The addition of sulfate to the methanogenic sites inhibited the methanogenesis, resulting in the persistence of 2,4,5-T. Adding organics to reduce sulfate levels stimulated methanogenesis, thus dehalogenation took place.

These results indicate that dehalogenation can take place in soils and the process is highly dependent on the activity of methanogens. It is interesting to note that halogenated compounds can be considered to be competitive to methanogens

because a part of the hydrogen has to be channeled to replace halogen atoms. However, the competitive potential of halogenated compounds, unlike sulfate, is less than methanogens (about a half in the case of 3-chlorophenol, according to the H<sub>2</sub> flow). Therefore, the activity of methanogens can still be maintained. In addition, sharing hydrogen flow between dehalogenating microorganisms and methanogens (or between CO<sub>2</sub> and halogenated compounds) benefits both microorganisms. The dehalogenated organics can be potential substrates for fermentative microorganisms and provide additional methanogenic substrates for methanogens. When sulfate is not limited, sulfate reducers can inhibit methanogenesis by consuming methanogenic substrates that are used as the source of reducing power for reductive dehalogenation. Since sulfate reducers have a high affinity to the reducing power (H<sub>2</sub>), all the hydrogen was channelled to sulfate reduction, resulting in blocking reductive dehalogenation.

## **2.5 TBA and MTBE degradation in subsurface systems**

TBA and MTBE have been reported to be relatively recalcitrant in previously uncontaminated anoxic/anaerobic subsurface systems (Goldsmith, 1985; White, 1986; Morris, 1988; Hickman, 1988; McBrayer, 1989). The degradation rates of TBA and MTBE were slow and depended on the ecological conditions in the subsurface. TBA was degraded in a zero order rate pattern but was found to follow a first order

response with respect to the initial concentrations (Goldsmith, 1985). MTBE was reported to have a similar degradation pattern to TBA (McBrayer, 1989).

Subsurface material from ten sites in six locations representing diverse environmental conditions was evaluated in order to develop a relationship between biodegradation rates and microbial density (Hickman, 1989). TBA biodegradation rates were positively correlated to bacterial density in soil samples from Blacksburg, Virginia; but the correlation was insignificant between soils from diverse locations. Although a positive correlation was observed in Blacksburg soils, the correlation coefficient was low, indicating a high variability in subsurface systems.

A considerable effort has been devoted to determine the roles of different anaerobic metabolic groups in the biodegradation of TBA. Table 2.12 shows the classification of sites based on the relative degradation rates, responses to electron acceptor/inhibitor amendments, and general environmental conditions (Hickman, 1988). The results of electron acceptor and inhibition studies indicated that more than one type of metabolic group was able to degrade TBA but the predominant groups responsible for TBA degradation varied with site. Soils were categorized to two different types based on TBA degradation rates (Hickman, 1989). The "Fast" soils had a background of high organic flux and denitrifiers, sulfate reducers and methanogens were all

Table 2.12 Effect of site variation on TBA degradation  
(Hickman, 1988).

	Fast Sites	Slow Sites
Representative Sites	WP, BB2, BB3, NN1	WL, DF, BB1, BB4, NN2
Relative Degradation Rates (Methanol and TBA)	High	Low
Nitrate Addition	Increases Rates	Decreases Rates (Low pH Sites) or No Effect (High pH Sites)
Nitrite Addition	Inhibitory	Inhibitory
Sulfate Addition	Increases Rates	Decreases Rates
Inhibit Sulfate Reduction (Molybdate Addition)	No Effect	Increases Rates
Inhibit Methanogenesis (BESA Addition)	No Effect	Decreases Rates
Apparent Important Metabolic Pathway (s)	Nitrate Respiration/ Denitrification, Sulfate Reduction, Methanogenesis	Methanogenesis, Other (?)

active. In "slow" soils, where denitrifying bacteria were relatively inactive, the activity of methanogenic bacteria determined the degradation rate of TBA. Morris (1988) proposed that the competitive exclusion of methanogenesis by the sulfate reducers (in the presence of sulfate) retarded TBA degradation. Hickman suggested that high organic flux and active microbial groups might be the reason for faster degradation rates. However, it is still unclear what particular components were in the "organic flux", how the organic flux influenced the microbial activity and the degradation and how much the degradation rate can be changed if the microbial activity is enhanced.

Morris (1988) observed that TBA degradation under anaerobic conditions exhibited a similar pattern to those of halogenated organic compounds. TBA degradation was significantly increased when molybdate, a metabolic inhibitor for sulfate reducers), was added in the microcosms containing low-organic soils. He proposed that:

- (1) TBA degradation required the replacement of methyl groups in TBA with a H before any further degradations occur; and
- (2) interspecies hydrogen transfer was the mechanism for the reductive demethylation of TBA.

Therefore, to enhance the degradation of TBA and maybe MTBE and ETBE in the subsurface, the interactions between sulfate reducers and methanogens must be understood.

## **Chapter 3 Methods and Materials**

### **3.1 General Approach**

One of the primary objectives of this study was to evaluate the impact of environmental factors, both biotic or abiotic, on biodegradation in the soils. Because of a lack of information on the oxygenates, especially MTBE and ETBE, and the complexity of soil systems, the first task was to identify the environmental factors which had the potential to influence biodegradation.

Previous biodegradation studies indicated that the degradation of TBA was related to chemical and biological characteristics at each site (Hickman, 1988). To identify the environmental characteristics which may have measurable impacts on the degradation of TBA and possibly MTBE and ETBE, the study consisted of two parts: (1) site characterization to identify site variables; and (2) microcosm studies with amendments of subsurface parameters identified in (1). The subsurface influence on biodegradation was then elucidated based on the results from (1) and (2).

### **3.2 Sites and Sampling**

Aquifer material was sampled from various depths at three sites. Site 1 was located in a wooded area at VA Tech in Blacksburg, Virginia. Soils at this site were mainly unsaturated clay. Soil samples from two different points about 100 ft away from each other were taken at this site.

Soils from the first sampling point were taken in the Fall of 1988 and were designated as Site 1(A). The second location, Site 1(B), was taken in the Spring of 1990. Site 2 was also at VA Tech but positioned in a low area which received runoff from a nearby feedlot. The groundwater table was about 2 feet below the surface. Soils at this site were sandy loam. Site 3 was in Newport News, VA. The groundwater table was about 3 feet below the surface. Soils here were mainly silty loam.

In addition to the site identification, the depth in feet where a soil sample was collected is placed after the site number. For example, Site 2-5 represents a soil sample from 5 feet below the surface at Site 2.

Soils from these sites have been extensively studied in the past (Goldsmith, 1985; Morris, 1988; Hickman, 1988). Site 1 has been characterized as a "slow" site in terms of degradation while Sites 2 and 3 have been categorized as "fast" sites.

Subsurface material was collected by hand auger and transferred to sterilized mason jars fitted with teflon caps. Soils were then transported to the laboratory and stored at 10 C (the average annual temperature in the subsurface).

All glassware was acid-washed with a 10 % HCl solution before use. The glassware and utensils used in microbial and microcosm studies were further sterilized by flame or autoclaving at 15 psi and 121 C for 20 minutes.

### **3.3 Chemical Characterization**

The soils were characterized as to their anaerobic microbial populations, soil anions (nitrate, nitrite, sulfate and phosphate), soil moisture content, organic content, nitrogen availability, ammonium fixation rate, and soil pH. These chemical and microbial characteristics were used to relate the intrasite variations to the biological response.

#### **Soil moisture and organic content.**

The soil moisture content was determined by drying soils at 104 C for 24 hours. The organic content was expressed as mg COD per kg dry soil, using the method described by Nelson and Sommers (1982). The degradable organic content was determined by measuring the soil organic content before and after incubation at room temperature for 30 days after addition of inorganic nutrients (N&P).

#### **Extractable anions**

Soil samples for nitrate, nitrite and phosphate analysis were mixed with distilled water at a 1:1 ratio of soil to water. The resulting solution was shaken for one hour and the supernatant was analyzed using a Dionex Model 2010i Ion Chromatograph with a Dionex HPIC-AS3 column. A mixture of sodium bicarbonate (2.8 mM) and sodium carbonate (2.2 mM) was used as the eluant and 0.025N H<sub>2</sub>SO<sub>4</sub> was used as the regenerant.

For sulfate analysis, soil samples were prepared by the same procedure except extraction was with a solution of 100

ppm phosphate as  $K_2HPO_4$  (Tabataba and Bremer, 1972).

### **Nitrogen availability and ammonium fixation**

Nitrogen availability and ammonium fixation tests followed the methods described by Keeney (1982) and Blasco and Cornfield (1966), respectively.

For nitrogen availability analysis, 10 grams of an autoclaved soil sample was mixed with 50 ml of a 1 M KCl solution for 1 hour. The soil slurry was then centrifuged and the supernatant was analyzed for ammonium nitrogen using the distillation method.

For the ammonium fixation test, 10 ml of an inorganic nutrient solution containing 0.3 g/l of  $NH_4Cl$  was added to 5 g of soil. The soil was then incubated at 4 C for 24 hour. The residual ammonium nitrogen was then measured using the same procedures described in the nitrogen availability tests. The percentage of ammonium fixed by soils was calculated as the concentration lost during incubation divided by the initial ammonium nitrogen in the nutrient solution (Blasco and Cornfield, 1966).

### **Soil pH**

Soil pH was determined by mixing 5 grams of soil with 5 ml of distilled water for 10 minutes and measuring the pH with a pH meter (McClean, 1982). The final pH in microcosms was measured using pH paper.

### **3.4 Microbial Characterization**

Bacterial densities were analyzed by the Most Probable Number (MPN) method (Alexander, 1982). Three anaerobic metabolic groups were enumerated: denitrifiers, sulfate reducing bacteria (SRB) and methanogens.

#### **Denitrifying bacteria**

The culture medium for denitrifying bacteria consisted of 8.0 g nutrient broth, 0.5 g  $\text{KNO}_3$  per liter of tap water. Positive tubes were determined by ion chromatography for disappearance of nitrate (Tiedje, 1982).

#### **Sulfate reducing bacteria**

Sulfate reducers were enumerated in a culture media consisting of 0.5 g  $\text{K}_2\text{HPO}_4$ , 1.0 g  $\text{CaSO}_4$ , 2.0 g  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 1.0 g  $\text{NH}_4\text{Cl}$ , 0.5 g  $\text{FeSO}_4(\text{NH}_4)_2\text{SO}_4 \cdot 6\text{H}_2\text{O}$  and 1.0 g yeast extract per liter of tap water. Lactate (6 g/l) and acetate (1 mM) were used to culture two separate groups (acetate-using and lactate-using SRB). The reduced environments were achieved by bubbling with  $\text{N}_2$  gas for 20 minutes and adding 1.0 g sodium thioglycolate as a reductant (Kaspar and Tiedje, 1982). Tubes with a black  $\text{FeS}$  precipitate indicated the presence of sulfate reducers.

#### **Methanogenic bacteria**

The culture medium for methanogenic bacteria included 0.54 g  $\text{KH}_2\text{PO}_4$ , 1.23 g  $\text{Na}_2\text{HPO}_4$ , 0.3 g  $\text{NH}_4\text{Cl}$  and 50 ml mineral solution (6 g  $\text{NaCl}$ , 2.2 g  $\text{CaCl}_2$ , 2 g  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ /l) in 1 liter of tap water. After the medium was sterilized and bubbled

with N<sub>2</sub> gas for 20 minutes, 4 g NaHCO<sub>3</sub>, 0.05 g cysteine, 0.024 g Na<sub>2</sub>S and 30 ml TiCl<sub>3</sub> solution (3.75 ml of 20% TiCl<sub>3</sub> and 0.0012 g sodium citrate dihydrate in 51.25 ml, neutralized by saturated Na<sub>2</sub>CO<sub>3</sub>) were added to the solution and the pH was adjusted to ~7.0. Positive tubes were identified by injecting 100 ul of headspace gas into a gas chromatography and testing for the presence of methane (Kaspar & Tiedje, 1982).

### **Aerobes**

Aerobes were enumerated by plate counts using soil extract or nutrient agar as the culture medium. The inoculation and incubation for total plate counts followed the methods described in The Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties (Page, 1982).

### **3.5 Microcosm Setup**

Biodegradation was evaluated in static soil/water microcosms. Microcosm preparation followed the procedures described by Goldsmith (1985) and Morris (1988). Aquifer material was aseptically transferred to septum-capped test tubes (13x100 mm) and then saturated with water which contained the oxygenate of interest. The initial concentration of organics was approximately 100 mg/l. Each microcosm contained about 5.0 grams (dry weight) of the appropriate soil. All microcosms were incubated in the dark at 20 C. Microcosms of a particular organic compound and

amendment condition were prepared in triplicate.

Two microliters of sample were periodically withdrawn from the aqueous phase by a heat-sterilized syringe. The analysis of TBA, MTBE and ETBE used a gas chromatography equipped with a flame ionization detector and a 6'x 1/8" stainless steel column packed with 0.2 % Carbowax 1500 on 80/100 mesh Carbopack C. The carrier gas was nitrogen (24cc/min) and the oven temperature was 120 °C for TBA and 140 °C for MTBE and ETBE. The injection port temperature was 150 °C and the detector temperature was 250 °C.

### **3.6 Amendment Studies**

Amendment assays were conducted to examine the degradation of these oxygenates by different metabolic pathways. Three major microbial processes were studied; aerobic respiration, denitrification, anaerobic respiration. To provide oxygen for aerobic respiration, 2 µl of 30% hydrogen peroxide was added to the microcosms every 7-10 days. The calculated concentration of hydrogen peroxide in the microcosms was 0.01 %, which is lower than the reported toxicity level of 0.05 % (Britton, 1985).

Five mM nitrate and two mM sulfate were added to stimulate denitrification and sulfate reduction, respectively. Carbonate, the electron acceptor for methanogenesis, was not considered to be limiting in the microcosms.

The effects of environmental factors were evaluated for

each metabolic pathway. Chemical oxidation by hydrogen peroxide and the influence of inorganic nutrients were studied in the aerobic degradation process. The inorganic nutrient solution consisted of 0.54 g/l  $\text{KH}_2\text{PO}_4$ , 1.23 g/l  $\text{Na}_2\text{HPO}_4$ , 0.3 g/l  $\text{NH}_4\text{Cl}$  and 50 ml/l of mineral solution (6 g  $\text{NaCl}$ , 2.2 g  $\text{CaCl}_2$ , 2 g  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$  per liter of tap water). The factors examined in denitrification included the availability of inorganic nutrients and the presence of primary organic substrates. In addition to the influence of inorganic nutrients and primary organic substrates, microbial competition was also studied under conditions favoring sulfate reduction and methanogenesis. Two mM of sodium molybdate (a specific metabolic inhibitor for sulfate reducers) or 1 mM of 2-Bromoethanesulfonic acid (BESA, an inhibitor of methanogenesis) were added in the microcosms to inhibit the activity of sulfate reducers or methanogens, respectively (Smith and Mah, 1978; Zehnder and Brock, 1981; Smith and Klug, 1981; Winfrey and Ward, 1983).

### **3.7 Estimation of Degradation**

After the microcosms were prepared, concentrations of the added oxygenates were monitored for more than 250 days. Studies have shown that TBA exhibits a long lag phase followed by a faster zero-order degradation pattern (Goldsmith, 1985; Morris, 1988; Hickman, 1988). Therefore, fast degradation rates do not necessarily indicate that

compounds will disappear in a short time. To have a more complete picture of the biodegradation of oxygenates in the subsurface, three parameters were used in the evaluation of degradation: degradation rate during the period where degradation is most active, lag time, and time for 80 % of the compound to be degraded ( $T_{80}$ ).

Degradation rates were calculated from the slope of a portion in the concentration-time plot that showed active degradation. The slope was then divided by the calculated dry weight of soil in each individual microcosm (Fig. 3.1). Due to soil heterogeneity, it is not uncommon to see different degradation patterns in triplicate microcosms using the same soil and incubated under the same conditions. Figure 3.1 shows an example of a soil with a variable degradation response. Usually the reported value is the average of three microcosms. However, when no degradation was evident over the study period in one of the three microcosms, data for the non-degrading microcosms was not included.

### **3.8 Volatilization Losses**

Throughout the experiments with MTBE and ETBE, volatilization was always a potential problem. Controls were used to monitor and quantify the volatilization losses in the system. These contained soils which were autoclaved once a day for five consecutive days at 120 °C and 15 psi pressure for 30 minutes. The soils were then transferred to the

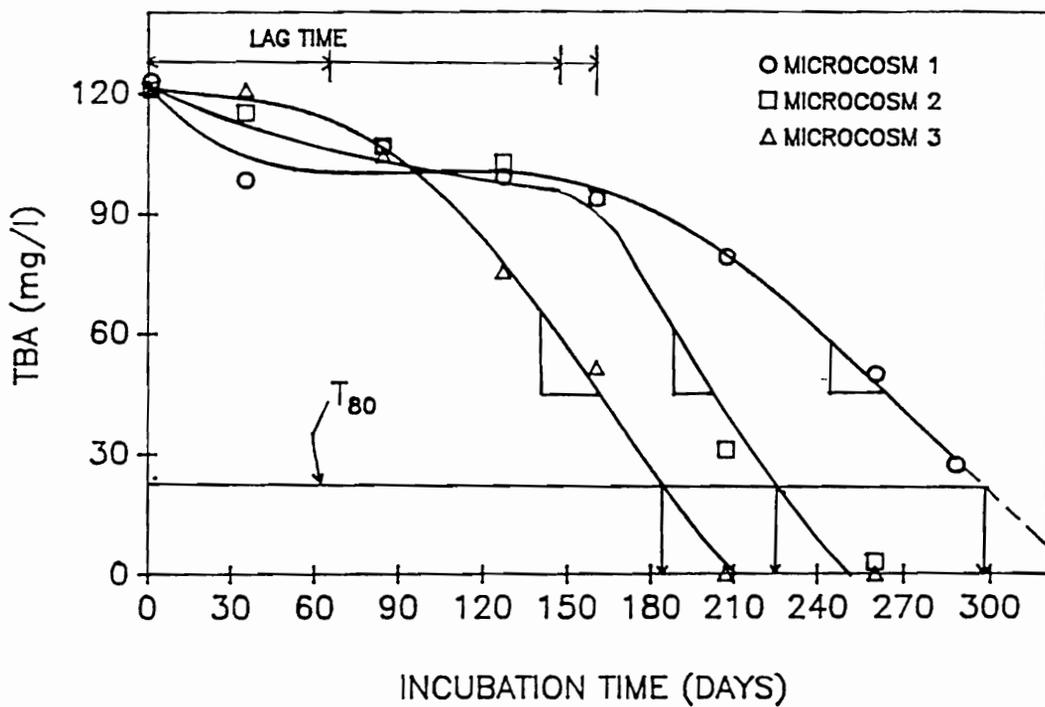


Figure 3.1 Degradation responses and determination of degradation in microcosms

microcosms and autoclaved two more times.

Figure 3.2 shows that little MTBE and ETBE were lost during sampling and incubation. These results indicate the system provided a satisfactory way to avoid volatilization losses. However, throughout the experiments, some microcosms still showed headspace and posed a potential possibility of volatilization loss. These microcosms were discarded.

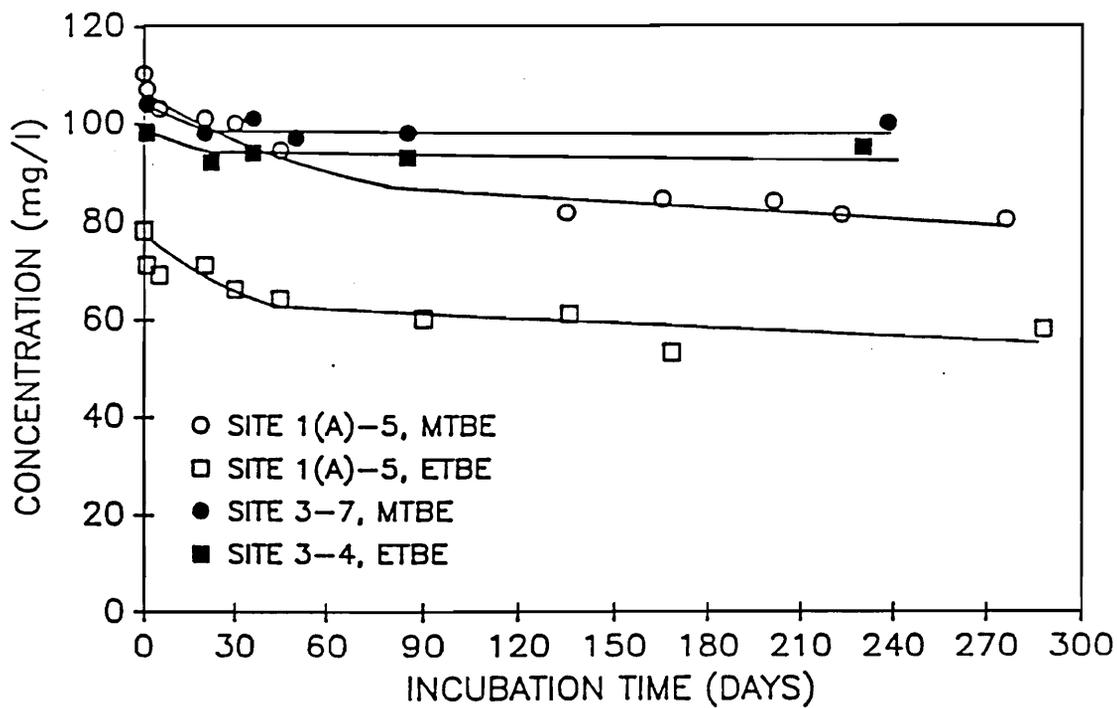


Figure 3.2 Volatilization losses of MTBE and ETBE in control microcosms.

## Chapter 4 Site Characterization

Soil characteristics for the sites are listed in Table 4.1. Except for the surface soils, the soils at the three sites were moderately acidic with a pH between 5.0 and 6.0. The degradable organic matter, nitrogen availability and ammonium fixation rates were reflective of the background at each site. In the soils from Sites 1 and 2, where the organic flux came from surface infiltration, organic matter, nitrogen and sulfate contents were high in the upper layers and decreased with depth. Site 2 surface soil, which received runoff from a feedlot, was very high in organic matter. At Site 3, organic matter entered from both the surface and through moving groundwater, so the concentration of organic in the soil from the unsaturated zone decreased with depth and then increased in the saturated zone.

One distinct characteristic of the Site 1 soils is that soils at this site fixed a significant quantity of ammonium (Table 4.1). More than 30% of added ammonium was absorbed in one day. The Site 1 soils were predominantly clays (for example, 72 % in Site 1(B)-2). Although the types of clay minerals at this site were not characterized, it is possible that large portions of the clay at Site 1 were the type of clay minerals which can fix ammonium (the 2:1 type).

The fixing capacity also depends on the amount of ammonium to which a soil has been exposed. Site 1 had less input of nitrogen and the direction of nitrogen input was from the

Table 4.1 Chemical and physical characteristics of soils.

Soil	% Moisture	Soil pH	Organic (mg/g as COD)		NH <sub>4</sub> <sup>+</sup> - N (mg/kg)		Major Anion (mg/l) <sup>b</sup>		
			Total	Degradable	Available	% Fixation	NO <sub>3</sub> <sup>-</sup>	PO <sub>4</sub> <sup>-3</sup>	SO <sub>4</sub> <sup>-2</sup>
1-2 <sup>a</sup>	26	5.0	10800	3700	14.0	53.8	0.2	0.07	34.0
1-5	29	4.8	3300	<100	8.4	7.7	0.3	0.03	19.0
1-10	27	5.1	3100	400	3.0	15.4	<0.1	0.40	5.0
1-14	30	5.0	1600	<100	3.0	69.2	1.0	0.03	5.0
2-0	23	6.8	66500	14800	51.4	0	15.0	0.14	40.0
2-5	31	5.0	4200	<100	26.6	0	7.0	0.14	24.0
2-10	30	5.1	1700	200	8.7	0	8.7	0.14	20.0
2-13	26	5.2	1100	<100	7.0	0	7.0	0.14	22.0
3-0	6	7.2	9000	2200	38.7	30.8	0.6	0.26	99.4
3-2	8	6.8	10400	2900	8.1	15.4	4.1	0.29	65.4
3-4	17	5.2	2900	<100	22.6	0	0.5	0.12	7.8
3-7	22	5.7	6500	3200	19.5	0	0.2	0.21	20.0
3-10	41	6.2	155000	11000	72.9	0	<0.1	0.05	230.0

a - average value of site 1(A) and Site 1(B).

b - in 1:1 soil extract

surface. Therefore, deeper soils tended to fix more ammonium than the upper soils. Little ammonium was fixed in the Site 2 soils. At Site 3 where the groundwater provided nitrogen, the subsurface soils absorbed less ammonium than the surface soils. Site 2 and 3 soils were thought to contain significant amounts of the 2:1 type clay minerals. However, because these soils had long-term exposures to an organic flux which was rich in nitrogen, the ammonium fixing capacity of these soils might have been exhausted and this may be the reason for less ammonium fixation.

The significant fixing capacity of ammonium in some soils could have profound impacts on the bioavailability of added nitrogen. Nitrogen is usually applied in the form of ammonium salts. Once absorbed on clay minerals, the availability of added ammonium is significantly reduced (Nommick & Vahtras, 1982). Therefore, the organic-poor but clay-rich soils will have less readily available nitrogen than the organic-rich and low-clay soils when the same amounts of ammonium nitrogen are applied.

The microbial populations reflected the organic and nutrient status at each site. The densities of anaerobic groups at the sites are given in Figure 4.1. Substantial numbers of anaerobic microorganisms were found at these sites. In general, microbial populations decreased with depth. Soils from Sites 2 and 3 had microbial populations 2 orders of magnitude higher than Site 1. Denitrifiers and

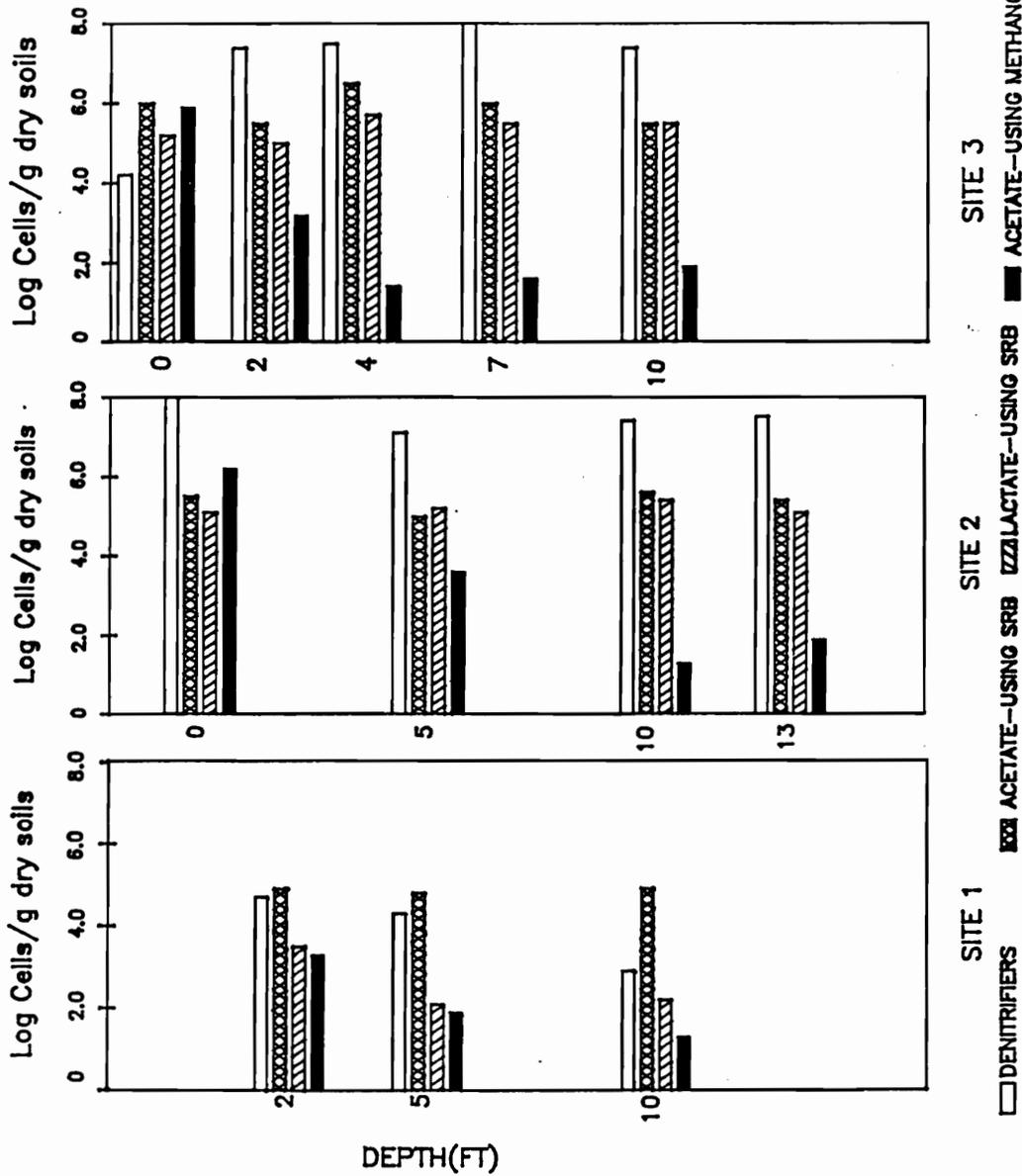


Figure 4.1 Distribution of anaerobic microorganisms in soils.

sulfate reducers were the dominant groups for all soils while methanogens were much fewer in the subsurface.

Studies have reported that the denitrifiers are the most dominant groups among the anaerobic microorganisms in anaerobic environments (Tiedje, 1988). The enumeration results for the denitrifiers are consistent with those results. The high populations of lactate-using and acetate-using sulfate reducers in soils are not surprising. Soils from these sites contained considerable amounts of sulfate which could be used by sulfate reducers. Sulfate reducers are also able to act as fermenters and survive when sulfate is depleted (Colberg, 1988).

Among the anaerobic groups, methanogenic bacteria were the fewest, even in the soils at Site 2 and 3. Acetate-using methanogens were only found in the top soils, indicating that soil environments determine the distribution of methanogenic microorganisms. Methanogens require environments of low redox potentials (Oremland, 1988). Except for the soils from the surface where abundant organics provided enough organic substrates and ensured anaerobic microsites, other soils were usually low in organic matter and active in sulfate reduction. Therefore the methanogenic microorganisms are expected to be relatively inactive in such environments.

The chemical and biological characteristics indicate that soils from Site 1 were relatively oligotrophic compared to

the others. Site 2 soils were the most eutrophic with respect to organic and nutrient contents.

## **Chapter 5 Degradation in Unamended Soils**

This phase of the study dealt with natural or unamended microcosms where the soils were incubated without addition of any external amendment to stimulate or inhibit the indigenous subsurface microorganisms. Because oxygen and nitrate were limited and sulfate and carbon dioxide were abundant in the system, the possible metabolic pathways involved in the biodegradation in the unamended microcosms would be fermentation, sulfate reduction and methanogenesis. The results from the unamended microcosms were used not only to obtain background information on biodegradation under natural conditions but also as a set of controls to be used to evaluate the effectiveness of the alternations imposed on the microcosms in the amendment studies.

### **5.1 TBA**

#### **Site 1**

TBA biodegradation data for the three sites are summarized in Table 5.1. Typical degradation patterns for three sites are shown in Fig. 5.1 and the complete set of data is contained in Appendix. In the absence of amendments, TBA was degraded very slowly in the Site 1 soils. In general, TBA degradation at Site 1 followed a zero degradation rate pattern with no obvious lag phase. These data are similar to those observation from other studies (Goldsmith, 1985; Morris, 1988; Hickman, 1988). TBA degradation rates at site 1

Table 5.1 TBA degradation in unamended microcosms.

Soil	Degradation Rate (mg/l/day/g dry soil)	Lag Time* (day)	T <sub>50</sub> (day)
1(A)-2	0.04	*	360
1(A)-5	0.02	*	>600
1(A)-10	<0.01	*	>600
1(B)-2	0.06	*	300
1(B)-5	0.03	*	>600
1(B)-10	0.05	*	>600
1(B)-14	0.04	*	>600
2-0	0	----	----
2-5	0.13	<30	110
2-10	0.11	110	200
2-13	0.12	40	160
3-1	0.06	short, <30	200
3-2	0.06	30	300
3-4	0.08	short, <30	200
3-7	0.15	short, <30	150
3-10	0.08	*	400

\* : Approximated values  
 \* - no clearly defined lag phase.

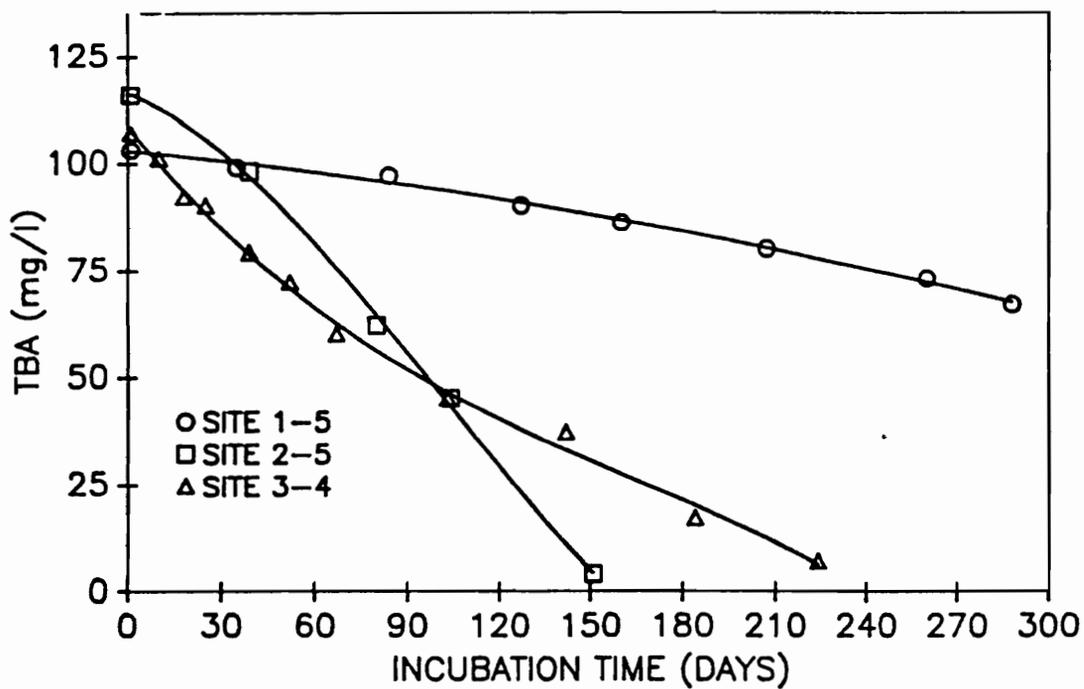


Figure 5.1 Typical TBA degradation patterns in unamended microcosms.

ranged from 0.01 to 0.06 mg/l/day/g dry soil and the projected  $T_{80}$  was more than 3 years. Soils from upper layers had slightly higher degradation rates. Similar results were obtained from Site 1(B) soils, indicating little variation in TBA degradation for separate soil samples from the same area.

### **Sites 2 and 3**

Except for the Site 2 surface soil (Site 2-0) in which a high concentration of methane was detected in the microcosms and no TBA degradation occurred, TBA degradation rates in Site 2 soils were two times higher than in the Site 1 soils and exhibited shorter lag phases. TBA degradation in the Site 3 soils was faster than in the Site 1 soils but slower than in the Site 2 soils except for the Site 3-7 soil which had a degradation rate similar to those for site 2.

The results from the unamended microcosms indicate that the chemical and microbial environments may have influenced the degradation of TBA. Site 2 and 3 soils contained organic matter and nutrients and had 2 orders of magnitude higher anaerobic microbial populations. Site 1, on the other hand, was low in microorganisms, organic matter, and nutrients. This implies that soils with relatively abundant organic matter and nutrients will develop more diverse and active microbial groups, resulting in faster TBA degradation.

## 5.2 MTBE and ETBE

### Site 1

No significant MTBE degradation was observed at any depths at Site 1 during the 250-day study period (Appendix A-2). Approximately 10 % of the MTBE was lost during the first 2 months of incubation, probably due to adsorption, and the concentrations in the microcosms remained steady after that.

In contrast to MTBE, ETBE was degraded in the 5 and 10 foot soils from Site 1 (Fig. 5.2). After about a 120-day lag, ETBE was metabolized to TBA, and this was confirmed by GC/MS (Fig. 5.3). Figure 5.4 shows the proposed degradation pathway for ETBE.

Cleavage of the ether bond as the first step in the degradation of methoxylated compounds has been reported in the literature. The cleavage of ether linkage produced an alcohol and an aldehyde. For example, the ether linkage in 2,4-D was cleaved, producing 2,4-dichlorophenol (Alexander, 1978). The mechanisms for aerobic and anaerobic demethoxylation are different. Aerobic demethoxylation requires the insertion of an oxygen atom on the methyl branch. The anaerobic demethoxylation was catalyzed by an acetogenic strain (Donnelly and Dagley, 1980; Bache and Pfenning, 1981; Schink, 1988).

Demethoxylation is also a step in the degradation of lignin-derived monoaromatic compounds under methanogenic conditions (Healty and Young, 1979; Colberg, 1988). Lignin

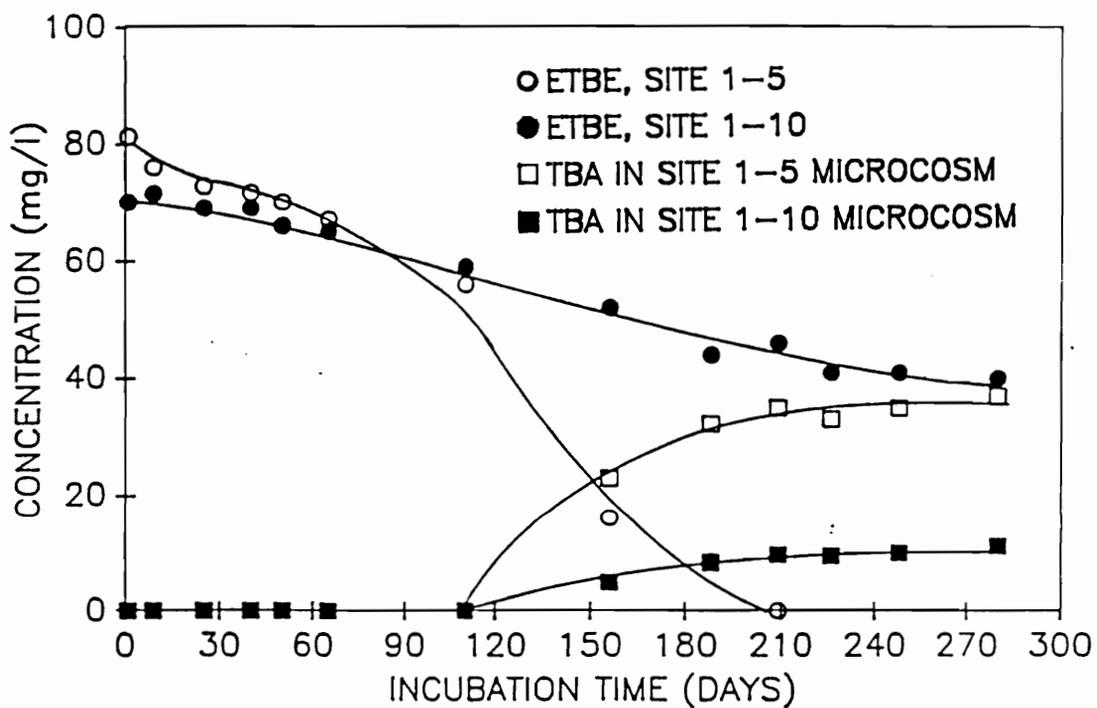


Figure 5.2 Degradation of ETBE in unamended Site 1(A) soils, showing TBA as an intermediate.

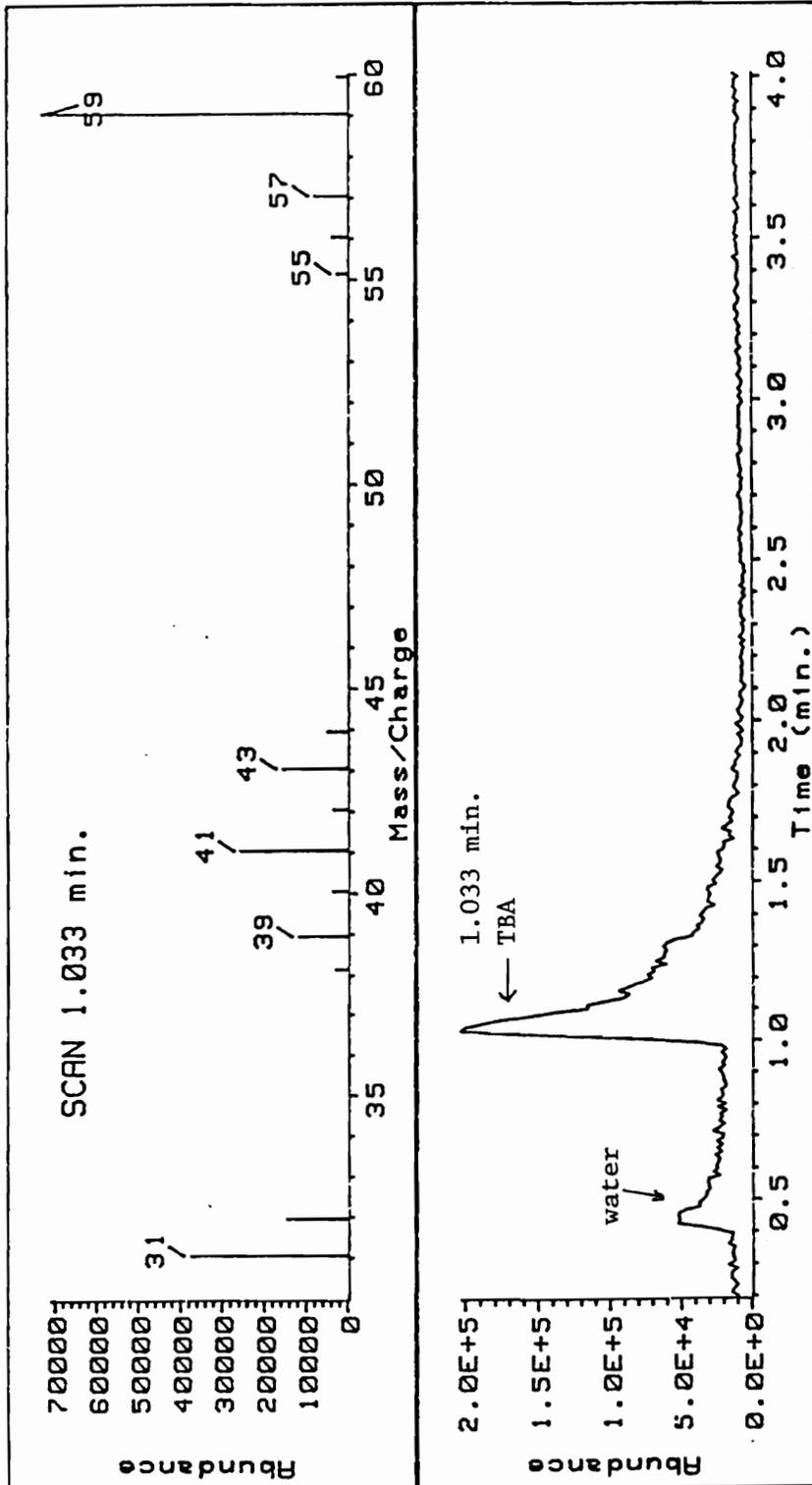
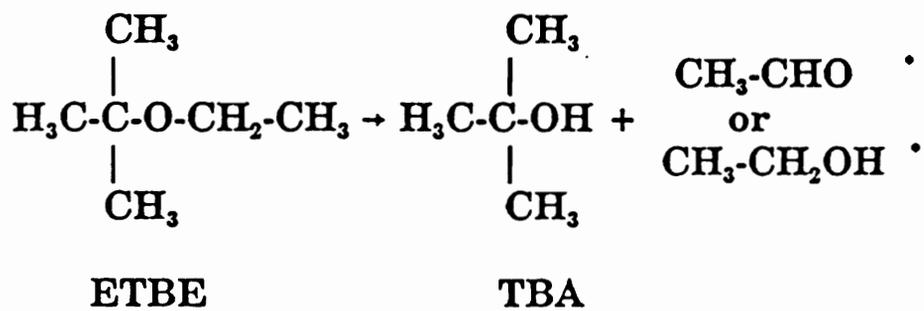


Figure 5.3 GC/MS confirmation of TBA produced in microcosms with Site 1(A)-5 soil after 200 days of incubation.



\* not detected by GC

Figure 5.4 Proposed pathway for anaerobic ETBE degradation.

is a natural substance in the soil. The removal of the methoxyl groups is one of the modification processes of lignin ongoing in the soil systems (Stevenson, 1982; Colberg, 1988). Studies have also shown that compounds with methoxyl groups such as methoxy-substituted phenols and benzoic acids are more resistant to biodegradation. The cleavage of the ether linkages must occur before degradation proceeds further (Boyd, et al., 1983; Taylor, 1983; Kaiser and Hanselmann, 1982).

The results from this study suggest that soil contains microorganisms which are capable of cleaving ether linkages but for the MTBE and ETBE this cleavage proceeds slowly. The conditions for cleavage of the ether bond in MTBE appears to be more stringent than for ETBE.

The accumulation of TBA suggests that breakage of the ether bond in ETBE is the first step in ETBE degradation. MTBE, if it were degraded, would be expected to undergo a similar reaction but at a much slower rate or with a very long lag phase. It is possible that MTBE degradation would have occurred if the degradation process was monitored beyond the study period of 250 days.

The moles of TBA accumulated per moles of ETBE degraded ranged from 0.4 to 0.8, depending on the relative degradation rates of ETBE and TBA (Table 5.2). For example, the degradation of ETBE was faster in the Site 1(A)-5 soil than in the Site 1(A)-2 soil. But the TBA degradation was slower

Table 5.2 Degradation of ETBE in the Site 1 soils

Soil	Disappearance of ETBE		TBA production	Final pH in ETBE microcosms	Final pH in TBA microcosms
	rate <sup>a</sup>	lag time <sup>b</sup>	rate <sup>a</sup>		
1(A)-2	0.02	120	0	4.7	5.8
1(A)-5	0.11	120	0.08	5.5	5.8
1(A)-10	0.03	120	0.02	6.0	6.1
1(B)-2	0	-	-	4.4	5.5
1(B)-5	0	-	-	4.7	5.8
1(B)-10	0	-	-	5.0	5.8
1(B)-14	0.04	160	0	5.4	5.8

a: mg/l/day/g

b: days

in the Site 1(A)-5 soil; therefore TBA accumulated in the Site 1(A)-5 soil but not in the Site 1(A)-2 soil. This indicates that rates of the ether bond cleavage and rates of TBA degradation are probably regulated by different organisms. Degradation of ETBE at Site 1(B) occurred at different depths from those at Site 1(A). The highest ETBE degradation rates were found at Site 1(A)-5 and Site 1(B)-14. In addition, even though ETBE was degraded in the Site 1(A) and Site 1(B) soils, degradation did not occur in each separate microcosm of the triplicates set. TBA degradation, on the other hand, occurred at similar rates for soils from both Site 1(A) and site Site 1(B) (section 5.1). This suggests that the ETBE degradation was subject to intersite variation more significantly than TBA degradation.

It was observed that the microcosms containing Site 1(A)-5 and Site 1(B)-14 soils had final pH of 5.5, suggesting that the pH might influence ETBE degradation (Table 5.2). In addition, the final pH in Site 1 microcosms containing MTBE and ETBE was lower than those with TBA. Both MTBE and ETBE are reported to be unstable in acidic solution (Budavari, 1989). Therefore, hydrolysis tests using solutions containing MTBE and ETBE were performed to evaluate the chemical hydrolysis of ETBE and MTBE in acidic and alkali environments. The effects of reducing environments were also evaluated. The appearance of TBA was used as an indication of the breakdown of ETBE and MTBE through hydrolysis.

The hydrolysis results are shown in Figure 5.5. ETBE is slightly more unstable than MTBE under reduced environments. However, in general, ETBE and MTBE are stable over the pH range from 4 to 10. Therefore, the decreases in ETBE in the soil microcosms were most likely the result of microbial activity.

#### **Site 2 and 3**

No degradation of MTBE and ETBE was observed over 250 days of incubation in Site 2 and 3 soils. These sites were microbially active and a variety of organic compounds including methanol, phenol and TBA were found to be rapidly degraded at these sites (Hickman, 1988; Morris, 1988). It might be that at microbially active and organically rich sites the population of microorganisms capable of synthesizing the enzymes to catalyze the breakage of ether bonds in MTBE and ETBE were outcompeted or the enzymes were repressed or inhibited.

The degradation results from unamended microcosms indicate that TBA can be biodegraded naturally in the subsurface and the degradation rates will vary over both the site and depth. MTBE, on the other hand, was recalcitrant to biodegradation. ETBE was biodegraded but this reaction was limited to soils which were least biologically active and contained the lowest levels of organic matter.

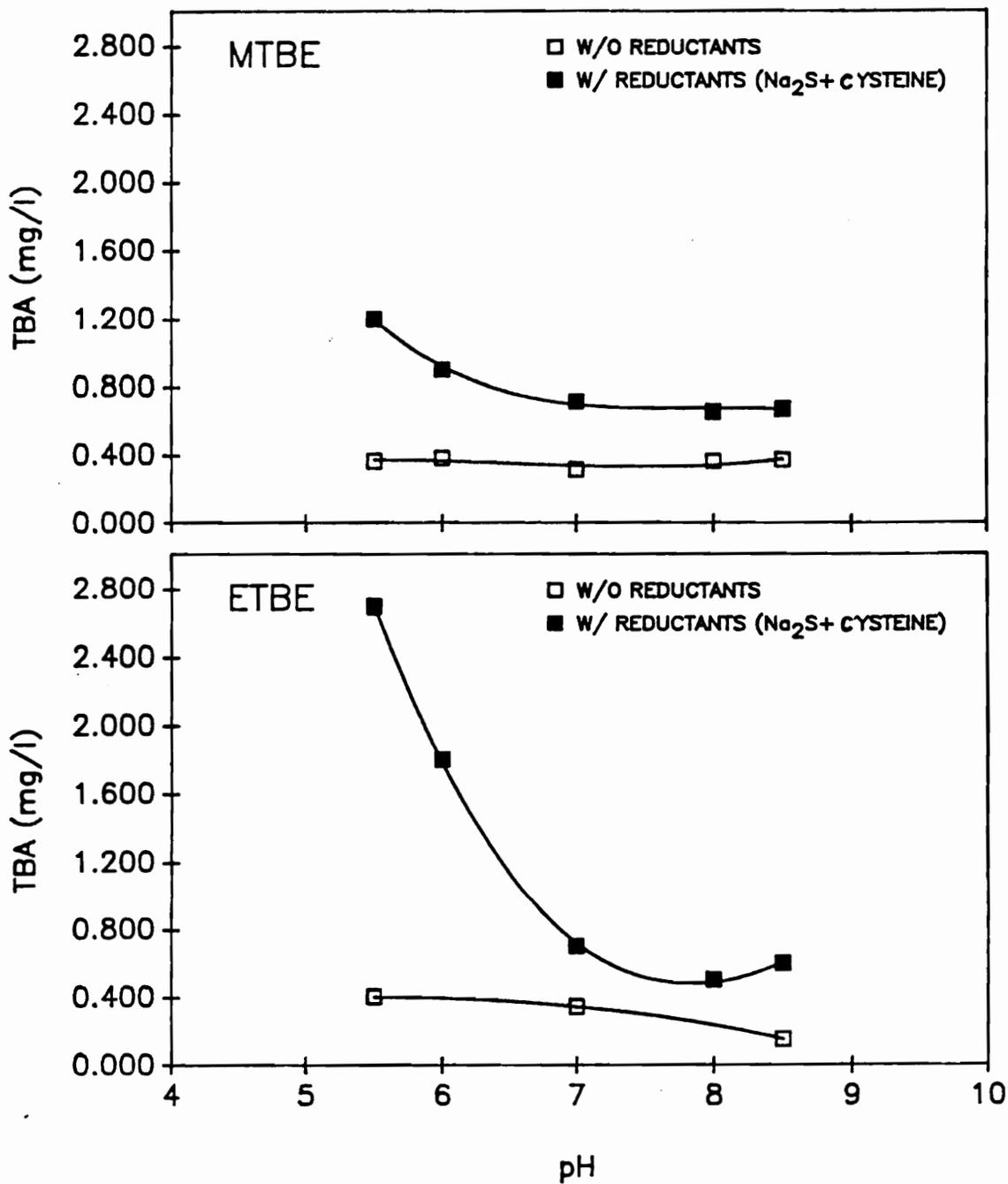


Figure 5.5 Effects of pH on the hydrolysis of MTBE and ETBE with and without the addition of reductants.

## **Chapter 6 Aerobic Degradation**

In this chapter, the aerobic degradation of TBA, MTBE and ETBE in soil samples from Site 1(B) and Site 3 is discussed. Hydrogen peroxide (2  $\mu$ l of 30%  $H_2O_2$  every 7-10 days) was added into the microcosms as an oxygen source. Chemical oxidation by hydrogen peroxide and the effects of nutrient availability are also evaluated.

### **6.1 Chemical Oxidation**

#### **Microcosms with autoclaved soils**

Because hydrogen peroxide is a strong oxidant, two sets of experiments were performed to evaluate the oxidation of the oxygenates in the presence of hydrogen peroxide. The first experiment used a solution system containing distilled water and the oxygenates. Figure 6.1 shows that none of the oxygenates were oxidized by hydrogen peroxide in the water solution.

The second set of the oxidation experiments used microcosms containing autoclaved soils. Two  $\mu$ l of hydrogen peroxide was added every 7-10 days. Figures 6.2 and 6.3 show decreases in TBA, MTBE and ETBE in the microcosms with autoclaved Site 1(B)-5 and Site 3-4 soils, respectively. Tables in Appendix B present the results of similar studies for the other soils.

The losses of TBA, MTBE and ETBE in the autoclaved soil microcosms were very significant. More than 50% of the total

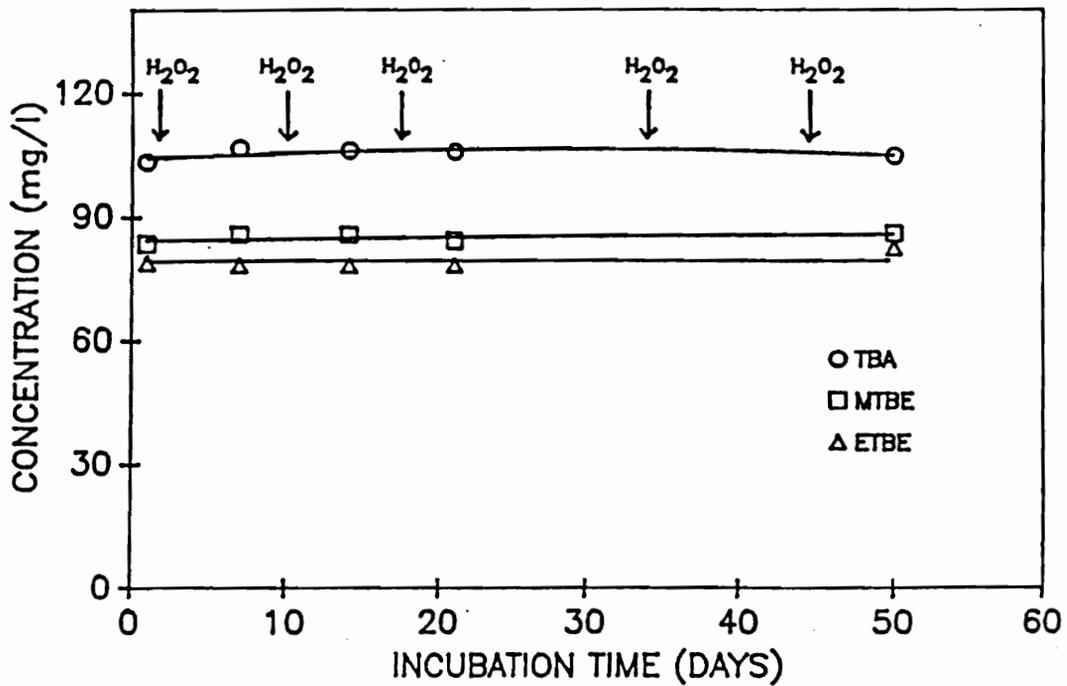


Figure 6.1 Losses of MTBE, ETBE and TBA in water solution with hydrogen peroxide addition.

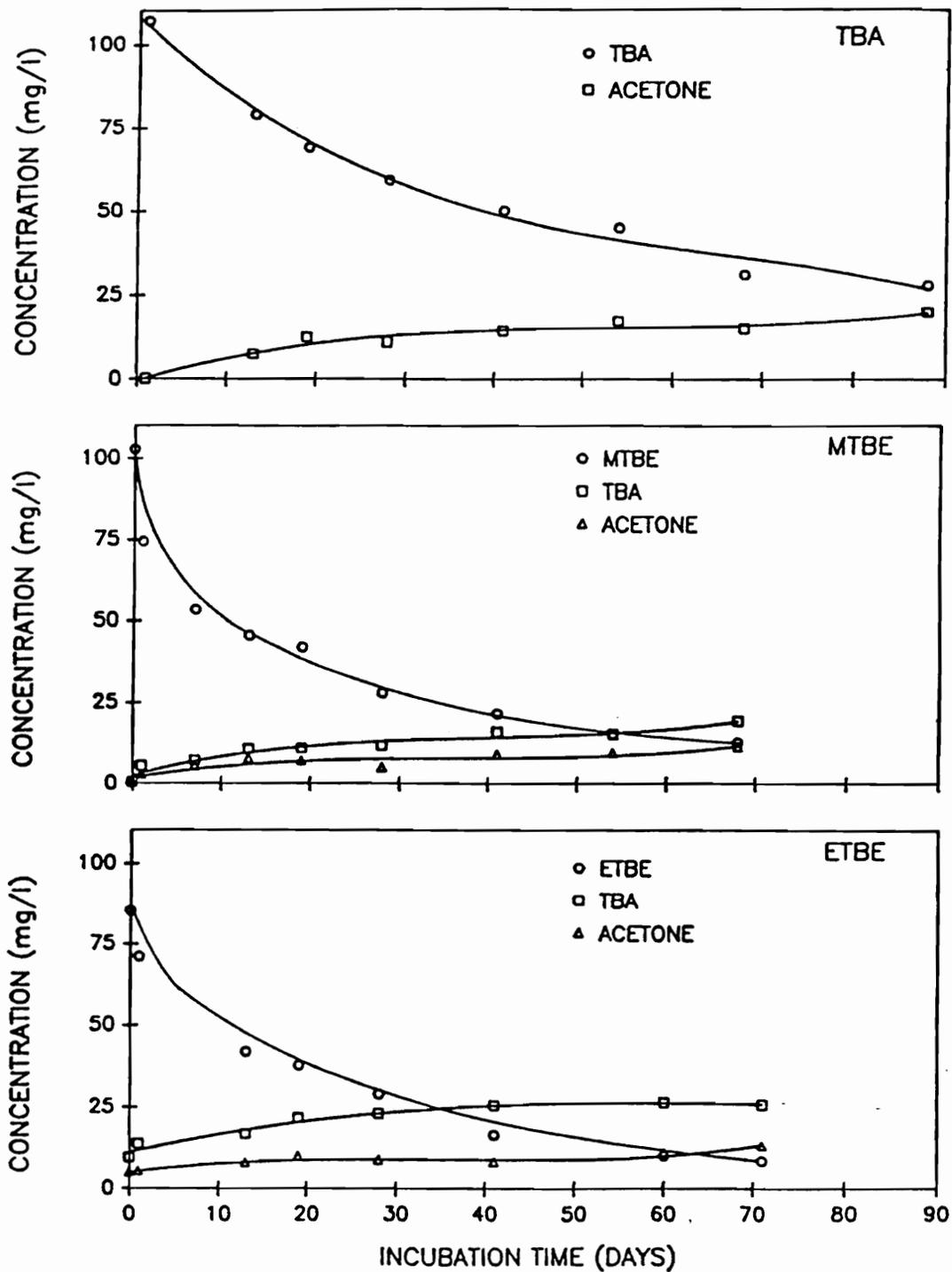


Figure 6.2 Losses of TBA, MTBE and ETBE in autoclaved Site 1(B)-5 soils with hydrogen peroxide addition.

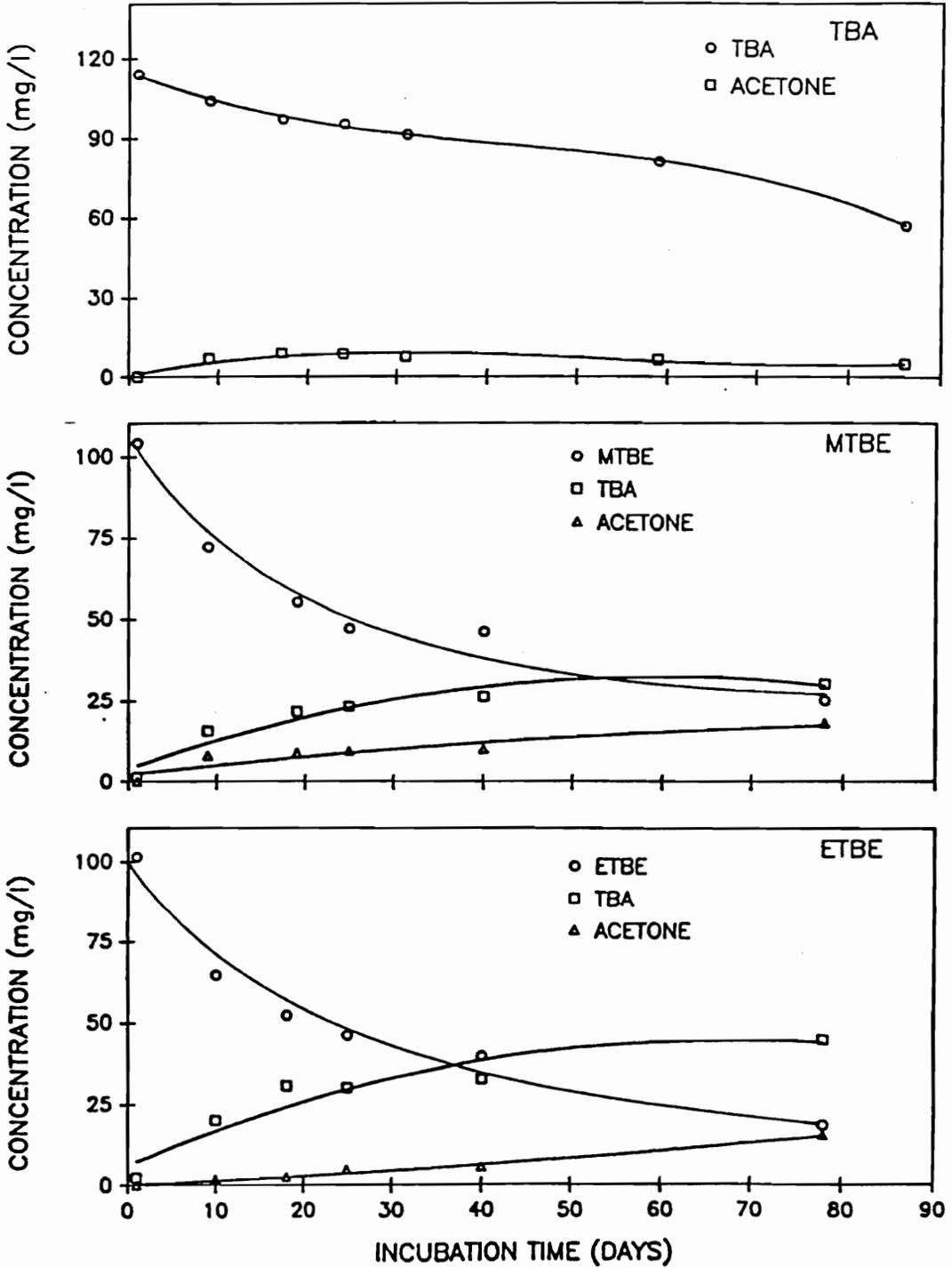


Figure 6.3 Losses of TBA, MTBE and ETBE in autoclaved Site 3-4 soils with hydrogen peroxide addition.

losses occurred in the first 20 days, the losses continued for one month and then stopped. Acetone appeared in the microcosms containing TBA, and acetone and TBA were detected in those containing MTBE and ETBE (based on the identical retention times to the standards). Other unidentified compounds also appeared, which were thought to be formaldehyde and acetaldehyde.

The extent of losses varied with the compounds added, site, and depth (Fig. 6.4). ETBE exhibited the most significant losses, followed by MTBE and TBA. Autoclaved Site 1(B) soils had greater losses than the autoclaved Site 3 soils.

The significant losses in the autoclaved soils raised the question of whether the conversions were due to bioactivity or chemical oxidation. The existence of active microbial cells in the autoclaved soil microcosms was confirmed by plate counts. A high population of aerobes (magnitude of  $10^7$  cells per g soil) was found in the soil samples in the autoclaved microcosms showing ETBE losses. The control (autoclaved soils incubated but without organic addition) had a cell count of  $10^2$ , indicating that the autoclaving process was able to significantly reduce cell populations but did not completely sterilize the soil. The large microbial population in the autoclaved soils after incubation with ETBE implies that the multiplication of microorganisms in the microcosms had occurred.

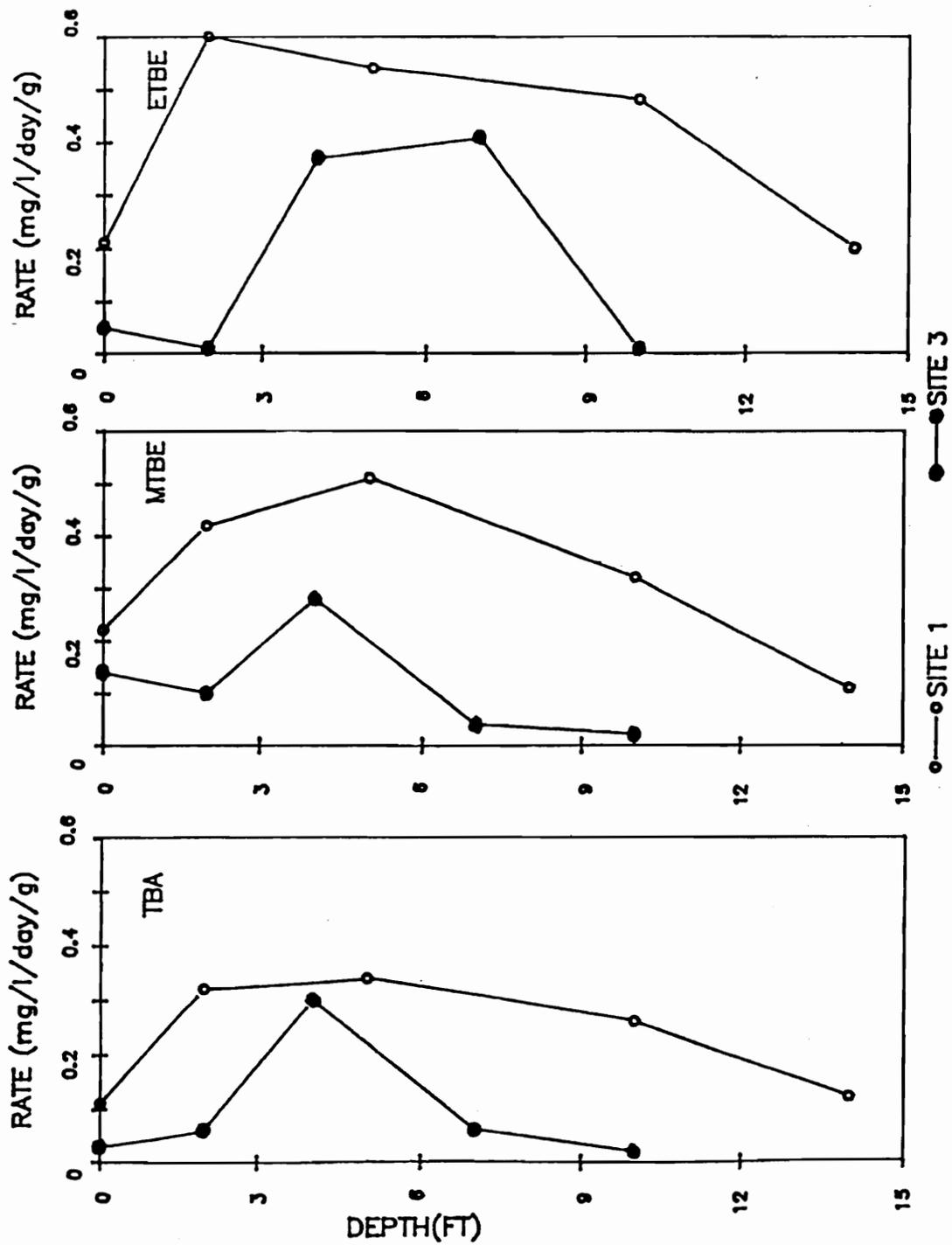


Figure 6.4 Depth profile of TBA, MTBE and ETBE losses in the autoclaved soils.

Although the autoclaving process is commonly used to sterilize liquids, its effectiveness for soil samples is still questionable. Therefore, it was not surprising to see the regrowth of microorganisms, especially the aerobes, in autoclaved soil samples. Many aerobes are known to form spores under extreme environments. The fundamental principle of consecutive autoclaving is that bacterial cells will regrow from the spore forms and then be killed in the next autoclave. However, it is possible that not all the spores grow to cells during the consecutive autoclaving. If soil environments are unfavorable for growth, which is usually the case, many cells will still remain in the form of spores. Contaminations during incubation and sampling are also possible.

Another set of experiments was performed to test the effects of autoclaving frequency on the losses of these oxygenates. If the microorganisms were involved in the losses of the oxygenates, more frequent autoclaving should result in fewer microorganisms and a lower chemical loss. The Site 3-7 soil was autoclaved one, three and five times and incubated with MTBE or ETBE. Figure 6.5 shows that increased autoclaving frequency did not decrease the rates of losses. This suggests that the losses are independent of the microbial populations.

A closer examination of the concentrations before and after  $H_2O_2$  addition to the microcosms with autoclaved soils

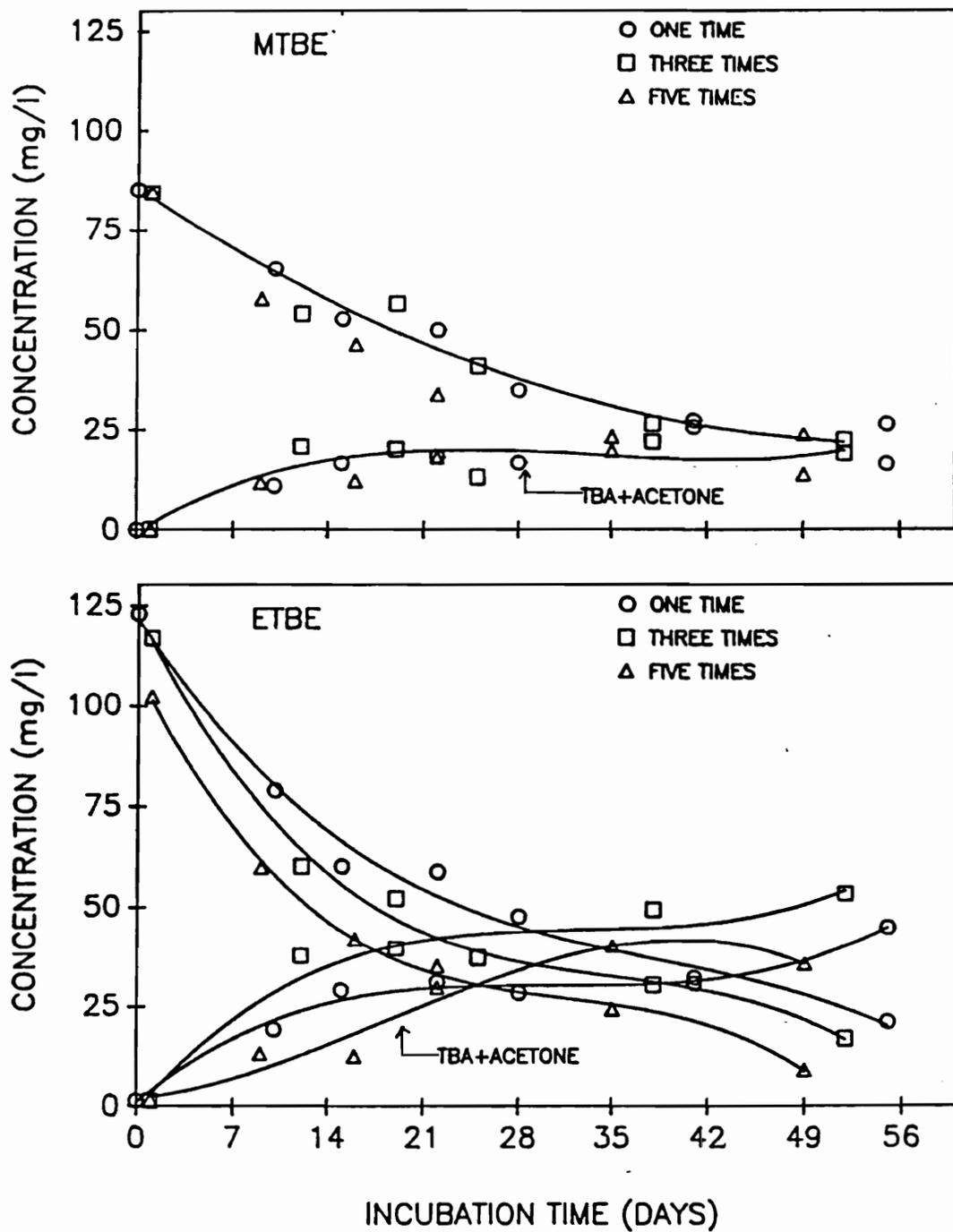


Figure 6.5 Effect of autoclaving frequency on MTBE and ETBE losses,  $H_2O_2 = 2 \mu l$  every 7 days.

suggested the occurrence of chemical oxidation. Figure 6.6 shows that the concentrations of MTBE and ETBE decreased immediately after the addition of H<sub>2</sub>O<sub>2</sub>. Acetone and TBA were produced from the oxidation of MTBE and ETBE.

The oxidation was 0.08 mM of MTBE and 0.09 mM of ETBE per mM of hydrogen peroxide in the first dose. Additional doses of H<sub>2</sub>O<sub>2</sub> further oxidized MTBE and ETBE. However, the ratio of mM of MTBE and ETBE oxidized per mM of H<sub>2</sub>O<sub>2</sub> decreased to 20% of that in the first dose.

As discussed earlier, chemical oxidation did not occur in the water solution systems. Therefore, the occurrence of chemical oxidation by hydrogen peroxide in the soil microcosms might result from some characteristics of the soils. The literature has shown that hydrogen peroxide is very unstable in soil systems. It is subject to decomposition chemically and biologically (Britton, 1985). One of the major mechanisms is through the catalytic action of ferrous iron. Aerobic microorganisms also carry enzymatic systems to detoxify H<sub>2</sub>O<sub>2</sub>. The hydroxyl radical, one of the intermediates in the decomposition of hydrogen peroxide, is a very strong non-specific oxidant. The use of the Fenton solution, a mixture of hydrogen peroxide and ferrous iron salts, to generate hydroxyl radical and oxidize organic compounds is well known (Bowers et al., 1989 and Murphy et al., 1989).

Soils, especially clays, contain abundant levels of iron.

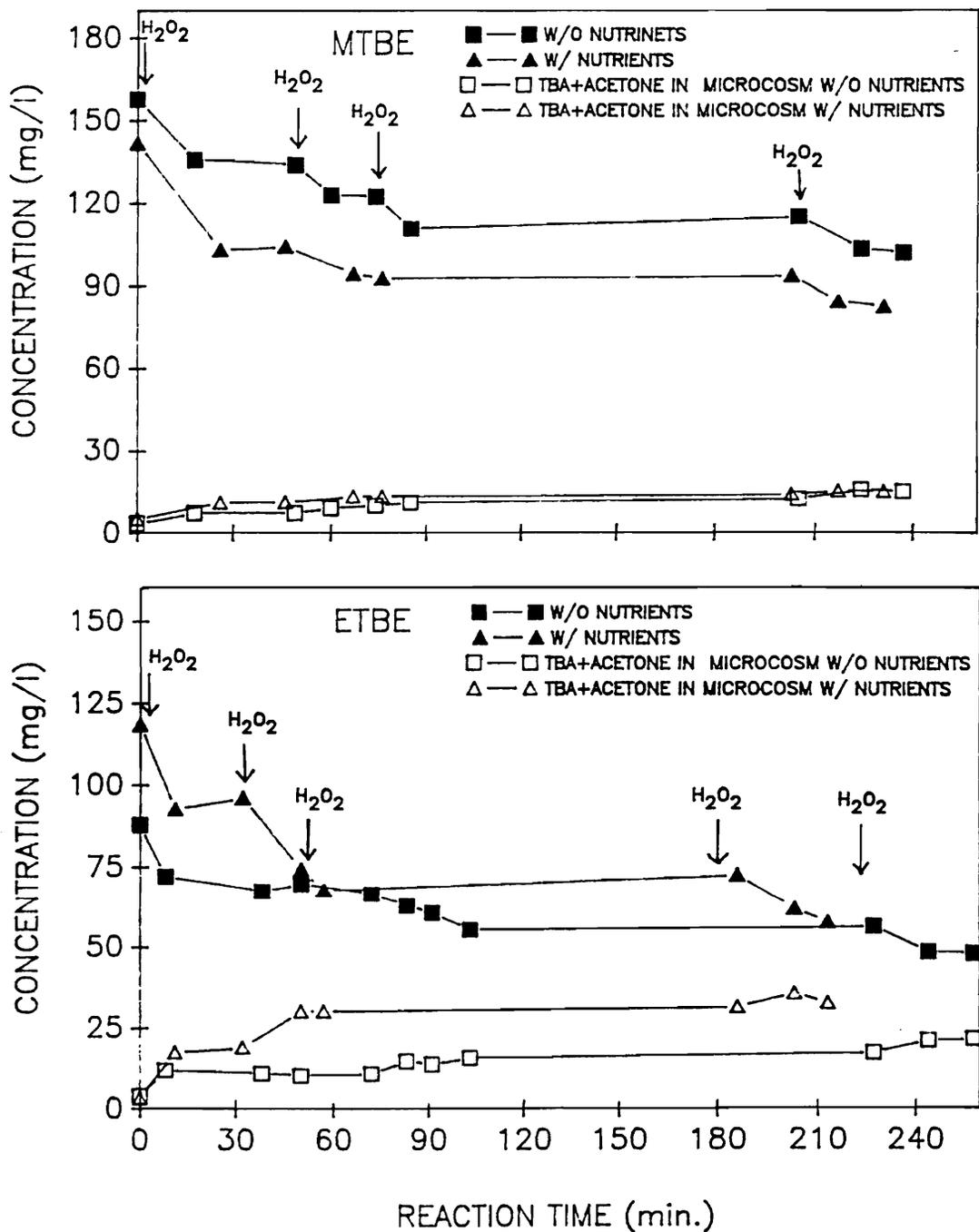


Figure 6.6 Oxidation of MTBE and ETBE by  $H_2O_2$ , producing TBA and acetone.

One of the major forms of soil iron is iron hydroxide. In aerobic environments, the solubility of iron hydroxide is very low. But when soils are saturated with water or when large amounts of organics are introduced into soil systems, Fe(+3) can be reduced to Fe(+2) by iron reducing bacteria (Brown et al. 1978). The Site 3 soils were organically rich and site 1 soils were predominantly clays (Chapter 4). Therefore, soils from these sites might contain iron that acts as a catalyst to release hydroxyl radicals.

#### **Chemical oxidation in iron solutions**

The chemical oxidation of TBA, MTBE and ETBE by hydrogen peroxide and the role of iron were confirmed in batch tests. Solutions containing iron salts ( $\text{FeCl}_2$  and  $\text{FeCl}_3$ ) and the oxygenates were prepared. The concentrations of the oxygenates were monitored before and after the addition of  $\text{H}_2\text{O}_2$ . The effects of types and concentrations of iron (Fe(+2)/Fe(+3)) and the presence of nutrients were measured.

Chemical oxidation of the oxygenates by hydrogen peroxide in the presence of iron was very fast. The reaction was completed within 1 minute (Fig 6.7). Similar to the observation in the microcosms with autoclaved soils, TBA, acetone, formaldehyde and acetaldehyde were products of the oxidation of MTBE and ETBE (Fig. 6.8). Oxidation of TBA produced acetone.

The oxidation of MTBE, ETBE and TBA by the hydroxyl radical was reported by Japar et al. (1991) and Wallington &

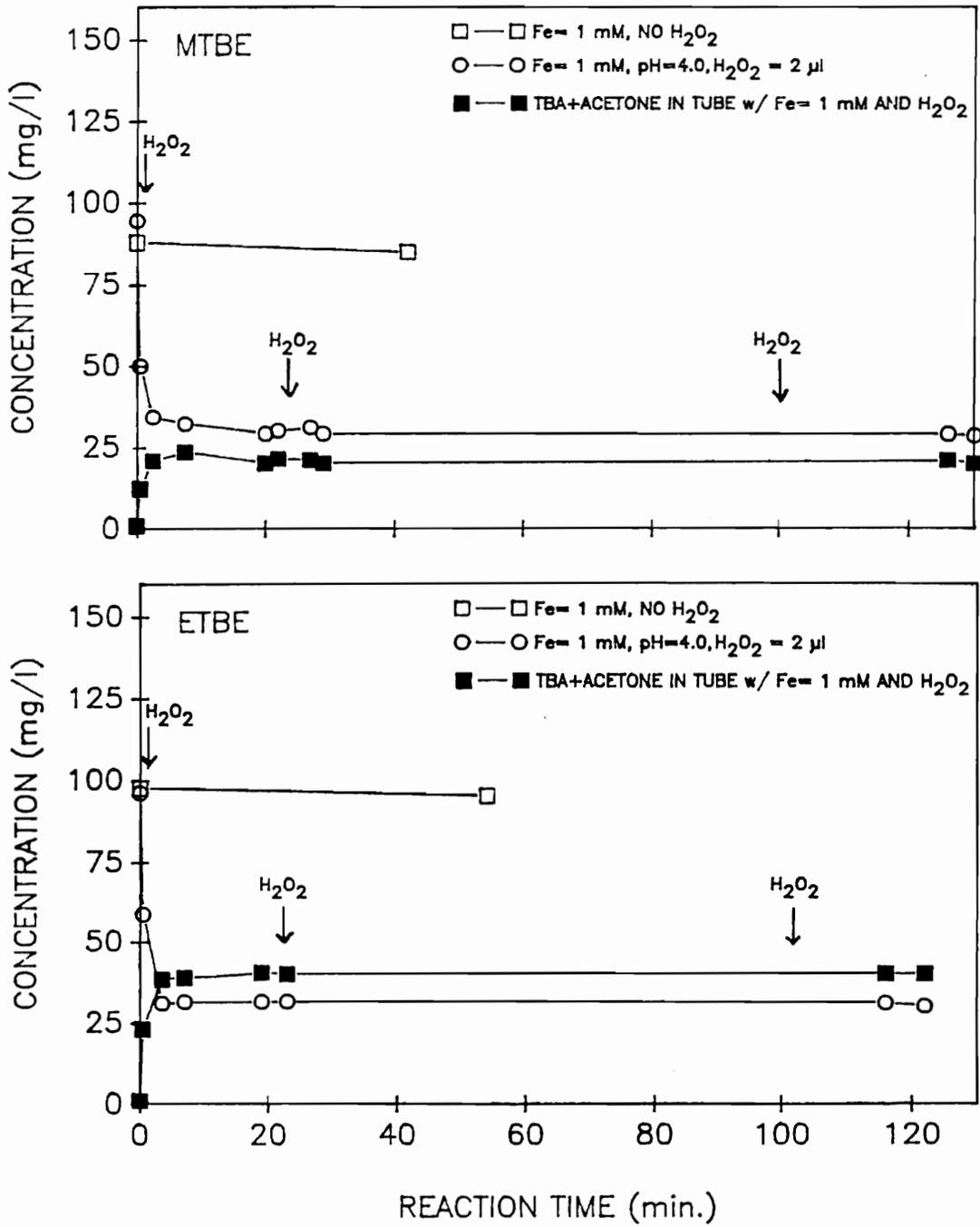


Figure 6.7 Chemical oxidation of MTBE and ETBE by H<sub>2</sub>O<sub>2</sub> in Fe(+2) solutions.

~~0.53~~ 0.28 (a) 1.21

[HP] 5880A MANUAL INJECTION @ 13:25 MAR 11, 1991  
ESTD

RT	AREA	TYPE	CAL	AMOUNT	NAME
0.53	5.67	VV	5	0.514	acetone
0.81	4.63	SB	3	0.373	TBA
1.21	854.84	SB	1	94.495	MTBE

~~0.53~~ 0.31 0.28 (b) 1.21  
~~0.81~~  
~~1.21~~

[HP] 5880A MANUAL INJECTION @ 13:35 MAR 11, 1991  
ESTD

RT	AREA	TYPE	CAL	AMOUNT	NAME
0.53	53.73	VV	5	4.868	acetone
0.81	95.11	VB	3	7.676	TBA
1.21	451.45	SB	1	49.903	MTBE

~~0.53~~ 0.31 0.28 (c) 0.81  
~~0.81~~  
~~1.21~~

[HP] 5380A MANUAL INJECTION @ 13:37 MAR 11, 1991  
ESTD

RT	AREA	TYPE	CAL	AMOUNT	NAME
0.53	56.33	BP	5	5.163	acetone
0.81	193.96	PB	3	15.653	TBA
1.21	309.11	SB	1	34.169	MTBE

Figure 6.8 intermediates, showing on gas chromatograms, from the chemical oxidation of MTBE and ETBE, (a) before H<sub>2</sub>O<sub>2</sub> addition; (b) 0.5 min. after H<sub>2</sub>O<sub>2</sub> addition; (c) 3 min. after H<sub>2</sub>O<sub>2</sub> addition. (H<sub>2</sub>O<sub>2</sub>=2ul, Fe(+2)=1 mM, initial pH=4.0).

Japar (1991). In their studies, the ozone-forming potential of oxygenates was evaluated. Their results indicated that ETBE, MTBE and TBA were degraded by an OH.-initiated atmospheric oxidation. The reactivity with hydroxyl radical is ETBE > MTBE >TBA. However, the degradation products observed in their study were different. They proposed that tertiary butyl formaldehyde is the major product from the oxidation of MTBE and ETBE, while in this study, TBA was the intermediate.

The amounts of MTBE and ETBE oxidized depends on the amounts of hydrogen peroxide in the first dose, the pH and the presence of nutrients (phosphate, ammonium and mineral salts) but were independent of the types and concentrations of iron. When more hydrogen peroxide was provided in the first dose, more MTBE and ETBE was oxidized (Tables 6.1-6.5). In addition, more MTBE and ETBE were oxidized by 2  $\mu$ l of H<sub>2</sub>O<sub>2</sub> in the solution system than in the soil/water microcosms. It might be that the slow diffusion or competition by soil organics limited the oxidation reaction in the soil system.

The chemical oxidation also occurred in the presence of ferric iron (Table 6.1, 6.2). However, ferrous iron catalyzed this chemical reaction faster than ferric iron. The solution with 10 mM of Fe(+3) needed a longer time (30 minutes) for reactions to be completed. The same amounts of MTBE were oxidized per  $\mu$ l of H<sub>2</sub>O<sub>2</sub> in the solutions containing Fe(+2) and Fe(+3), but less TBA and acetone were produced in

Table 6.1 Chemical oxidation of MTBE in iron solutions  
(pH = 4.0)

	Fe(+2) =10 mM			Fe(+3)= 10 mM	
	1 $\mu$ l H <sub>2</sub> O <sub>2</sub>	2 $\mu$ l H <sub>2</sub> O <sub>2</sub>	10 $\mu$ l H <sub>2</sub> O <sub>2</sub>	2 $\mu$ l H <sub>2</sub> O <sub>2</sub>	10 $\mu$ l H <sub>2</sub> O <sub>2</sub>
Rx. Time (min.)	<0.5	<0.5	<0.5	26	29
MTBE oxi- dized (mg)	0.2	0.4	0.73 <sup>a</sup>	0.44	0.87 <sup>a</sup>
acetone produced (mg)	0.03	0.04	0.04 <sup>a</sup>	0.03	0.06 <sup>a</sup>
TBA pro- duced (mg)	0.01	0.07	0.11 <sup>a</sup>	0.04	0.05 <sup>a</sup>

a: all MTBE in the tube were oxidized.

Table 6.2 Chemical oxidation of ETBE in iron solutions  
at Fe(+2) = 10 mM and pH = 4.0

	1 $\mu$ l H <sub>2</sub> O <sub>2</sub>	2 $\mu$ l H <sub>2</sub> O <sub>2</sub>	10 $\mu$ l H <sub>2</sub> O <sub>2</sub>
Rx. Time (min.)	<0.5	<0.5	<0.5
ETBE oxi- dized (mg)	0.55	0.89	1.1 <sup>a</sup>
acetone produced (mg)	0.05	0.07	0.11 <sup>a</sup>
TBA pro- duced (mg)	0.02	0.38	0.18 <sup>a</sup>

a: all the ETBE in the tubes were oxidized

the presence of Fe(+3).

Although the chemical oxidation occurred very rapidly in the iron solutions, the oxidation of TBA, MTBE and ETBE occurred only during the first dose of hydrogen peroxide. After the addition of hydrogen peroxide, the color of the iron solution changed from light yellow to yellowish brown. Further additions of H<sub>2</sub>O<sub>2</sub> did not oxidize the remaining MTBE, ETBE or TBA.

Iron is subject to oxidation by oxygen (Snoeyink & Jenkins, 1980). Therefore, iron was thought to be oxidized to Fe(OH)<sub>3</sub> or FeOOH in oxidized environments. It is speculated that once the oxidation of the iron occurs, the chemical oxidation of the oxygenates will not take place. This might be because that iron hydroxides precipitated or that iron hydroxide can not catalyze the production of hydroxyl radicals.

The concentration of iron salts in the system had little effect on the amount of MTBE and ETBE oxidized, confirming the catalytic role of iron. Similar ratios of the amounts of MTBE and ETBE oxidized per uL of H<sub>2</sub>O<sub>2</sub> were observed at 0.2, 1 and 10 mM of Fe(+2) (Table 6.3). A slightly longer time was needed for the reaction to be completed (2 min. more) at lower Fe(+2) concentrations but overall the oxidation was still very fast.

Phosphate, especially monophosphate, was reported to be able to stabilize the hydrogen peroxide solution (Britton,

Table 6.3 Chemical oxidation of ETBE and MTBE at various Fe(+3) concentrations  
(H<sub>2</sub>O<sub>2</sub> = 2 µl, pH=4.0, no nutrients)

	Fe(+2) = 0.2 mM		Fe(+2) = 1 mM		Fe(+2) = 10 mM	
	MTBE	ETBE	MTBE	ETBE	MTBE	ETBE
Rx. Time (min.)	5.0	3.0	2.5	3.0	<0.5	<0.5
MTBE/ETBE oxidized (mg)	0.42	0.70	0.56	0.60	0.45	0.80
acetone produced (mg)	0.01	0.01	0.05	0.04	0.04	0.07
TBA produced (mg)	0.02	0.02	0.14	0.32	0.07	0.39

1985; Lee et al. 1988). It is suggested that phosphate can be preinjected to the subsurface to control the decomposition of hydrogen hydroxide by iron (Lee et al. 1988). However, the results in the iron solution systems containing monophosphate show that the chemical oxidation was not stopped by phosphate (Table 6.4). In the presence of nutrients, the oxidation of ETBE was slower but MTBE oxidation was not changed. The nutrient solution also contained other cations such as calcium and magnesium which might interact with phosphate, thereby reducing the impact of phosphate on the release of hydroxyl radicals.

On the other hand, the pH has a significant effect on the oxidation. Oxidation is favored under acidic conditions (Table 6.5). It is possible that at low pH values some of the iron remained as Fe(+3) or Fe(+2) instead of being converted to iron hydroxide, the inactive form.

## **6.2 Degradation of MTBE and ETBE in microcosms with non-sterile soils**

The fate of the oxygenates in the microcosms with non-sterile soils was different from those with autoclaved soils. In the same manner as the autoclaved soil microcosms, the microcosms with non-sterile soils were dosed with 2  $\mu$ l of hydrogen peroxide every 7-10 days. While the site 1 autoclaved soils exhibited the same or even faster chemical oxidation than site 3 autoclaved soils, active degradation of

Table 6.4 Chemical oxidation of ETBE and MTBE with and without nutrient solutions  
(H<sub>2</sub>O<sub>2</sub>= 2 μl, pH=4.0, Fe(+2)= 1 mM)

	without nutrients		with nutrients	
	MTBE	ETBE	MTBE	ETBE
Rx. Time (min.)	3.0	5.0	3.0	<0.5
ETBE oxidized (mg)	0.45	0.80	0.5	0.5
acetone produced (mg)	0.04	0.07	0.03	0.02
TBA produced (mg)	0.07	0.39	0.02	0.03

Table 6.5 Chemical oxidation of ETBE and MTBE at various pH  
 ( $H_2O_2 = 2 \mu l$ ,  $Fe(+2) = 1mM$ , with nutrients)

	pH = 4.0		pH = 5.0		pH = 6.5	
	MTBE	ETBE	MTBE	ETBE	MTBE	ETBE
Rx. Time (min.)	3.0	0.5	3.0	3.0	0.5	30
ETBE oxi- dized (mg)	0.5	0.5	0.40	0.35	0.03	0.33
acetone produced (mg)	0.03	0.02	0.02	0.02	0.01	0.01
TBA pro- duced (mg)	0.02	0.03	0.02	0.03	0.01	0.01

MTBE and ETBE was only observed in microcosms with non-sterile Site 3 soils (Fig. 6.9, 6.10 and Tables in Appendix B). In the MTBE and ETBE microcosms with non-sterile Site 3 soils, TBA and acetone appeared in the early stage of incubation, possibly due to chemical oxidation. However, the confirmation of chemical oxidation was not performed in these microcosms. The Site 3 soils were organically rich saturated soils, while Site 1 soils were mainly unsaturated clays. Therefore, Site 3 non-sterile soils would contain active forms of iron, while most of the iron in Site 1 soils would be iron hydroxide. As discussed previously, oxidized iron might not be able to catalyze the chemical oxidation. Thus, the Site 3 soils would provide a more favorable environment than the Site 1 soils for the occurrence of chemical oxidation.

Chemical oxidation in microcosms with non-sterile soils was confirmed in the microcosms with Site 3-3 soils taken in April of 1991 during the wet season (Fig. 6.11-6.13). TBA and acetone immediately appeared in the soil microcosms containing MTBE and ETBE after the addition of hydrogen peroxide. Chemical oxidation was slower in the non-sterile soils than in the autoclaved soils. Acetone produced from chemical oxidation began to be degraded on the second day of incubation.

It is possible that chemical oxidation of MTBE and ETBE in the microcosms with non-sterile Site 3 soils provided

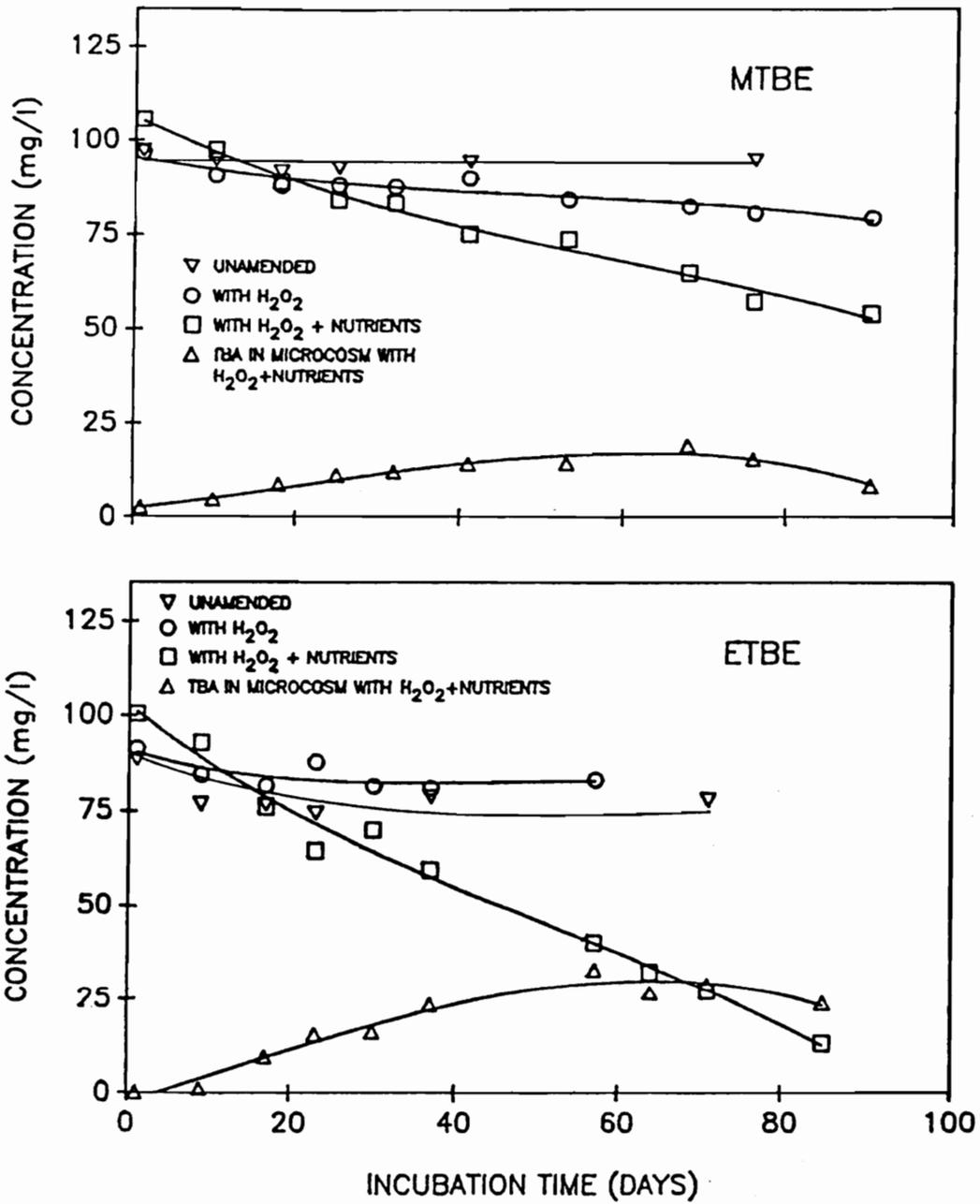
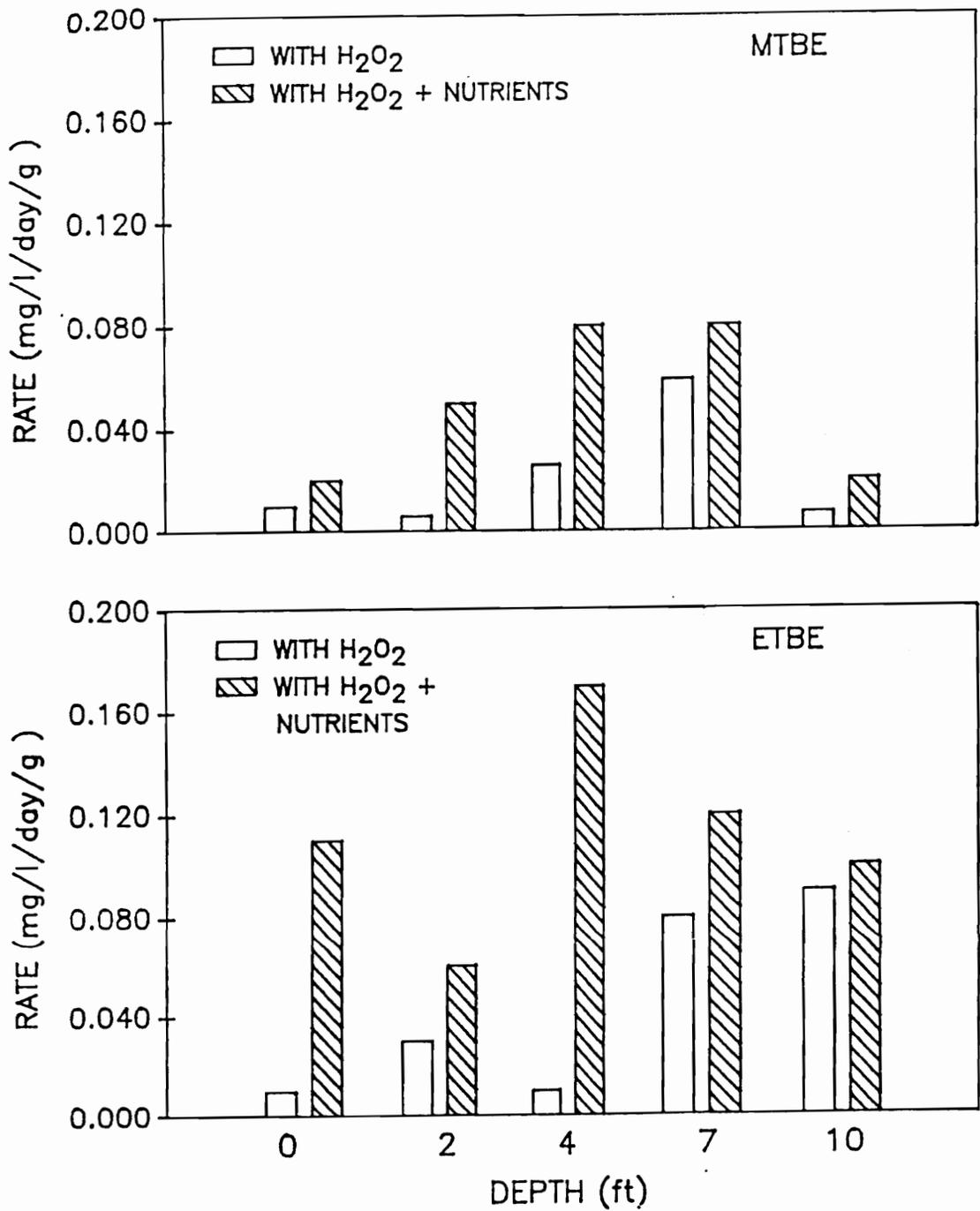


Figure 6.9 Decreases of MTBE and ETBE in Site 3-4 non-sterile soil with 2  $\mu$ l of H<sub>2</sub>O<sub>2</sub> addition every week.



**Figure 6.10** Depth profile of MTBE and ETBE degradation with H<sub>2</sub>O<sub>2</sub> amendment at Site 3.

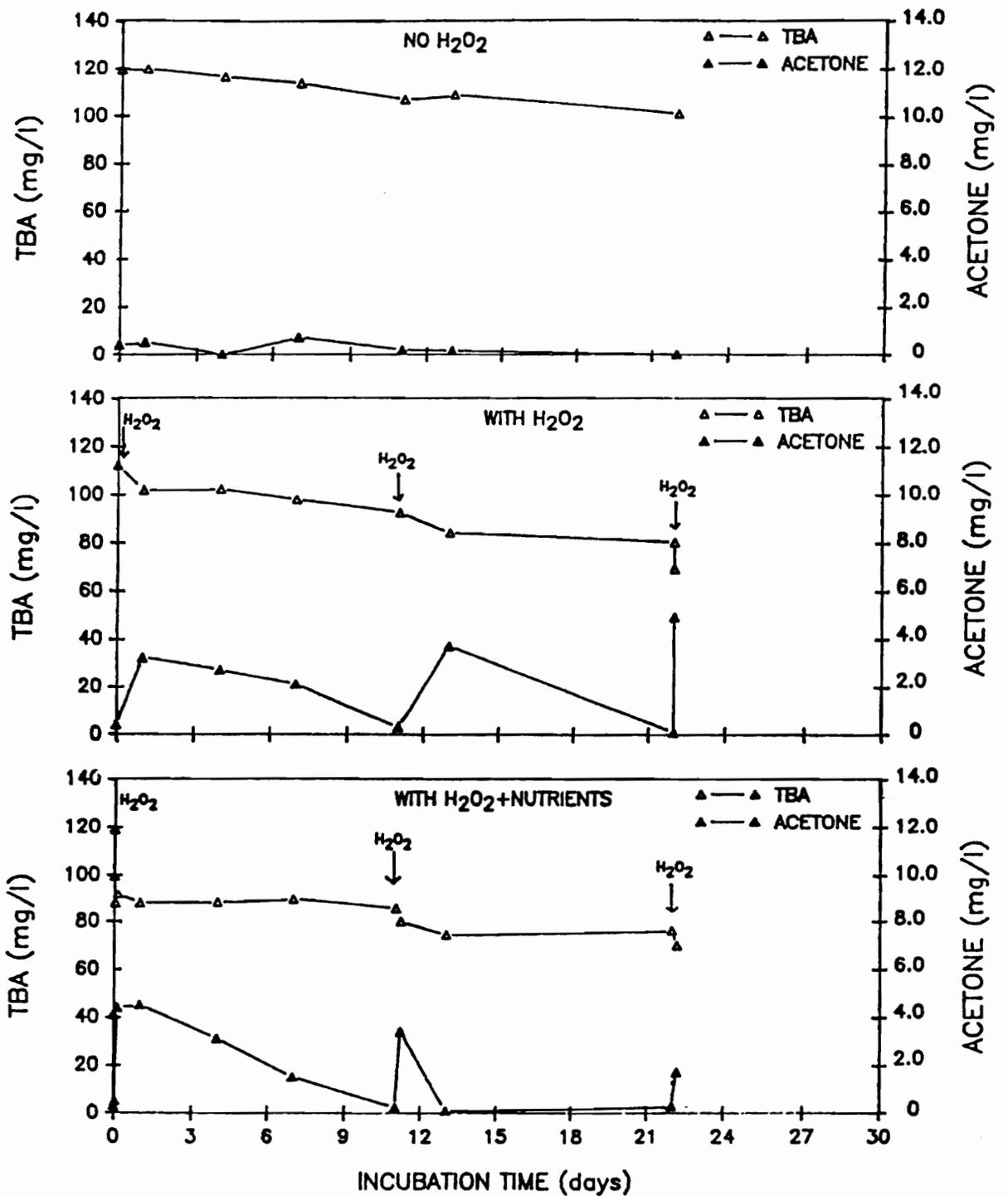


Figure 6.11 Chemical oxidation of TBA in the microcosms with non-sterile Site 3-3 soils, showing the production of acetone immediately after H<sub>2</sub>O<sub>2</sub> addition.

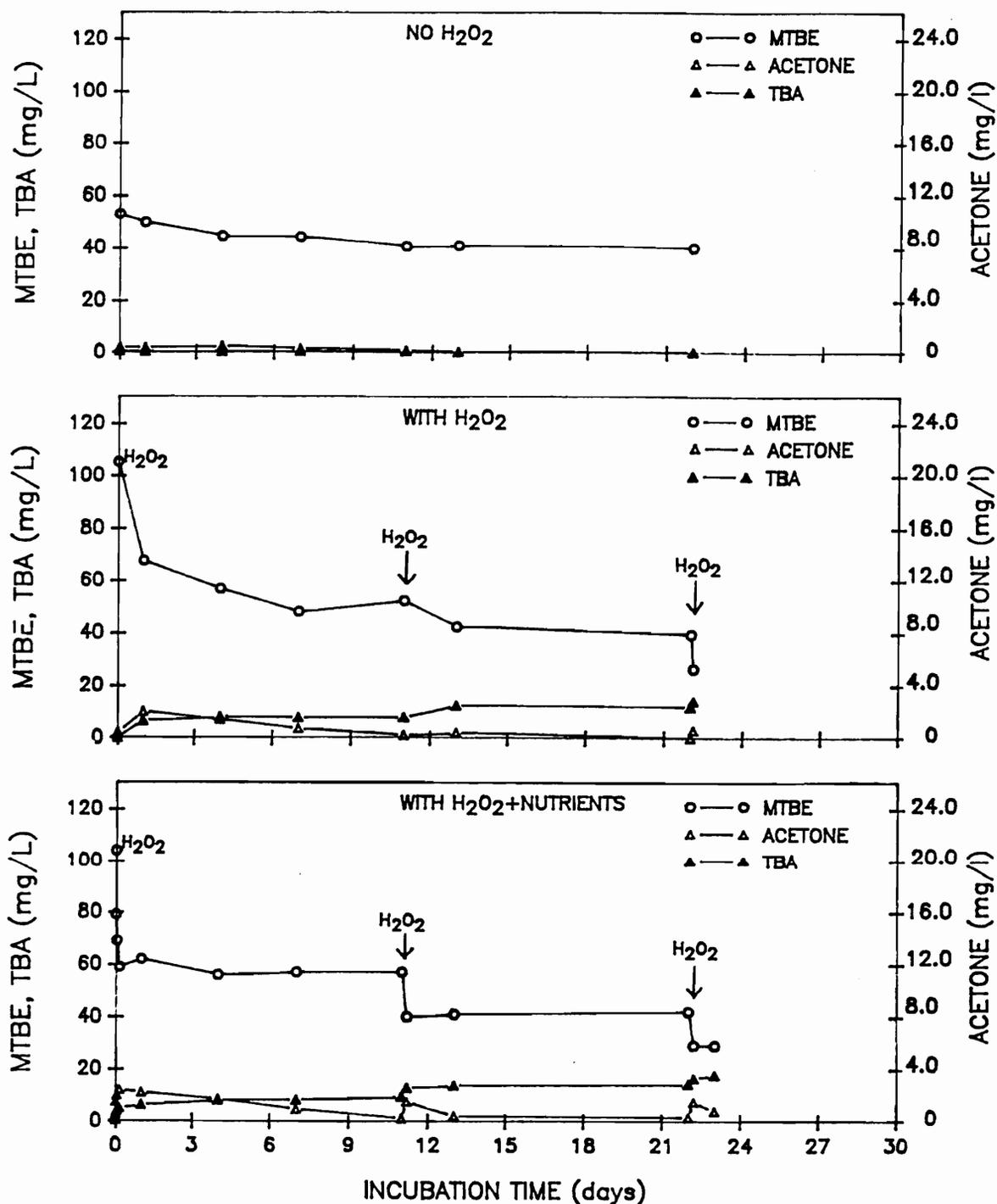


Figure 6.12 Chemical oxidation of MTBE by H<sub>2</sub>O<sub>2</sub> in the microcosms with non-sterile Site 3-3 soils, showing the production of acetone and TBA after H<sub>2</sub>O<sub>2</sub> addition.

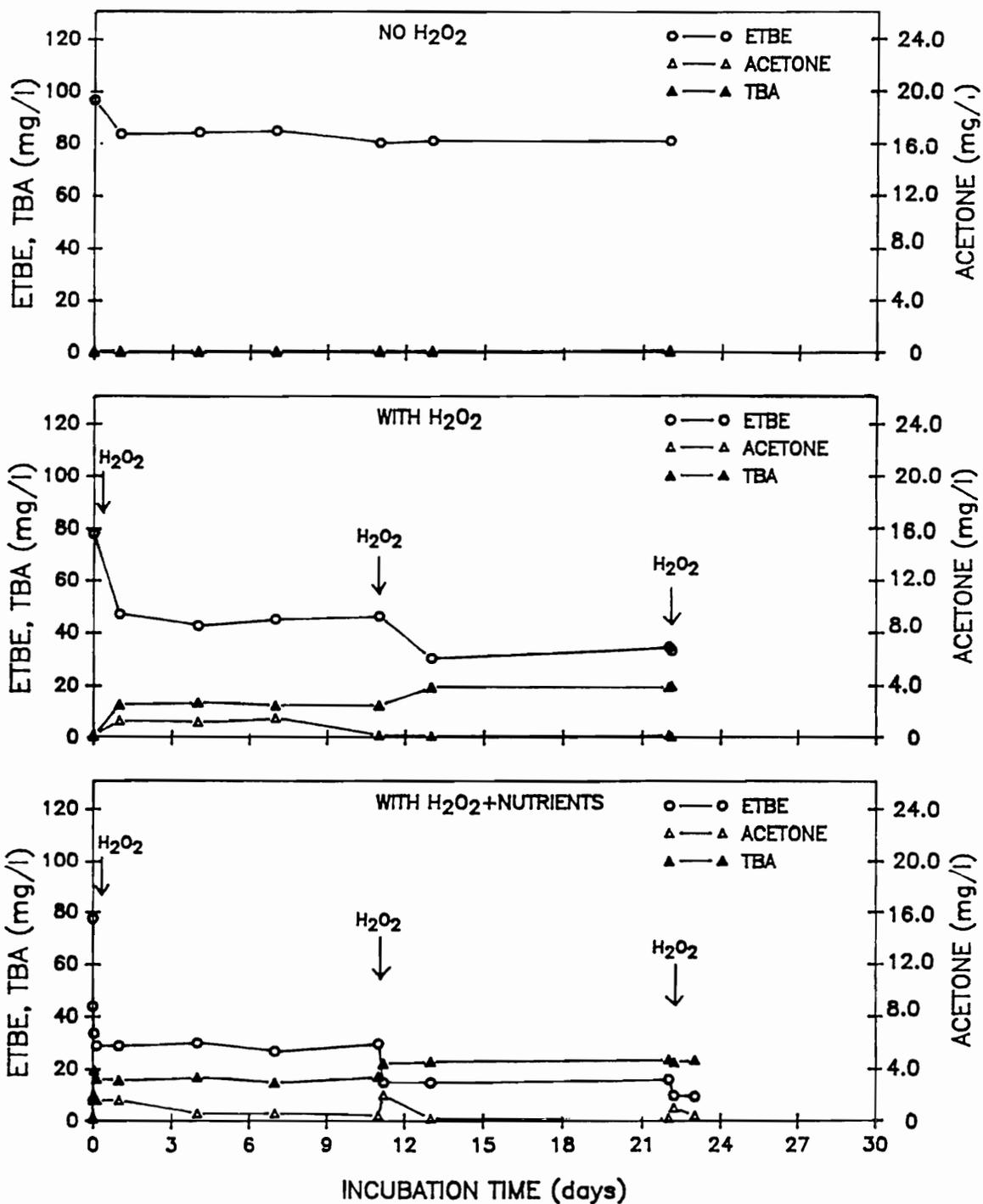


Figure 6.13 Chemical oxidation of ETBE in the microcosms with non-sterile Site 3-3 soils, showing the production of acetone and TBA after H<sub>2</sub>O<sub>2</sub> addition.

acetone, formaldehyde, acetaldehyde and other easily degraded organic substrates for soil microorganisms. The degradation of these intermediates might have depleted oxygen and created anaerobic environments which favored the iron reduction. Thus an aerobic-anaerobic cycle could have occurred where ETBE and MTBE were degraded through the involvement of chemical oxidation followed by microbial degradation (Fig. 6.14).

The insignificant degradation in the Site 1(B) soils might be due to the lack of iron in the beginning. Trace amounts of TBA and acetone were detected but the reactions stopped after one month. Because less acetone and other organics were produced and the microbial activity was low in the Site 1(B) soils, the soil environments might have stayed aerobic and iron remained at the oxidized phase.

A relationship between the soil pH and the oxidation of MTBE and ETBE was developed for the soils showing active chemical degradation (autoclaved site 1(B), Site 3 and non-sterile Site 3 soils). Figure 6.15 shows that similar to the observation in the solution systems, faster chemical degradation occurred in soils with lower pH.

Since the extent of chemical oxidation over the 90 day incubation was not evaluated in the microcosms, it is unclear if microbial breakage of the ether linkages in ETBE and MTBE was active at the same time when chemical oxidation occurred. Because of the abundance of acetone and other easily degraded organics, microorganisms might not have developed the

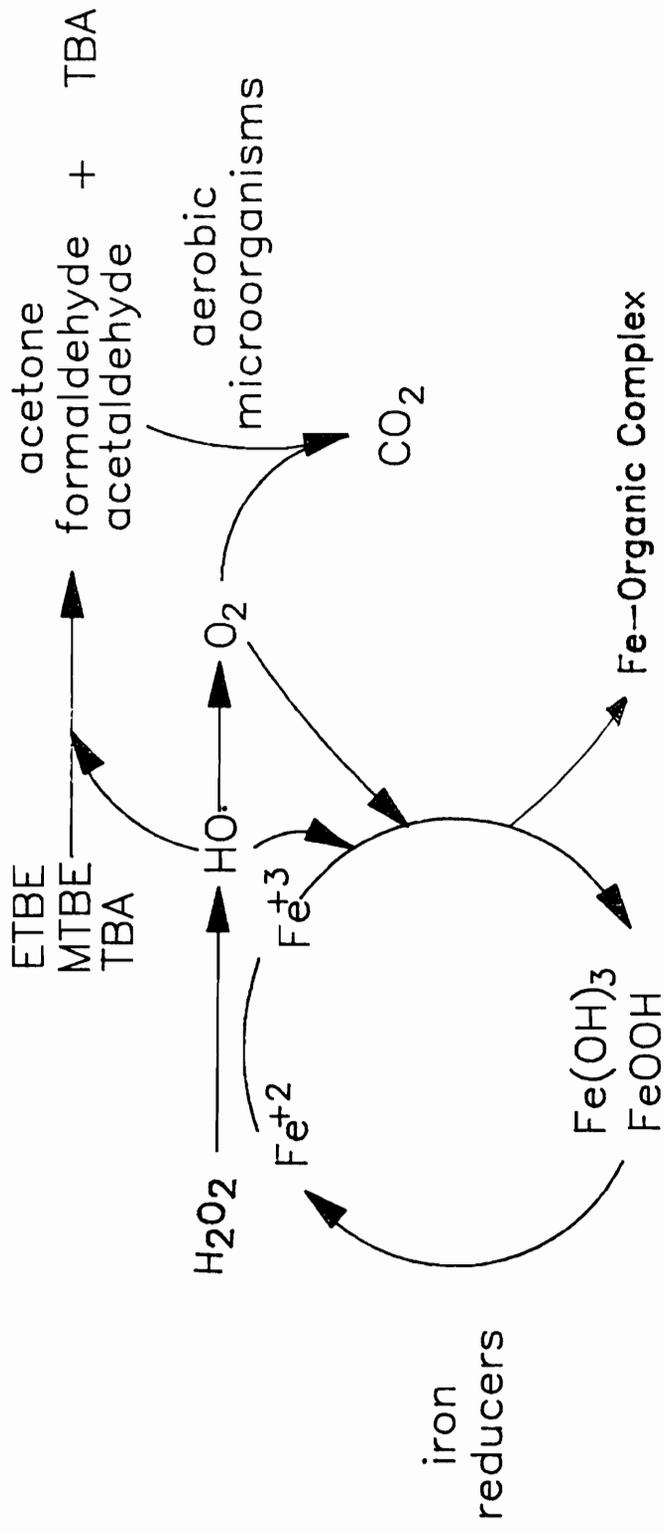


Figure 6.14 Proposed chemical oxidation/microbial degradation of MTBE and ETBE in soils rich in iron.

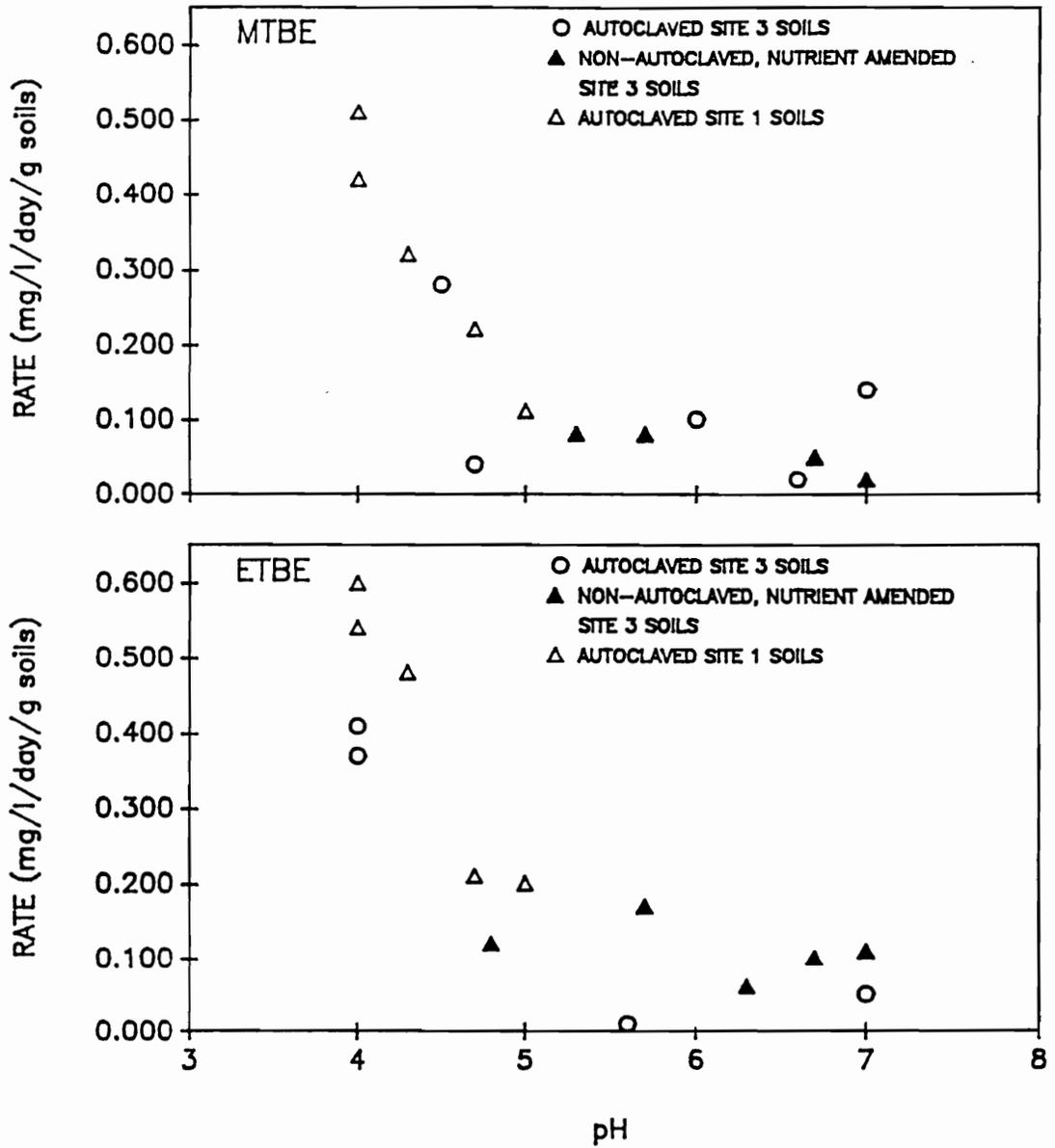


Figure 6.15 Effect of pH on the chemical oxidation of MTBE and ETBE in soil microcosms.

necessary enzymatic systems to catalyze the cleavage of ether bonds. In addition, they might have been outcompeted by the microorganisms growing better on the intermediates produced from the chemical oxidation.

### **6.3 Biodegradation of TBA, MTBE and ETBE**

#### **TBA**

Aerobic biodegradation seemed to be the dominant process in the decrease of TBA in the non-sterile soils from Site 1(B) and Site 3. The degradation patterns of TBA in regular soils were different from those in the autoclaved soils (Fig 6.16, Tables in Appendix B). Unlike the more than 50% of total decreases in the early stage of incubation in the autoclaved soils, the degradation of TBA in the non-sterile soils in the first 20 days was very slow. Fast degradation began after 30 days of incubation. Site 1(B) soils required a longer lag phase but followed the same patterns as in the Site 3 soils (Table 6.6). No accumulation of acetone was observed.

#### **ETBE**

As discussed previously, chemical oxidation might be the dominant reaction in the microcosms with non-sterile Site 3 soils and biological cleavage of the ether bond in MTBE and ETBE might not occur or might be insignificant. The question which must be answered, therefore, is, "can aerobic microorganisms cleave the ether linkages in MTBE and ETBE".

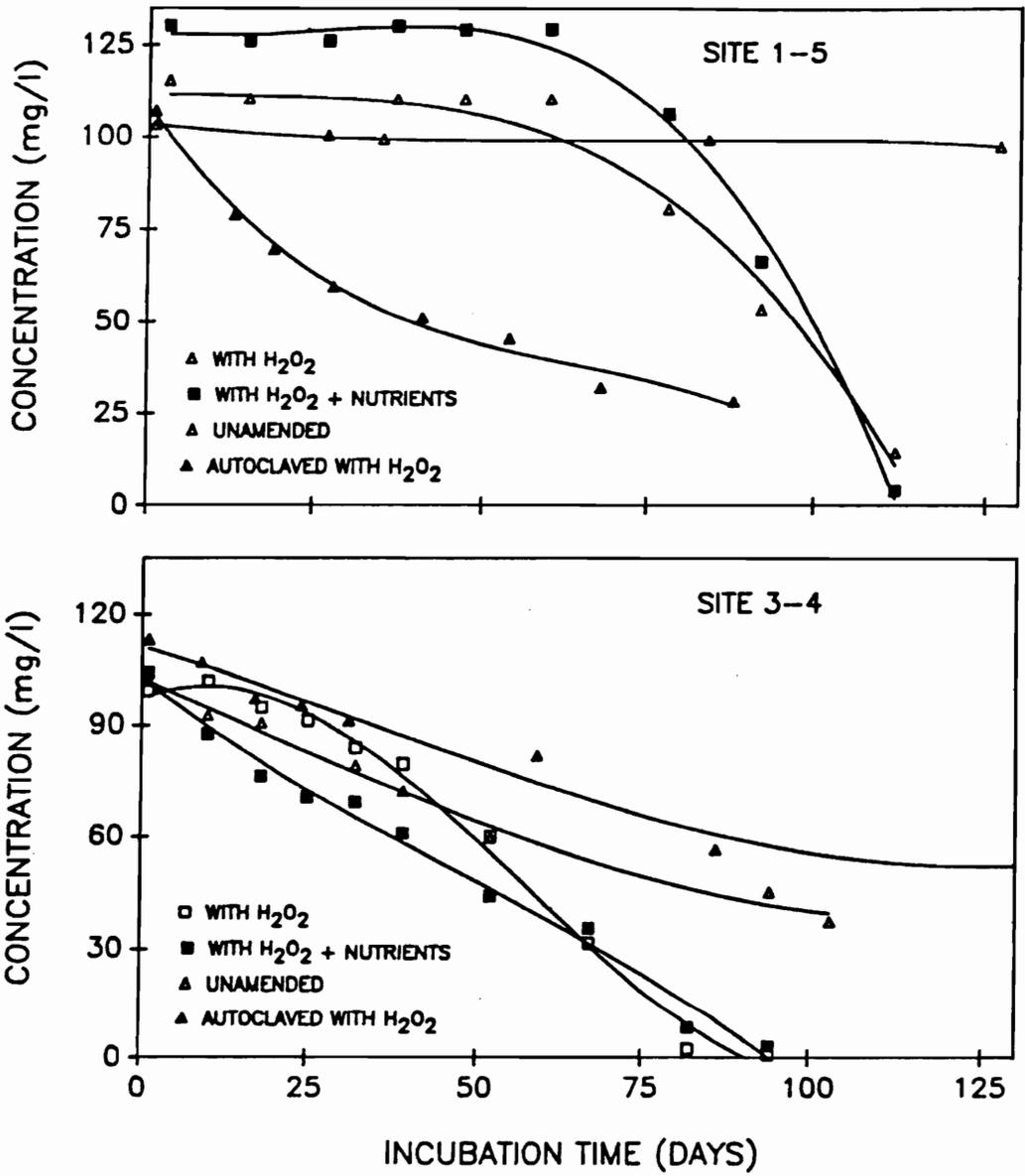


Figure 6.16 Aerobic biodegradation of TBA in soil microcosms.

Table 6.6 Aerobic degradation of TBA

Soil	w/o nutrients			w/ nutrients		
	rate <sup>a</sup>	lag time <sup>b</sup>	T <sub>80</sub> <sup>b</sup>	rate <sup>a</sup>	lag time <sup>b</sup>	T <sub>80</sub> <sup>b</sup>
1(B)-2	0.37	60	120	0.67	60	90
1(B)-5	0.26	60	110	0.32	60	110
1(B)-10	0.23	70	150	0.07	100	160
1(B)-14	0.24	80	120	0.34	50	100
3-0	0.19	20	80	0.22	20	70
3-2	0.20	20	80	0.19	20	90
3-4	0.25	20	70	0.16	10	80
3-7	0.41	10	60	0.27	10	80
3-10	0.15	30	170	0.21	10	150

a: mg/l/day/g dry soil

b: days

To test this, chemical oxidation must be stopped or avoided to encourage biological activity of microorganisms. Because hydrogen peroxide and iron was the probable controlling parameters in the chemical oxidation, two approaches were used to study the biodegradation of MTBE and ETBE by microorganisms. The first approach used sacrificial microcosms containing 1.5 g of Site 3-3 soil and 2 ml of nutrient solutions. The overall air space was about 5 ml. The calculated amount of O<sub>2</sub> in the head space was expected to be sufficient for the biodegradation of ETBE or MTBE in the microcosms.

After 90 days of incubation, no degradation of MTBE and ETBE was observed in non-sterile soils as compared to autoclaved ones (Fig 6.17). TBA, on the other hand, was degraded. Trace amounts of acetone and TBA were detected in both autoclaved and non-sterile soils but it is not certain whether this was due to biological or chemical reaction. The monitoring was stopped after 100 days of incubation.

Another approach was to add 5 µl of hydrogen peroxide to soil microcosms every 5 days for 20 days prior to the addition of oxygenates. By doing so, iron would be oxidized and the occurrence of chemical oxidation would be minimized.

Figure 6.18 shows the occurrence of biodegradation of ETBE in the site 3-3 soil which was pretreated with hydrogen peroxide. Some chemical oxidation still occurred after the addition of hydrogen peroxide, probably due to the reducing

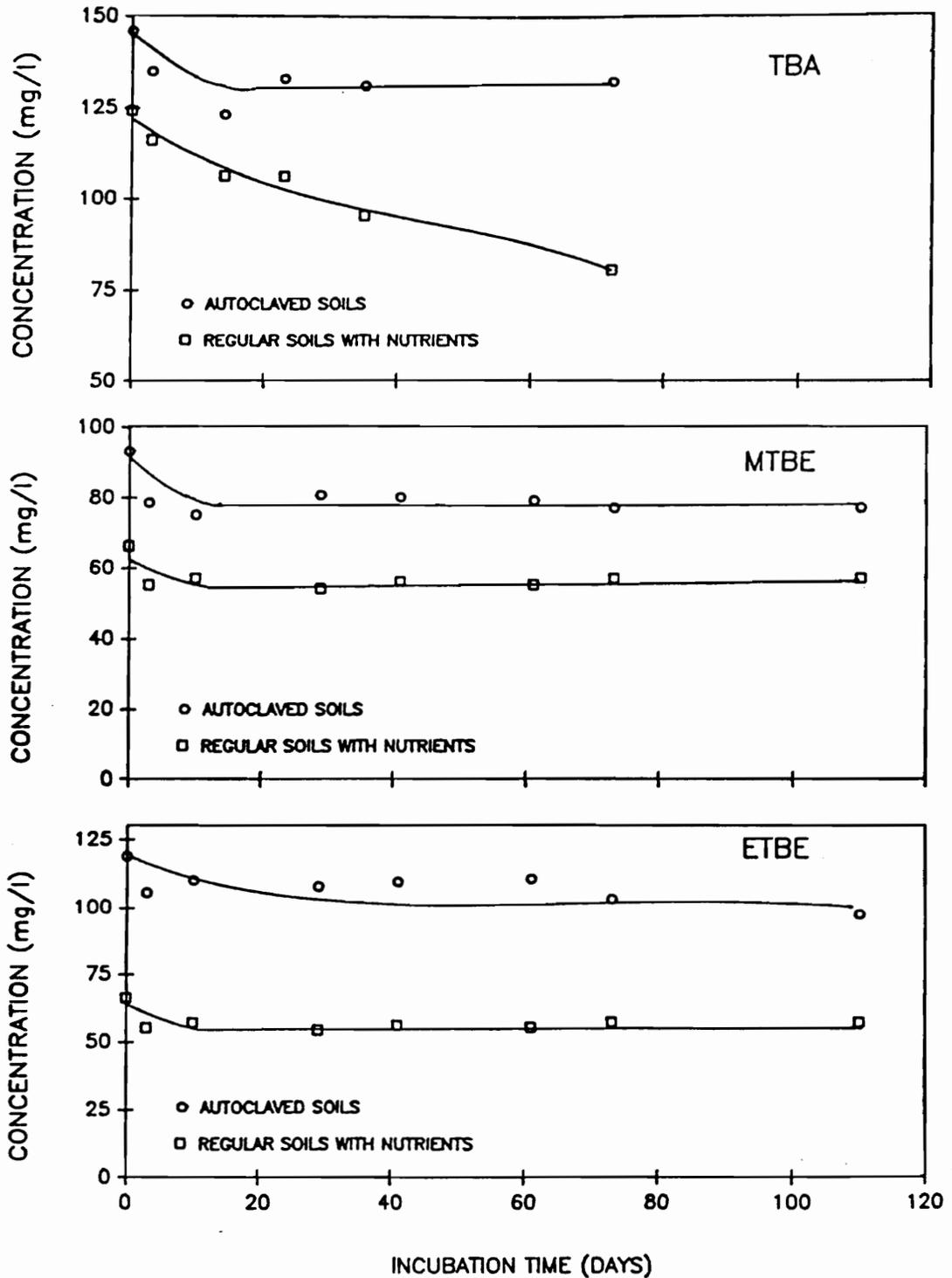


Figure 6.17 Degradation of TBA, MTBE and ETBE in sacrificial microcosms with air as an oxygen source.

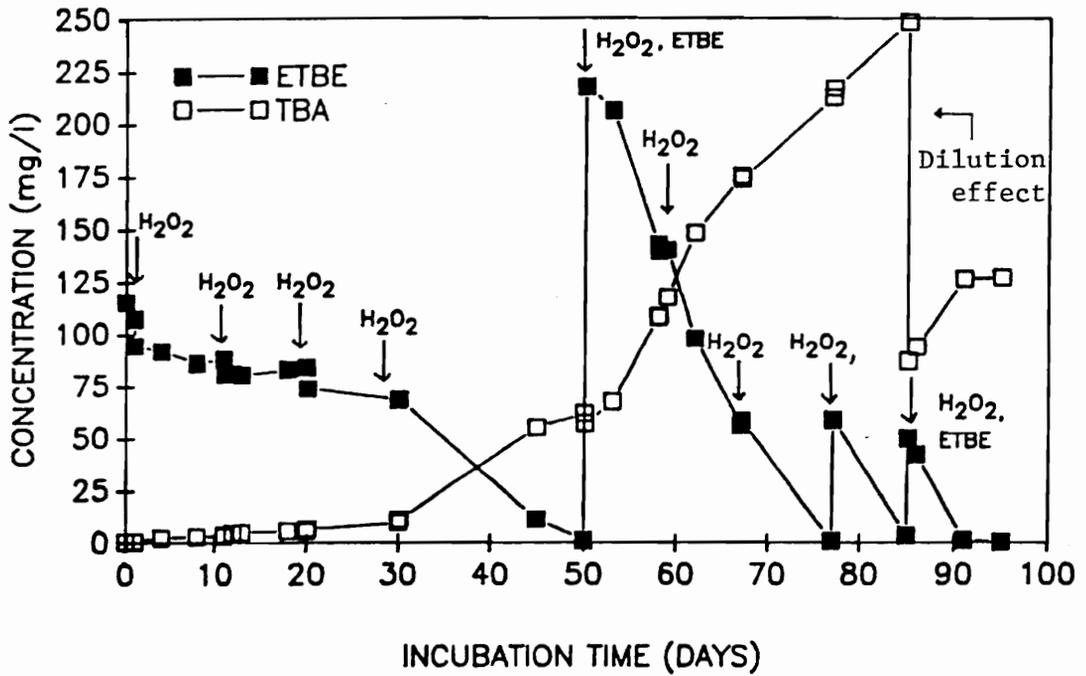


Figure 6.18 Biodegradation of ETBE and production of TBA in microcosms with Site 3-3 soils pre-oxidized with H<sub>2</sub>O<sub>2</sub>.

character of this soil. However, the extent of chemical oxidation was less than that in the non-sterile and non-pretreated soils (Fig. 6.11). After 30 days of incubation, the concentration of ETBE in one of the microcosms amended with nutrients decreased very rapidly and TBA was the major intermediate. The other ETBE microcosms amended with nutrients started showing slow degradation in the 80th day of incubation (Appendix B). Repeated doses and disappearance of ETBE indicate that this was a biological reaction. The results suggest that aerobic microorganisms were able to directly cleave the ether bond in ETBE and use the aldehyde produced from ETBE as organic sources.

The rapid degradation of ETBE in the microcosms with preoxidized soils after a 30-day lag phase confirmed the speculation concerning the non-preoxidized soil microcosms. As discussed in section 6.2, microbial cleavage of the ether linkages might be insignificant due to active chemical oxidation in the systems. The degradation pattern of the biological cleavage showing in Fig. 6.18 indicates that once the enzymatic systems had developed, the degradation of ETBE was very fast. Therefore, if the microbial cleavage of the ether linkages had occurred significantly in the non-preoxidized soils, the degradation patterns and degradation rates of ETBE would have been similar to those observed in the preoxidized soils.

The occurrence of biodegradation of ETBE in the microcosms

supplied with hydrogen peroxide but not in the sacrificial microcosms implies that hydrogen peroxide might be involved in the biodegradation. Aerobic microorganisms carry enzymatic systems to detoxify hydrogen peroxide generated from the reduction of  $O_2$  during aerobic respiration (Block, 1989). The hydroxyl radical is the intermediate from the enzymatic degradation of hydrogen peroxide. The hydroxyl radical is very toxic to microbial cells and must be removed. It is possible that hydroxyl radicals were one of the reactants used by microorganisms to degrade ETBE. This might explain that trace amounts of acetone and TBA were observed in the sacrificial microcosms supplied with air. However, during the respiratory processes, only a small amount of hydrogen peroxide was generated, resulting in production of a small amount of hydroxyl radicals and little degradation of ETBE. However, when the system was provided with significant amounts of external hydrogen peroxide, hydroxyl radicals were produced in large quantities which could have been used by microorganisms to degrade ETBE.

#### **MTBE**

Similar experiments were performed on MTBE but no biodegradation was observed, possibly because the enzymatic systems for MTBE degradation required a long lag phase to develop. While ETBE degradation occurred in the microcosms with preoxidized soils, no MTBE degradation was observed over 100 days of incubation. To test whether this was due to

unfavorable soil environments, 1 ml of soil slurry from the microcosms which had shown active ETBE biodegradation was transferred to the MTBE microcosms. However, no MTBE degradation was observed over 1 month of incubation. This indicates that the enzymatic systems for ETBE degradation are different from those for MTBE degradation. Different types of enzymes might be required for MTBE degradation or the structure of ETBE-degrading enzymes might need to be adjusted and such adjustments require a longer incubation time.

## Chapter 7 Degradation Under Denitrifying Conditions

In this chapter, experiments were designed to evaluate whether degradation of TBA, MTBE and ETBE would occur under denitrifying conditions and if so, to determine the rates of degradation. One of the primary objectives in this study is to identify the major subsurface environmental factors that can enhance or inhibit the degradation. Hickman (1988) found that the addition of nitrate significantly increased TBA degradation at the organically rich sites but not at the organically poor sites. He suggested that the organic flux at the organic-rich sites might be the reason for faster TBA degradation. According to these conclusions, one of the objectives in this chapter was to further evaluate how the ingredients in the organic flux influence the degradation in the organically poor soils.

According to the results from site characterization in chapter 4, the organically rich sites (Site 2 and 3) contained much higher concentrations of nitrogen and degradable organic matter. Therefore, the influences of nutrient availability and the degradable organics were examined. Ethanol was chosen to represent the degradable organic matter because alcohol-type compounds are one of the intermediates during the anaerobic degradation of natural organic compounds and they are common contaminants in the subsurface.

In order to monitor nitrate and nitrite, a larger

microcosm system containing 15 gram of soil was used in some of the experiments. The soil to water ratio was kept the same as in the smaller microcosm systems.

## **7.1 TBA degradation**

### **Site 1**

TBA degradation at Site 1 was enhanced by providing nitrate as electron acceptor (Fig. 7.1, Tables in Appendix C). The most significant improvement was the reduction of the lag time and  $T_{80}$ . Nutrient enrichment along with nitrate further increased the degradation rate of TBA from 0.05 to 0.2 mg/l/day/g dry soil and the  $T_{80}$  was shortened to 140 days. The decreases in the nitrate concentration are shown in Figure 7.2 and Tables in Appendix C).

TBA degradation rates at all depths at Site 1 were similar under denitrifying conditions after addition of nitrate and nutrients (Fig. 7.1b). This suggests that one of the primary factors controlling the TBA degradation rate at Site 1 is the nutrient availability in this soil. Denitrifiers are reported to be ubiquitous in soils (Tiedje, 1988). The enumeration data showed that denitrifiers existed at all depths but more at Site 2 and 3 (Fig. 4.1). Site 1 was categorized as an oligotrophic site in which nutrients were confined to the upper layers of soils and TBA degradation was slower in deeper soils. With proper nutrient amendment TBA degradation occurred at nearly the same rate at all

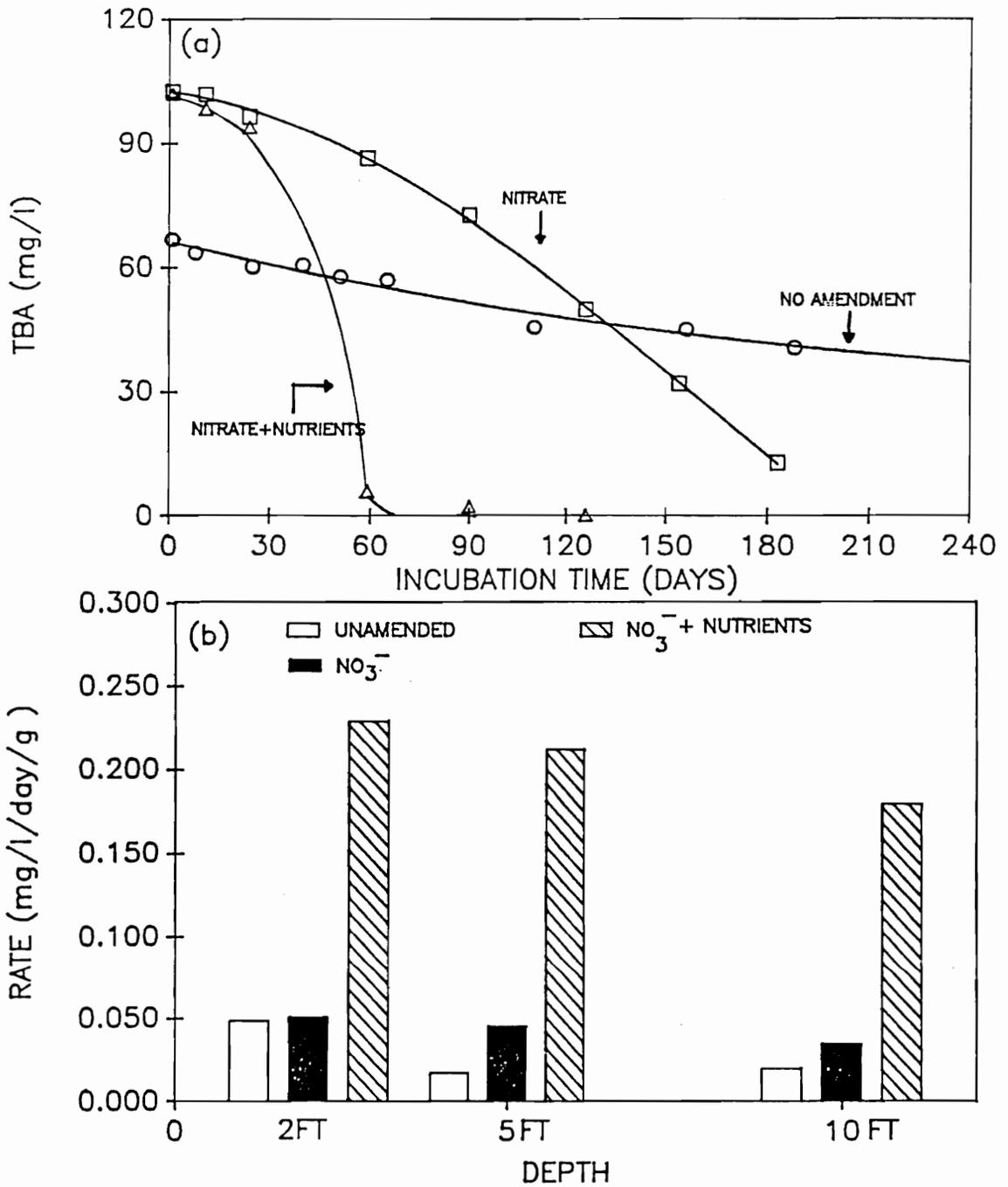


Figure 7.1 Degradation of TBA under denitrifying conditions; (a) degradation patterns; (b) depth profile of degradation rates.

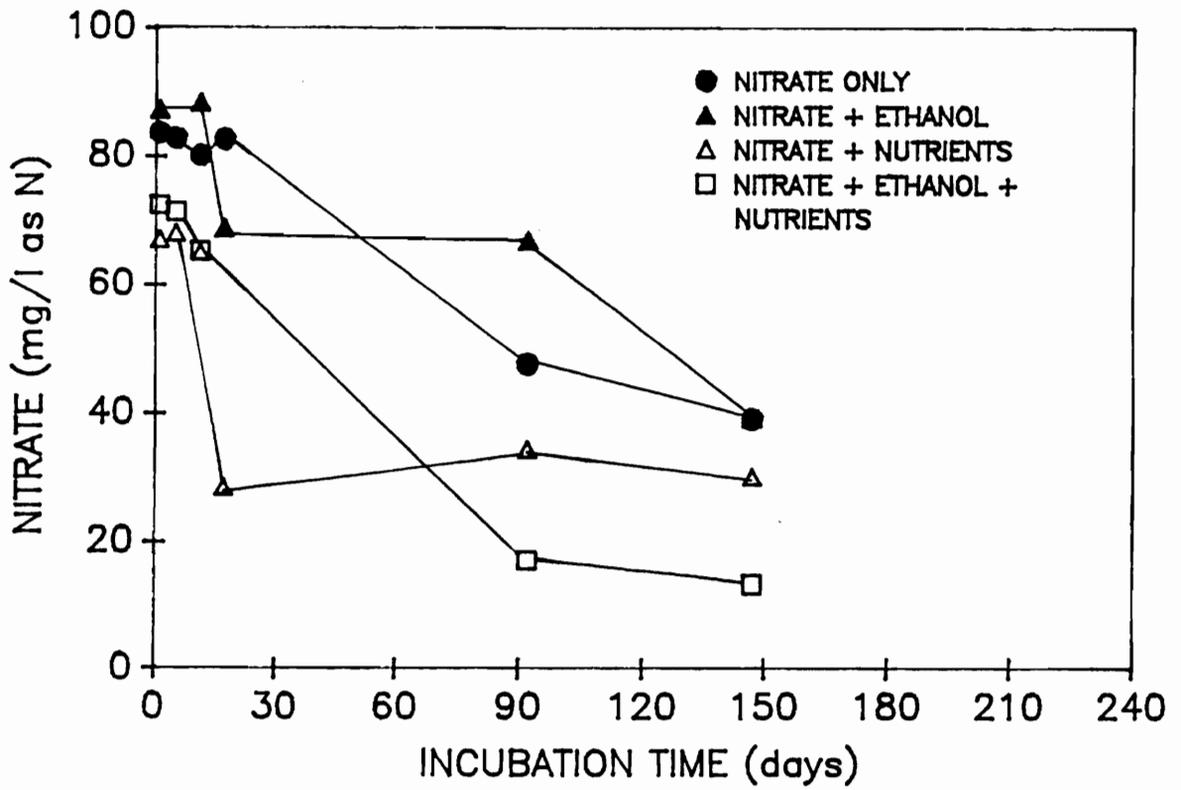


Figure 7.2 Decreases of nitrate in nitrate-amended TBA microcosms.

depths.

Since molybdate was a trace nutrient required by the denitrifiers and methanogens, the influence of molybdate availability on microbial activity was evaluated. Three mg/l of molybdate (the concentration used in cultural studies in the literature) was added along with nitrate and nutrients in the microcosms with Site 1(B) soils. The results are shown in Table 7.1. Addition of molybdate did not significantly increase TBA degradation under denitrifying conditions, suggesting that under the growth conditions provided, molybdate was not a limiting factor in these microcosms.

The addition of 100 mg/l ethanol retarded TBA degradation under denitrifying conditions in the Site 1 soils (Fig. 7.3). TBA degradation under denitrifying conditions stopped in the presence of ethanol. Ethanol was completely degraded within two weeks but unless nutrients were provided, TBA degradation did not occur. Nutrient enrichment stimulated TBA degradation when ethanol was present but the rate was not as fast as when no ethanol was provided.

It is not unusual that contamination in soil and groundwater systems involves mixtures of organic chemicals that exhibit a broad range of biodegradabilities. When exposed to multi-substrates, microorganisms can exhibit a variety of responses from diauxic growth to simultaneous use of several substrates. The relative concentrations of the

Table 7.1 Effects of molybdate on TBA degradation with nitrate/nutrinets amendment

Soil	w/o molybdate			+3 mg/l molybdate		
	rate <sup>a</sup>	lag time <sup>b</sup>	T <sub>80</sub> <sup>b</sup>	rate	lag time	T <sub>80</sub>
1(B)-2	0.22	30	140	0.22	30	140
1(B)-5	0.20	30	160	0.15	30	130
1(B)-10	0.18	30	140	0.21	30	140
1(B)-14	0.28	30	120	0.21	30	120

a: mg/l/day/g dry soil

b: days

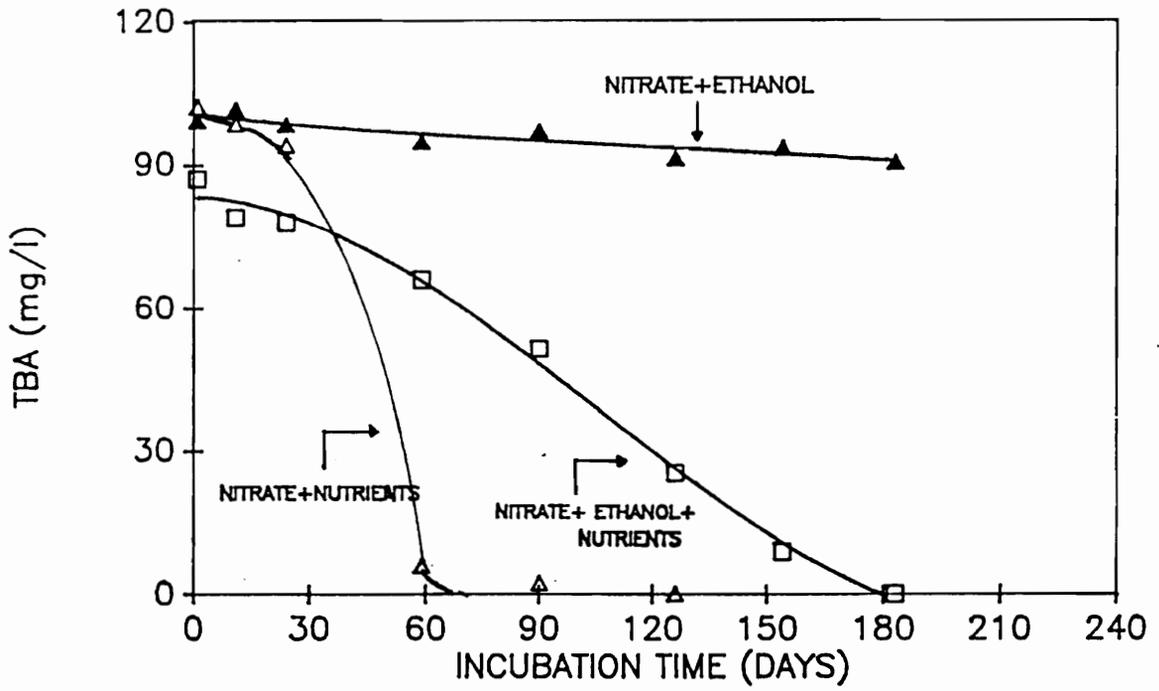


Figure 7.3 Effect of ethanol on TBA degradation under denitrifying conditions.

organics are critical in determining how they are degraded. Harder and Dijkhuizen (1982) concluded that when the concentrations of primary substrates are high, the degradation patterns of the secondary substrates will exhibit a diauxic pattern. On the other hand, if concentrations of the primary substrates are low, concurrent utilization will occur.

During the simultaneous utilization of several substrates, each individual compound is often degraded at a lower rate per cell but the overall cell growth rate is higher. LaPat-Polasko et al. (1984) demonstrated that the degradation of methylene chloride at low concentrations by a methylene chloride acclimated strain was enhanced in the presence of acetate at a relatively high concentration compared to methylene chloride. Degradation of acetate was slower but the degradation rate for methylene chloride was higher. However, the results shown in their study indicated that the effectiveness of a primary substrate on the degradation of the secondary substrate will diminish when the concentration of the primary substrate is increased to or beyond a certain level.

Most of the studies evaluating the secondary utilization phenomenon involved the use of microbial cultures which had acclimated to use specific substrates. In these cases, the microbial groups active in degrading the substrates were not only dominant in the system but also the enzymatic systems

had been developed. Therefore, adding a primary substrate at appropriate concentrations could help the degradation. In soils which are low in microbial activity and previously uncontaminated with the pollutants, the addition of a primary substrate might not enhance degradation of the secondary substrate. Competitions among microbial groups and substrates might play more significant roles in the subsurface than in the cultural study. The addition of ethanol might have stimulated the growth of different types of microorganisms which compete with the TBA degrading microorganisms. Also, ethanol might repress or inhibit the TBA degrading enzymes.

#### **Site 2 and 3**

TBA degradation in organic-rich soils (Sites 2 and 3) was not changed by nutrient addition (see Appendix C). In fact, the addition of nitrate plus nutrients resulted in higher degradation rates in the Site 1 soils than in the organic-rich Site 2 and 3 soils (Fig. 7.4). This is consistent with data from Site 1 where it was found that addition of 100 mg/l of ethanol retarded TBA degradation. Although the organic-rich soils have a higher nutrient content than the Site 1 soils, they also contained higher degradable organics and these appeared to decrease the degradation rate of TBA, possible because of competition between a primary substrate (degradable organic matter) and a secondary substrate, in this case, TBA.

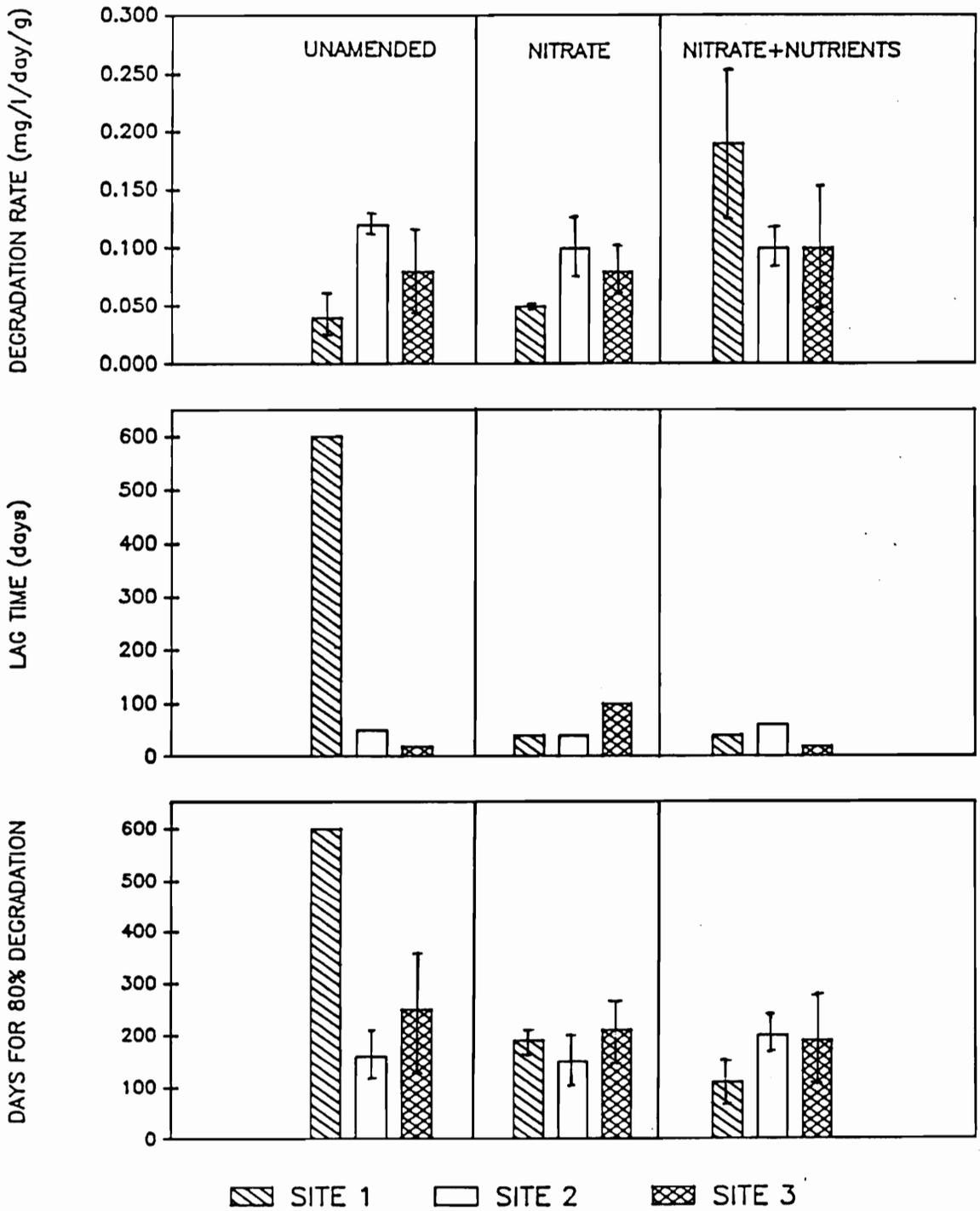


Figure 7.4 TBA degradation under denitrifying conditions at three sites.

## 7.2 MTBE and ETBE degradation

### Site 1

MTBE degradation under denitrifying conditions was not observed at any depth at Site 1. ETBE degradation under denitrifying conditions was observed only in the Site 1(A)-5 and 1(B)-14 Soils (Fig. 7.5, Tables in Appendix C). No TBA accumulation was observed in the microcosms undergoing active ETBE degradation.

The amendment with nitrate was carried out in larger microcosms containing soils 3 times as much as the unamended ones. Although the degradation curves of ETBE degradation with nitrate and nitrate/nutrients amendment were similar to those without amendment (Figure 7.5), when the amount of soil was taken into account the degradation rate of ETBE under denitrifying conditions was actually lower. Taylor (1983) reported that vanillic acid and other methoxyl-aromatic compounds could be degraded by denitrifying species. This study suggests that either denitrifying bacteria degraded ETBE at a slower rate or they are not able to catalyze the cleavage of the ether bond in ETBE. Instead, they degraded TBA produced from ETBE degradation by other soil microorganisms.

Degradation of ETBE under denitrifying conditions was very sensitive to the existence of other organic compounds (Fig 7.5b). While TBA degradation could be stimulated by nutrient addition to the system after ethanol was degraded,

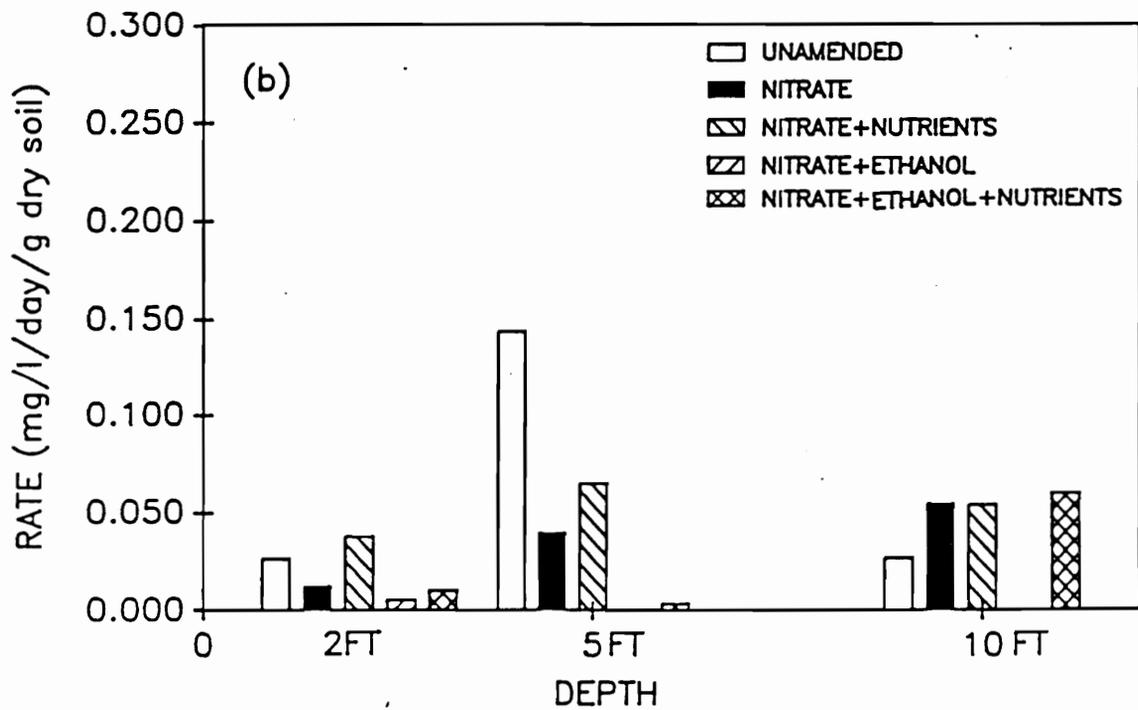
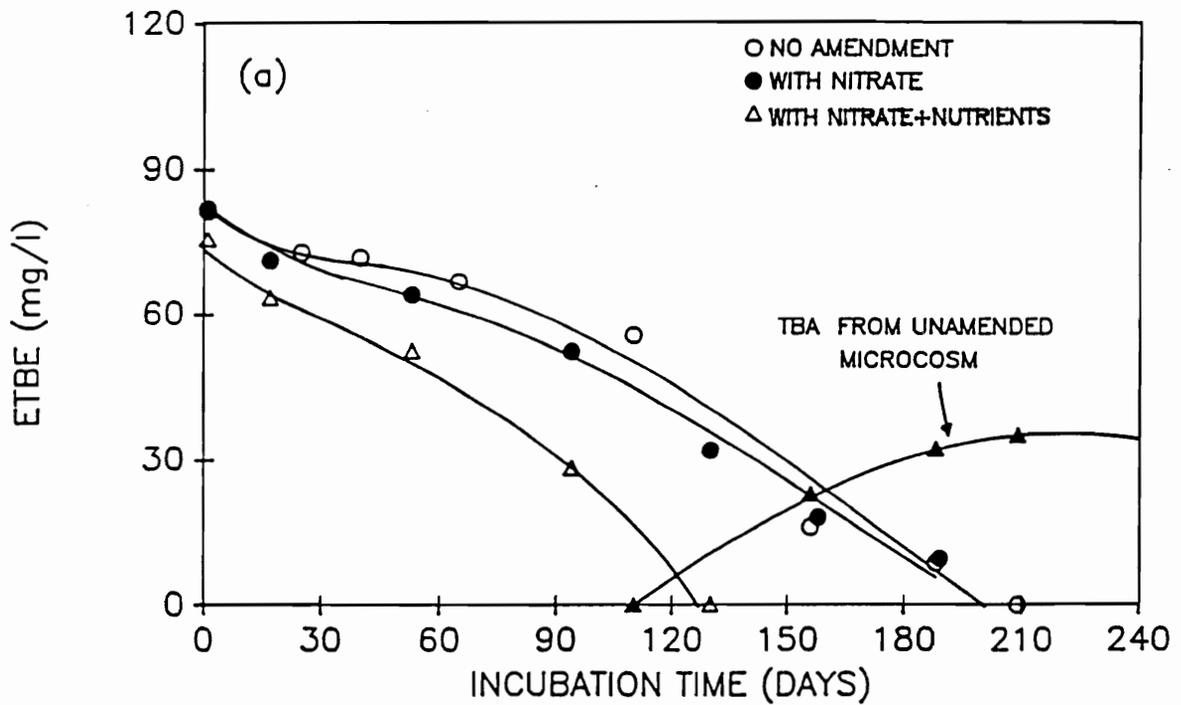


Figure 7.5 Degradation of ETBE under denitrifying condition: (a) degradation patterns in Site 1(A) soils; (b) effects of ethanol.

ETBE degradation did not occur even after all the ethanol was degraded.

### **Sites 2 and 3**

This same phenomenon might also account for the lack of degradation of MTBE and ETBE in the organic-rich Site 2 and 3 soils. Neither MTBE nor ETBE was degraded in soils from Site 2 and 3. It is still possible that MTBE and ETBE could be degraded but required incubation time beyond the 250 day study period.

The lack of competitive ability of the microorganisms capable of degrading ETBE and MTBE may explain the severe inhibition by ethanol of ETBE degradation. As discussed previously, the addition of easily degraded organics to the organic-poor soils and the degradable organic matter in the organic-rich soils decreased TBA degradation. However, TBA degradation still took place under such situations but at slower rates. In the cases of MTBE and ETBE, the easily degradable substrates might have selectively enriched certain groups of microorganisms that were not capable of degrading MTBE and ETBE but could outcompete the ETBE degraders.

Other highly water-soluble compounds such as methanol or ethanol may be blended together with TBA, MTBE and ETBE in the reformulated fuels. These compounds, because of their high solubility, will exist with the oxygenates in

groundwater contaminated by these fuels. The degradation data clearly show that MTBE and ETBE were very resistant to biodegradation in organic-rich soils. Although they can be anaerobically degraded in oligotrophic soils, when other easily-degraded organic compounds were present the degradation was inhibited. Therefore, the biodegradation of MTBE and ETBE can be expected to be difficult in almost all fuel contaminated systems. Single substrate degradation studies should be interpreted cautiously because results may differ significantly when mixtures of organics are likely to occur.

The addition of nitrate to promote the activity of denitrifiers and enhance the degradation of organic pollutants is a potential alternative for the subsurface systems in which the supply of oxygen is limited. Many gasoline components have been reported to be degraded under denitrifying conditions. However, for the gasoline oxygenates, the study indicates that enhancing the activity of denitrifiers will not promote degradation of MTBE and ETBE significantly.

## **Chapter 8 Degradation under Anaerobic Conditions**

The results of degradation under anaerobic conditions are discussed in this chapter. To stimulate anaerobic conditions, the solution was purged with oxygen-free nitrogen gas, and then reductants (cysteine and sodium sulfide) were added along with the inorganic nutrients. Because of a limited supply of nitrate, it is likely that fermentation, sulfate reduction and methanogenesis would be the dominant anaerobic metabolisms in the microcosms.

Degradation of TBA under anaerobic conditions was studied intensively in the past (Morris, 1988; Hickman, 1988). Their studies suggested that inhibition by active sulfate reducers was the main mechanism for the slow degradation of TBA at the organic-poor sites. At the organic-rich sites, on the other hand, the activity of sulfate reducers had no effect on TBA degradation. However, it is not clear how the interaction between sulfate reducers and methanogenic microorganisms influenced TBA degradation. Their results pointed out the importance of methanogenic activity and the direction of hydrogen flow, but did not answer: (1) why active sulfate reduction inhibited TBA degradation at the organic-poor sites but did not at the organic-rich sites; and (2) could the inhibition be changed so that the degradation would occur at any site. In this phase of the study, nutrient availability and the presence of organic matter, two major characteristics in the organically rich soils and important factors in TBA

degradation under denitrifying conditions, were evaluated. In addition, the degradation of MTBE and ETBE under fermenting conditions was evaluated.

### **8.1 TBA Degradation**

There are indications in the literature that interspecies hydrogen transfer processes were involved in the degradation of chlorinated organic compounds under anaerobic conditions (see Chapter 2). Adding a hydrogen donor to promote the activity of methanogenic bacteria has achieved some success in the degradation of chlorinated compounds. Morris (1988) observed significant increases in TBA degradation in the organic-poor soils by inhibiting the activity of sulfate reducers with molybdate. Therefore, he proposed that hydrogen during interspecies hydrogen transfer might be required for TBA degradation. The results of Hickman's study suggested that the organic-rich sites might provide enough hydrogen and the competition between sulfate reducers and methanogens for hydrogen was less significant in these soils.

Experiments were set up to study the mechanisms of the interactions between sulfate reducers and methanogenic microorganisms and their effects on TBA degradation. The parameters studied included the availability of primary hydrogen donors and nutrients, and the activity of sulfate reducers and methanogenic microorganisms inhibited or stimulated by electron acceptors or metabolic inhibitors

(molybdate and BESA).

### **Nutrient availability**

Figure 8.1 and Tables in Appendix D show typical degradation patterns of TBA at three sites with nutrient amendment. Figure 8.2 compares the effectiveness of nutrient amendment in the degradation rates, lag time and  $T_{80}$  of TBA at three sites. Similar to the responses observed under denitrifying conditions, the most significant enhancement of TBA degradation by nutrient addition occurred for the Site 1 soils. The results also show that by supplying nutrients, TBA degradation would occur at nearly the same rates at all sites, indicating that one of the controlling factor for TBA degradation under fermenting conditions was nutrient availability.

Table 8.1 compares the results of nutrient amendment in this study to those reported by Morris (1988) at the initial concentration of TBA as 100 mg/l. It was found that without amendment, TBA degradation rates at Site 1 and Site 3 in this study are close to those for Blacksburg soils (slow) and Newport News soils (fast) in Morris's study, respectively. But when nutrients were added in the Site 1 soils, TBA degradation rates were increased to those observed for the Newport News site in Morris's study.

### **Amendment with molybdate**

The molybdate amendment study was carried out by adding molybdate to the microcosms which had been incubated for 250

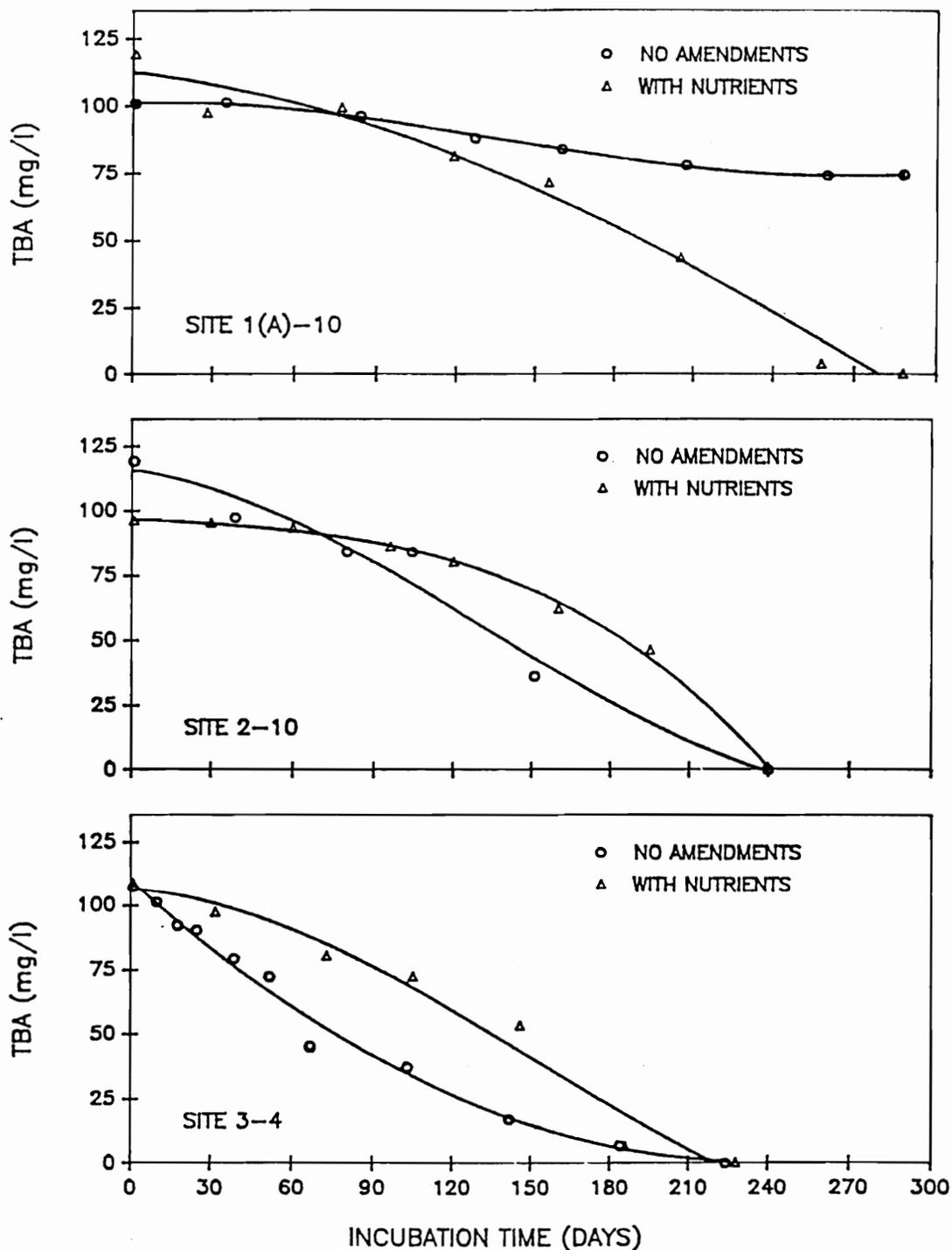


Figure 8.1 Anaerobic degradation of TBA with and without nutrient amendment in soils from three sites.

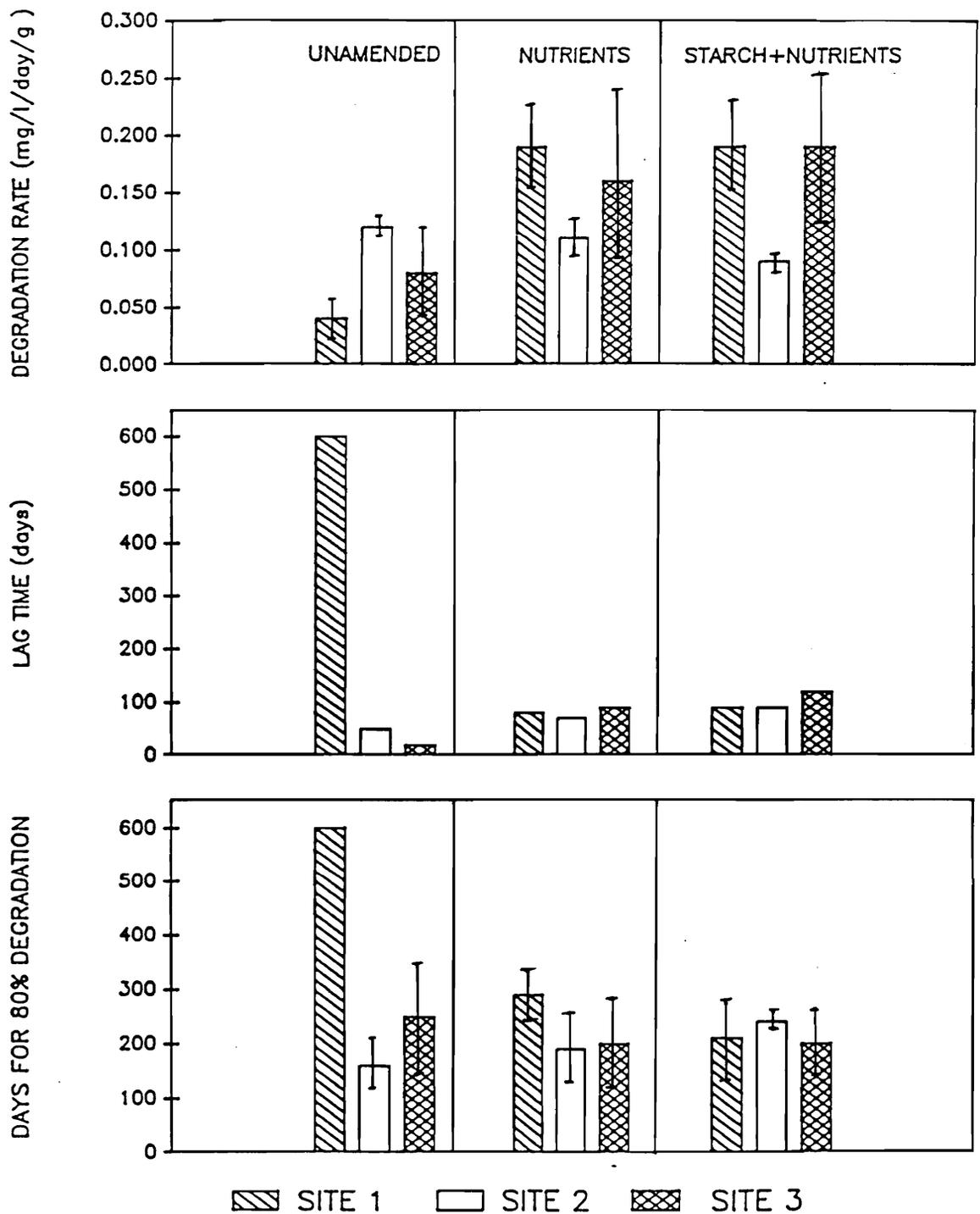


Figure 8.2 Effect of nutrients and starch/nutrients on anaerobic degradation of TBA.

Table 8.1 Comparison of anaerobic TBA degradation rates<sup>a</sup> in this study and in the study by Morris (1988)

Amendment	Slow sites		Fast sites	
	Site 1 <sup>b</sup>	Blacksburg <sup>c</sup>	Site 3 <sup>b</sup>	Newport News <sup>c</sup>
No amendment	0.01	0.007	0.08	0.2
Molybdate	0.01	0.10	0.11	0.2
Nutrients	0.20	-	0.16	-

a: mg/l/day/g dry soil at initial TBA conc.= 100 mg/l

b: Sites in this study, average of all depths

c: Sites in Morris's study, value of one depth

days with TBA (the unamended microcosms). It was found that the degradation of TBA at Site 1 was not significantly enhanced by molybdate (see Tables in Appendix D). The degradation patterns and rates are similar to those without amendment. This suggests that the activity of sulfate reducers was not the only factor in TBA degradation. A lack of hydrogen donors or depletion of nutrients in the microcosms might be other factors.

#### **Amendment with primary substrates**

To test the requirement for a hydrogen donor, ethanol or starch was added in the microcosms. Ethanol was selected because it is a common intermediate in the methanogenic fermentation process. Starch represents the cellular-like natural compounds existing in soil systems.

Ethanol addition resulted in inhibition of TBA degradation under anaerobic conditions (Fig. 8.3). Ethanol (200 mg/l) was degraded in two weeks and the addition of ethanol was repeated every two weeks to maintain a source of hydrogen donor in the system. TBA degradation did not occur over the entire 200 day incubation period, regardless of stimulation or inhibition of sulfate reduction or methanogenesis. However, these results were obtained from systems without nutrient amendment, so the lack of TBA degradation could have been due to the depletion of nutrients by ethanol degraders. Ethanol might also have caused similar detrimental effects on TBA degradation, as discussed in Chapter 7.

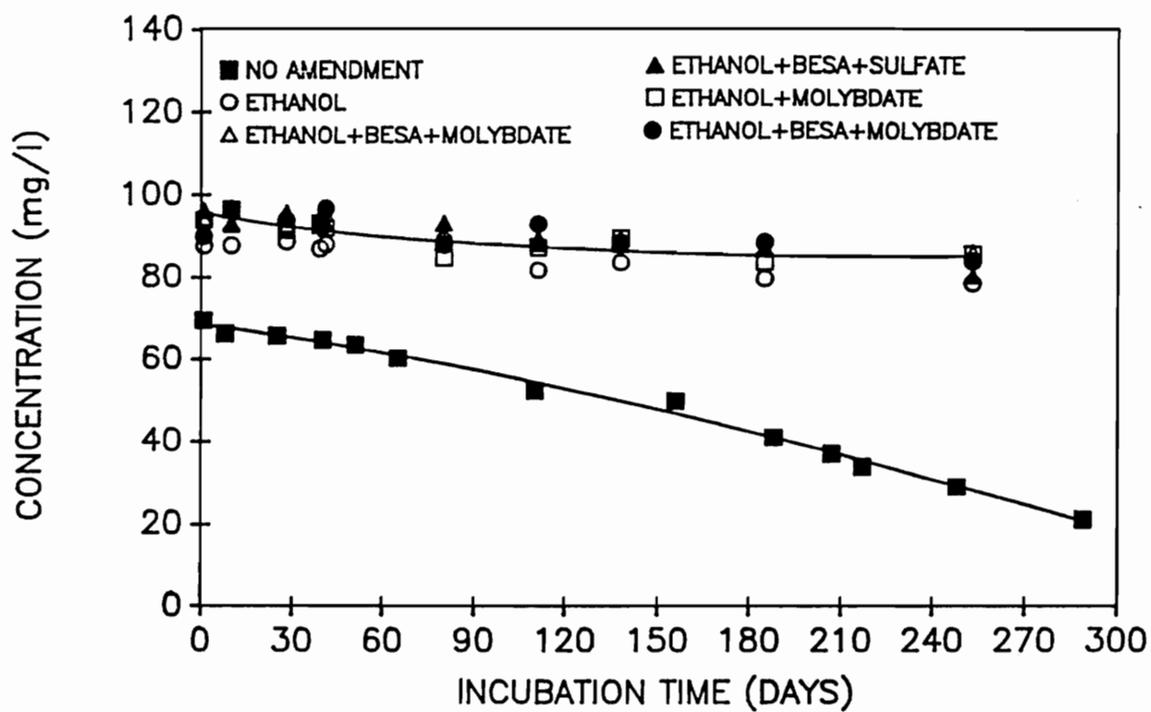


Figure 8.3 Effect of ethanol on TBA degradation in Site 1(A)-2 soil.

Because of the ineffectiveness of ethanol in promoting TBA degradation, starch was used as a source of hydrogen. One possible advantage of starch is that starch is usually degraded by groups of soil microorganism and thus might not cause similar inhibitory effects as seen in the ethanol amendment experiments. However, the concentration of starch could not be monitored.

It was observed that starch alone (200 mg/l, one dose) did not enhance TBA degradation in the Site 1 and Site 2 soils (Fig. 8.4). When nutrients were added along with starch, TBA degradation was significantly increased in site 1 soils but not for Site 2 soils. However, when the TBA degradation by nutrient/starch amendment at Site 1 was compared to that with only nutrient amendment, it was found that the degradation patterns and rates were similar (Fig. 8.1, 8.2). This indicate that nutrients were more important than starch in enhancing TBA degradation at Site 1 under the starch/nutrient amendment.

One extreme case of the effects of degradable organics on TBA degradation was observed for the Site 2 surface soil. Methane was detected early in the incubation period in the microcosm with this soil and no degradation of TBA was observed during 2 years of incubation. As discussed in Chapter 7, the relative concentration of the primary substrate to the target substrate concentration is important in the degradation pattern of the secondary substrates.

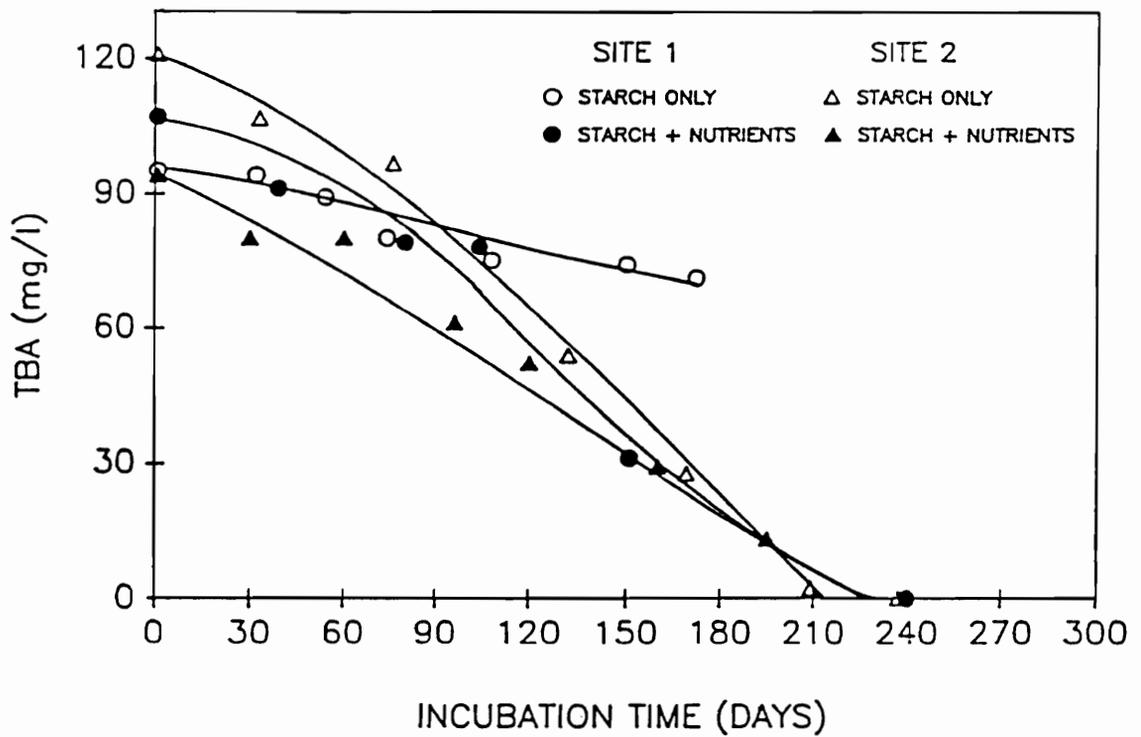


Figure 8.4 Effect of starch on TBA degradation in Site 1(A) and Site 2-5 soils.

Therefore, in the soils with high degradable organic compounds such as the Site 2 surface soil, TBA degradation might have been inhibited by the readily degradable organics.

As observed in the previous experiments, nutrients significantly increased TBA degradation at Site 1 and the addition of starch along with nutrient did not significantly change TBA degradation. To study the influence of the activity of sulfate reducers on TBA degradation, the next experiments were to add molybdate or sulfate along with starch and nutrients. Figure 8.5 shows that TBA degradation was not significantly changed by stimulation (Sulfate addition) or inhibition of sulfate reducers (molybdate addition). This suggests that in the Site 1 soils, inhibition of sulfate reduction on TBA degradation might not occur when nutrients were present. Once nutrients were added to the organic-poor soils, TBA degradation responded in similar patterns to those in the organic-rich soils.

One of the major factors limiting the activity of sulfate reducers is the availability of sulfate (Widdle, 1988). All the soils in this study contained measurable sulfate (Table 4.1). In addition, the population of sulfate reducers in these soils was high, in part because they have a high affinity for nutrients and can survive by acting as fermenters when sulfate is limited (Widdle, 1988). Therefore, sulfate reduction would be expected to be occurring in the soil.

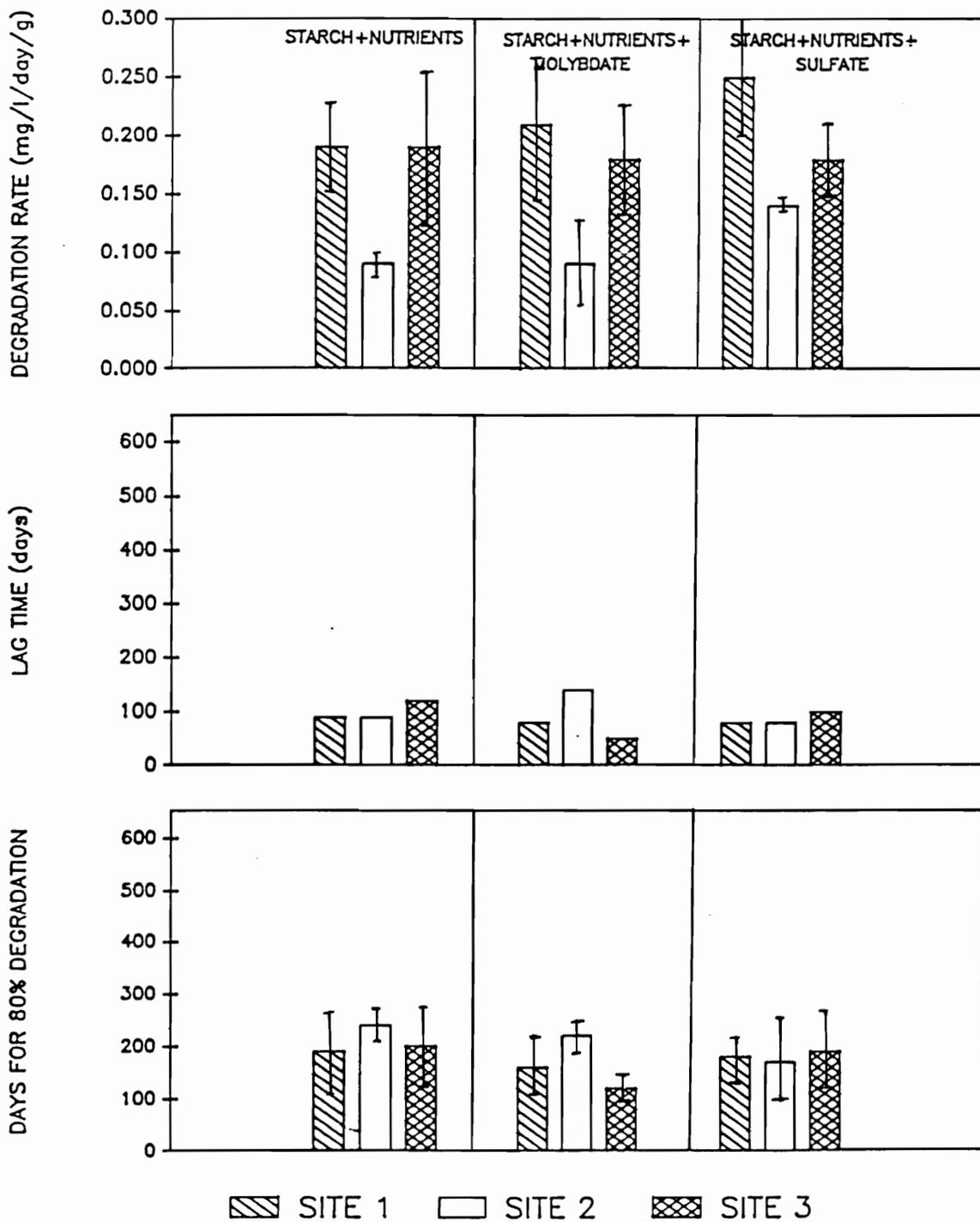
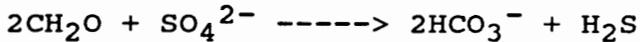


Figure 8.5 Effect of the activity of sulfate reducers on TBA degradation.

One question that needed to be answered is whether the lack of inhibition from sulfate reducers on TBA degradation in the presence of starch and nutrient amendment was due to the depletion of sulfate. The added 200 mg/l starch would consume 106 mg/l of sulfate, based on the equation below:



Therefore, if all the hydrogen generated from the degradation of 200 mg/l of starch went to sulfate reducers, sulfate might be depleted, and the sulfate reducers would be inactive because of a lack of sulfate. However, literature reported that sulfate reducers can only use monomers and it is likely that sulfate reducers would have to rely on fermenters to hydrolyze the added starch.

To further determine if sulfate was depleted by starch, the final concentration of sulfate in the microcosms was analyzed. It was found that there were adequate residual sulfate concentrations (greater than 10 mg/l) in the unamended microcosms with site 1 soils, indicating that sulfate reducers were not limited by lack of sulfate. Residual sulfate in the microcosms amended with nutrients/starch/sulfate indicates that less than 10% of the added sulfate was consumed. This also implies that sulfate reduction was inactive in the nutrient amended microcosms.

A further investigation on the effect of sulfate reducers was performed using an amendment matrix consisting of various combinations of sulfates, molybdate and BESA. The results

are summarized in Table 8.2. The data suggest that methanogenic activity was important in controlling TBA degradation in Site 1(B) soils. The addition of BESA increased the lag time and  $T_{80}$ , regardless of the inhibition or stimulation of sulfate reducers with molybdate or sulfate. However, TBA degradation reoccurred after a much longer lag phase. This could be due to inefficient inhibition by BESA in soil systems (Widdle, 1988; Oremland, 1982; Smith, 1983; Zinder et al., 1984).

The results of the inhibition/stimulation experiments suggested that sulfate reducing processes did not significantly inhibited TBA degradation when nutrients were abundant. The only significant enhancement of TBA degradation resulting from inhibition of sulfate reducers occurred in the Blacksburg soils (Site 1) when they were amended with molybdate but received no nutrient addition (Morris, 1988). Studies in the literature reported that, in addition to sulfate, interactions between sulfate reducers and methanogenic microorganisms also depends on types of substrates (see Chapter 2). Sulfate reducers do not compete well for substrates such as methanol and trimethylamine to which methanogenic microorganisms have a higher affinity. Therefore, in a system containing these non-competitive substrates, methanogens are active regardless of the activity of sulfate reducers.

It is possible that in a low-organic and low-nutrient

Table 8.2 Effect of methanogenic activity on TBA degradation.

Amendment <sup>b</sup>	Rate (mg/l/day/g dry soil)	Lag Time (days)	T <sub>80</sub> (days)
S + N	0.23	100	210
S + N + Mo	0.25	120	200
S + N + SO <sub>4</sub> <sup>2-</sup>	0.25	80	180
S + N + Mo + BESA	0.22	220	290
S + N + BESA + SO <sub>4</sub> <sup>2-</sup>	0.18	200	270

a : Each number is the average value for all depths

b : S - 200 mg/l starch, N: inorganic nutrients, Mo - 1 mM Na<sub>2</sub>MoO<sub>4</sub>  
 SO<sub>4</sub><sup>2-</sup>: 2 mM, BESA: 2 mM

system where sulfate reducers are the dominant species, hydrogen sources and nutrients might be controlled by the sulfate reducers. At Site 1, the population of the TBA-degrading microorganisms stayed low because nutrients were limited and sulfate reducers outnumbered methanogens by three orders of magnitudes in the nutrient-poor sites. Inhibiting sulfate reducers by adding molybdate to the nutrient-poor sites will channel the nutrients to TBA-degrading microorganisms. However, the organic-poor soils were still low in nutrients; therefore TBA degradation exhibited a non-growth degradation pattern (zero-order degradation).

In the organic-rich soils or the organic-poor soils amended with nutrients, because nutrients were abundant, competition was less significant. Therefore, inhibiting sulfate reducers under such a condition had no significant effects on TBA degradation. Figure 8.6 describes the proposed competitive mechanism between TBA-degrading microorganisms and sulfate reducers in the degradation of TBA.

It is not clear in this study that TBA was degraded by methanogens or through the interactions between fermenters and/or methanogens. No methane or fermentation products such as fatty acids were detected from TBA degradation. It might be that the degradation of 100 mg/l of TBA did not generate methane at concentrations high enough to be detected by FID. Also, the GC column used to measure oxygenates will not

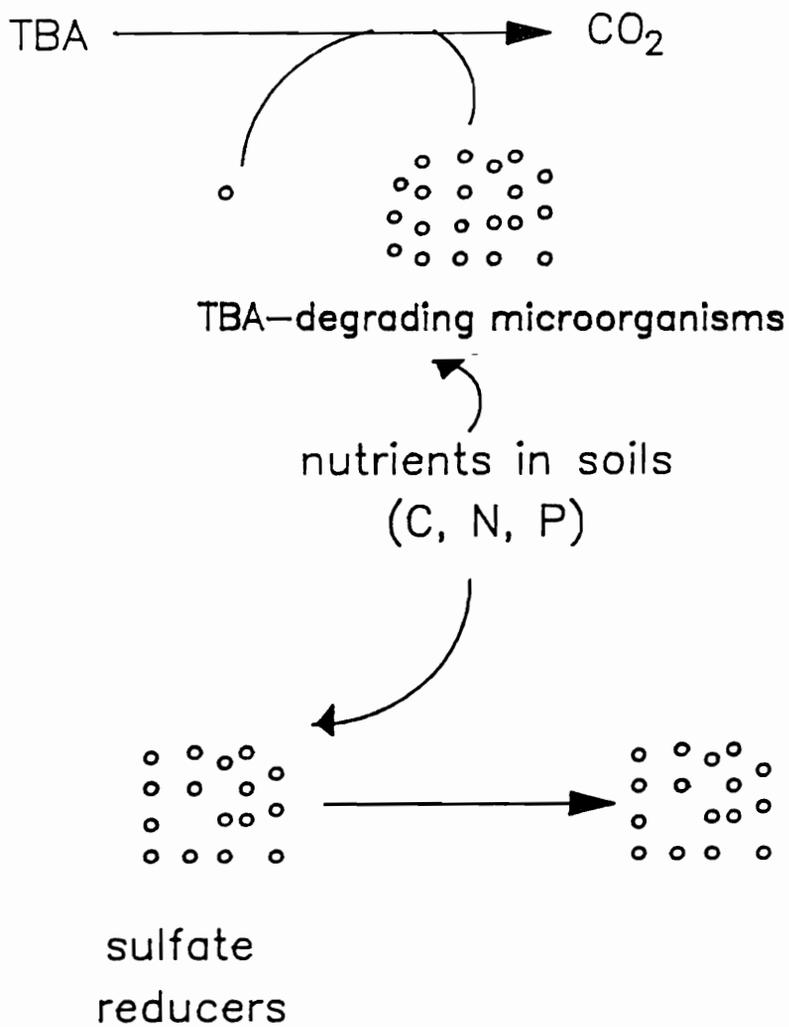


Figure 8.6 Proposed competition mechanisms between TBA-degraders and sulfate reducers in TBA degradation.

detect fatty acid.

## **8.2 MTBE and ETBE Degradation**

The repeated addition of ethanol under anaerobic conditions inhibited ETBE degradation (Fig. 8.7). ETBE was degraded in the unamended microcosms for Site 1 soils, but when ethanol was added, no degradation was observed.

The addition of nutrients enhanced ETBE degradation at Site 1 (Fig. 8.8a). However the stimulation resulting from nutrient addition did not occur in every depth of soil at Site 1, implying that nutrients were important but not the only factor limiting the degradation of ETBE. Previous results in the unamended microcosms indicated that ETBE degradation may be inhibited by low soil pH. A plot of ETBE degradation rates as a function of pH reveals the important influence of soil pH (Fig. 8.8b). ETBE degradation was observed at microcosms with a pH between 5.0 and 6.0 and the optimum pH for fastest ETBE degradation was around 5.5. Site 1 soils are rich in clays which contribute to soil acidity. Although the nutrient solution added into the microcosms contained a phosphate buffer and had a pH of 7, the clay-rich soil was able to decrease pH to near its original value and retarded degradation when that pH was less than 5.0.

Degradation of MTBE was observed under anaerobic conditions (Fig. 8.9a). Degradation of MTBE at Site 1 under anaerobic conditions was the only site and conditions where

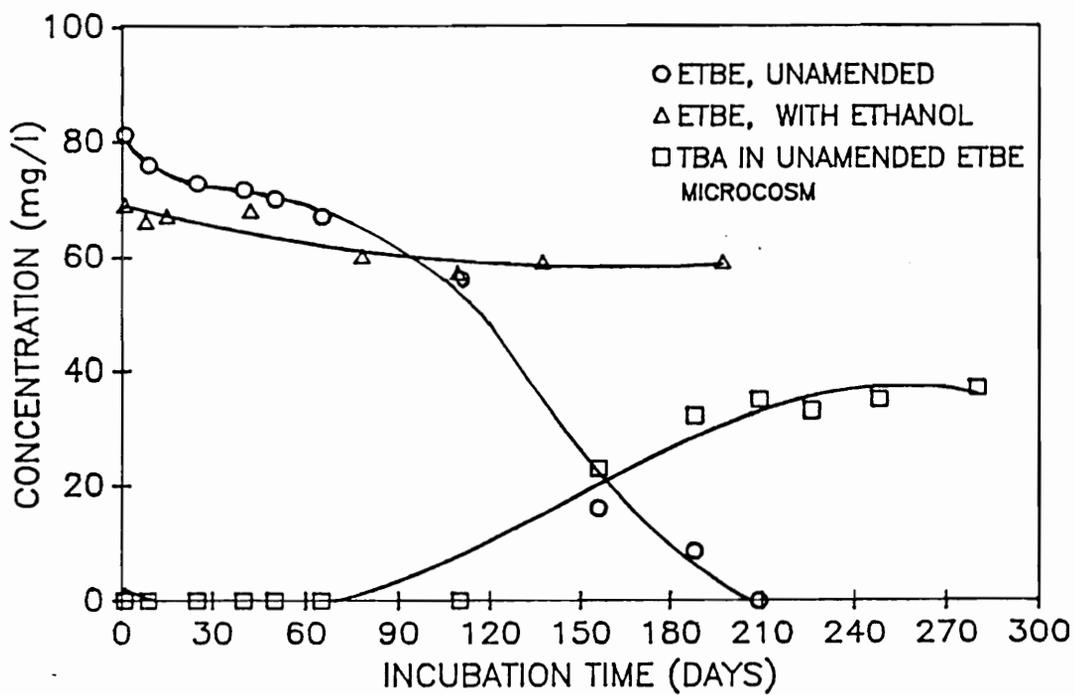


Figure 8.7 Inhibitory effect of ethanol on ETBE degradation.

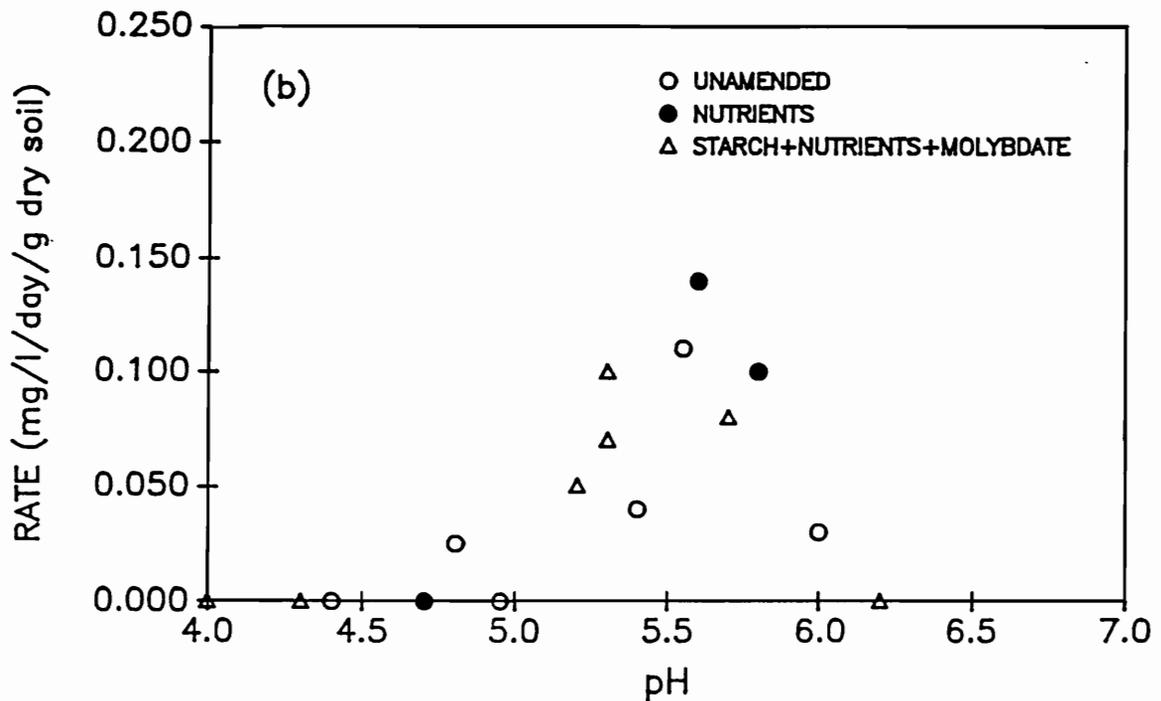
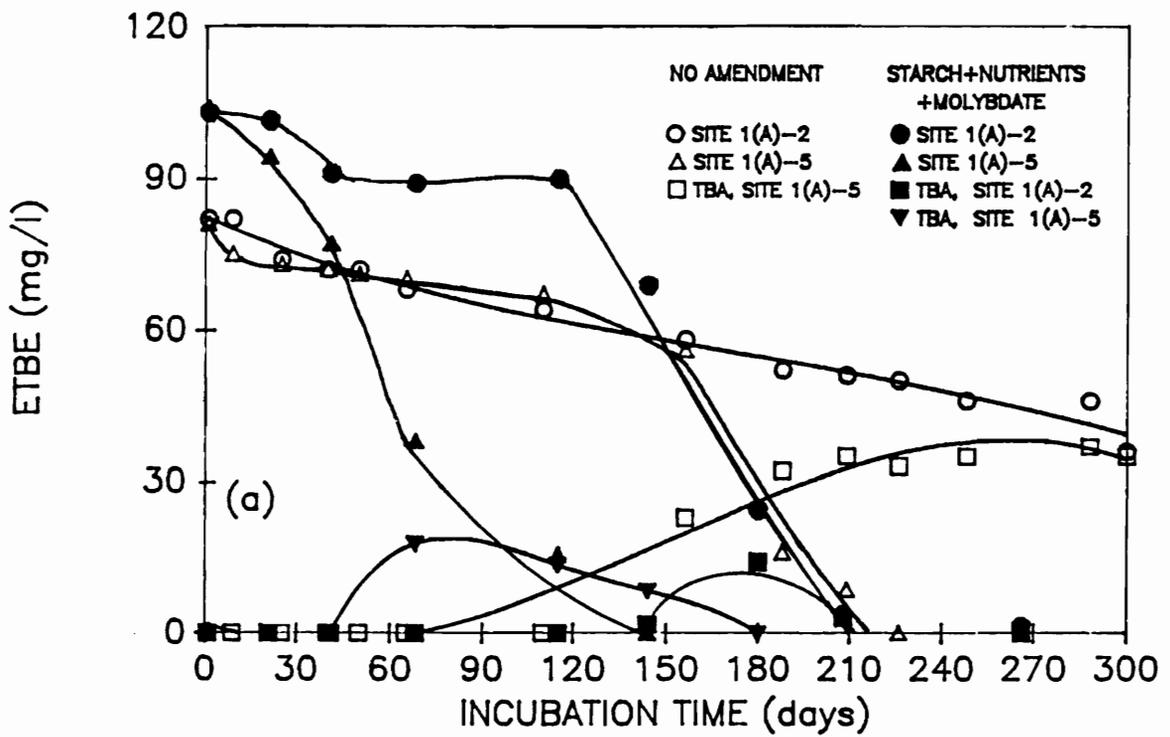


Figure 8.8 Degradation of ETBE under anaerobic conditions: (a) degradation patterns; (b) effect of pH.

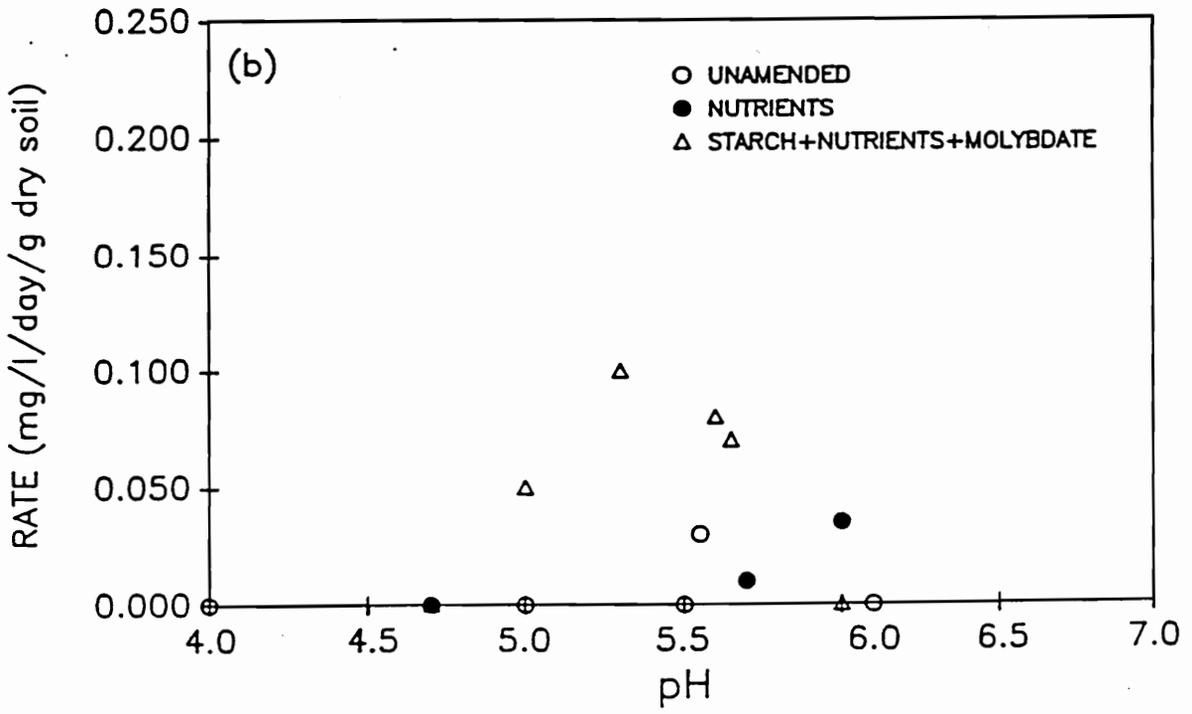
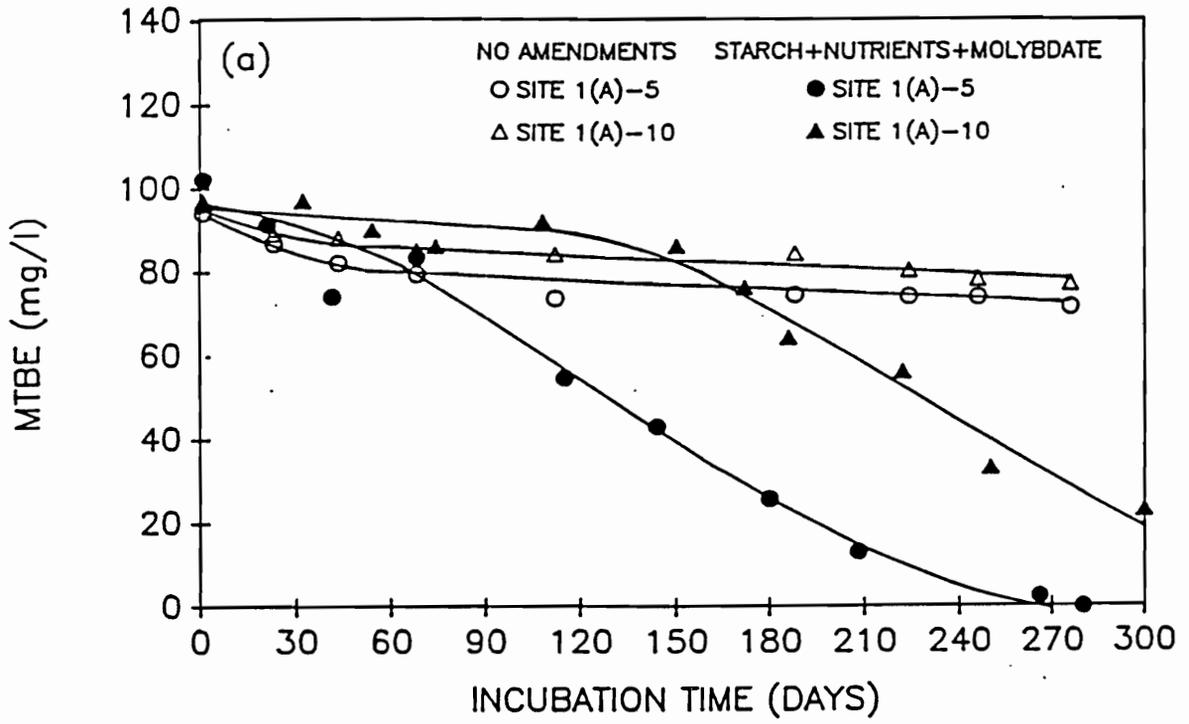


Figure 8.9 Degradation of MTBE under anaerobic conditions: (a) degradation patterns; (b) effect of pH.

MTBE degradation was observed. However, the degradation rates was still much slower than those for TBA and ETBE under similar conditions. In addition to nutrients, pH also appears to influence MTBE degradation in the Site 1 soils (Fig. 8.9b).

How the soil pH affected the microbial activity and the degradation in soils microcosms is unknown. In general, the optimum pH for microbial growth is thought to be around 7.0. However, some microorganisms are still active in acidic environments. Because the original soil pH at Site 1 was acidic, it is reasonable to suggest that the soil microorganisms at Site 1 were able to grow under acidic environments. However, the growth of soil microorganisms are controlled by many factors which are influenced by soil pH. Soil pH might be just an indicator of the physical, chemical and biological interactions in soils that influence the biodegradation.

Because of the long incubation time required to monitor MTBE and ETBE degradation, studies on the influence of sulfate reducers on MTBE and ETBE degradation were not performed. Therefore, it is not clear whether sulfate reducers will influence the degradation of either MTBE or ETBE. However, the results in Fig. 8.8(b) and Fig. 8.9(b) show that addition of molybdate did not always increase the degradation of MTBE and ETBE. It is possible that neither sulfate reducers nor methanogens were actually involved in

cleaving the ether linkages in MTBE and ETBE. The literature has reported that an acetogenic species was able to demethoxylate methoxylated compounds (Bache and Pfenning, 1981; Schink, 1988). Similar types of anaerobes might be responsible for this reaction.

No degradation of MTBE and ETBE was observed at Site 2 or Site 3, indicating that MTBE and ETBE can be very recalcitrant in organic-rich soils. The recalcitrant characteristics of MTBE and ETBE in organically rich soils will have profound impacts on bioremediation efforts for MTBE and ETBE cleanups. As previous stated, it is likely that other organic compounds will be present with these oxygenates in a groundwater system contaminated by fuels. Thus the degradation of MTBE and ETBE can be expected to be difficult even though microbial degradation of both MTBE and ETBE can occur under methanogenic conditions.

## Chapter 9 Conclusions

The purpose of this work was to determine the biodegradation potential of MTBE, ETBE and TBA in soils under aerobic, denitrifying and anaerobic conditions. The influence of subsurface environments on the degradation under each condition was studied. Based on the results obtained in this study, the conclusions were:

1. In the unamended microcosms, the biodegradation potential of MTBE, ETBE and TBA varied substantially with site and depth. TBA was the easiest compound to biodegrade. MTBE was very recalcitrant in the subsurface. ETBE was degraded only in the organic-poor soils, producing TBA.
2. TBA degradation was slower at organic-poor sites but relatively faster at organic-rich sites.
3. The addition of hydrogen peroxide led to the chemical oxidation of MTBE and ETBE in the microcosms with organic-rich soils. The reduced iron in the organic-rich soil acted as a catalyst for the generation of hydroxyl radicals which oxidize MTBE and ETBE. ETBE was chemically oxidized faster than MTBE.
4. In soil-free solutions containing reduced irons, chemical oxidation of MTBE and ETBE by hydroxyl radicals depended on

the dose of hydrogen peroxide and pH, but was independent of the reduced iron concentration over a range of iron concentration from 0.2 mM to 10 mM.

5. ETBE could be biologically degraded by aerobic soil microorganisms. MTBE, on the other hand, was not aerobically biodegraded. The biological degradation of ETBE was insignificant in the soil microcosms when chemical oxidation occurred. Aerobic microbial degradation was the primary mechanism for TBA degradation in soil microcosms.

6. With nitrate and nutrient addition, TBA degradation under denitrifying conditions could be enhanced to the same level at all sites. The presence of ethanol hindered TBA degradation under denitrifying conditions.

7. ETBE degradation was not increased by nitrate and nutrient amendment. MTBE was not degraded at any site under denitrifying conditions.

8. The co-existence of other easily degraded organic compounds had a detrimental effect on ETBE degradation under denitrifying conditions. No ETBE degradation was observed in organic-rich soils. When ethanol was added in the organic-poor soils, ETBE degradation did not occur.

9. Under anaerobic conditions, nutrient addition enhanced TBA degradation to the same level at all sites.

10. The repeated addition of ethanol without nutrient amendment had inhibitory effects on TBA degradation.

11. MTBE and ETBE were anaerobically degraded at the organic-poor site. The degradable organics had significant influences on the degradation. Ethanol inhibited MTBE and ETBE degradation. In the organic-rich soils, the presence of degradable organic matter inhibited MTBE and ETBE degradation.

12. An optimum pH of around 5.5. was observed for MTBE and ETBE degradation.

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## **Appendix A**

- . Sterile Control Data
- . Degradation in Unamended Microcosms

TBA, Sterilized, Site 1(A)

Day	2ft		5 ft		10 ft
	microcosm		microcosm		microcosm
	1	2	1	2	1
1	85.4	84.1	83.6	86.4	92.0
20	82.2	84.2	80.7	80.0	86.5
45	77.5	77.4	75.9	80.2	84.4
90	74.3	72.0	73.4	72.9	78.3
136	76.3	71.1	75.5	75.7	79.8
168	74.8	70.2	72.2	78.2	79.3
198	73.0	64.3	71.0	74.4	76.6
228	72.4	62.2	71.6	70.4	70.4
268	70.2	65.3	67.5	67.7	75.7

TBA, Site 2, Sterile control

Day	0 ft			5 ft			10 ft		13 ft	
	microcosm			microcosm			microcosm		microcosm	
	1	2	3	1	2	3	1	2	1	2
1	110.8	102.9	109.1	108.9	111.8	105.1	113.8	101.1	110.9	100.1
39	105.2	105.8	102.7	100.0	102.2	95.1	108.5	104.2	100.8	102.3
80	101.0	106.2	103.8	98.2	101.8	95.3	107.4	105.1	104.0	-
104	101.0	102.4	105.7	100.2	102.1	94.2	104.8	104.9	100.5	98.9
151	101.6	101.3	103.3	94.7	106.5	97.2	104.1	97.2	102.7	-
240	104.7	109.2	107.2	104.4	108.4	92.2	109.7	108.2	102.7	105.0
280	99.2	102.0	107.8	103.3	115.5	90.6	107.6	99.2	103.6	95.2

MTBE, sterilized, Site 1(A)

Day	2ft		5 ft		10 ft
	microcosm		microcosm		microcosm
	1	2	1	2	1
1	119.5	116.7	115.8	107.0	118.0
30	111.6	110.6	106.4	100.6	116.2
89	102.6	104.0	101.0	88.3	106.4
135	103.5	100.1	98.2	81.8	104.9
165	99.8	99.2	94.7	84.6	102.2
201	96.6	98.9	94.6	84.1	103.0
223	97.5	102.1	88.6	81.3	100.6
253	92.0	94.6	88.8	80.4	96.9

MTBE, Site 2, Sterile control

Day	0 ft		5 ft	10 ft	13 ft
	microcosm		microcosm	microcosm	microcosm
	1	2	1	1	1
1	120.3	96.9	83.7	139.9	104.8
25	117.9	89.2	72.1	139.0	96.9
77	115.0	88.3	74.0	137.2	80.4
110	117.4	87.8	74.5	142.9	87.8
231	116.2	82.3	78.8	145.8	74.0
261	117.5	88.0	75.5	144.5	77.7

ETBE, sterilized, Site 1(A)

Day	2ft	5 ft	10 ft
1	79.5	78.2	66.0
30	78.2	71.2	70.0
90	72.1	65.8	67.9
136	69.1	64.0	62.2
168	66.8	61.6	63.1

ETBE, Site 2, Sterile control

Day	0 ft		5 ft		10 ft
	microcosm		microcosm		microcosm
	1	2	1	2	1
1	85.9	89.0	102.3	106.6	91.6
30	70.8	75.2	89.7	88.1	66.7
66	75.9	75.4	93.7	86.8	73.1
112	71.8	77.6	86.4	94.8	68.1
224	75.6	80.0	99.8	102.2	65.2
274	73.2	81.5	95.3	90.4	69.3

TBA, Site 3, sterilized

Day	0 ft		2 ft		4 ft		7 ft		10 ft	
	microcosm		microcosm		microcosm		microcosm		microcosm	
	1	2	1	2	1	2	1	2	1	2
1	138.3	140.9	130.1	121.4	49.0	101.6	118.6	106.6	97.0	107.8
21	140.0	132.5	131.1	127.9	51.9	115.5	120.6	115.1	105.7	108.8
35	131.3	137.8	132.9	126.5	51.8	113.7	118.4	115.0	104.0	108.0
49	136.2	135.1	128.1	125.8	50.7	110.7	116.8	111.8	101.5	104.1
77	129.4	128.0	112.1	121.0	46.9	107.9	102.3	109.9	93.1	100.4
113	123.5	125.1	117.4	117.8	50.5	109.1	116.2	95.1	101.1	99.7
152	131.5	134.3	128.4	122.7	49.2	108.0	111.3	113.2	106.6	101.5
194	127.2	127.2	123.1	120.2	47.3	110.0	113.8	106.4	100.0	102.2
234	123.0	124.9	122.6	120.3	47.9	107.1	113.3	110.6	104.0	102.6
272	123.9	126.8	116.4	120.0	46.9	105.0	93.0	105.7	97.4	100.4

MTBE, Site 3, sterilized control

Day	0 ft		2 ft		4 ft		7 ft		10 ft	
	microcosm		microcosm		microcosm		microcosm		microcosm	
	1	2	1	2	1	2	1	2	1	2
1	84.5	142.8	143.3	146.7	116.5	93.7	103.5	123.2	96.6	155.9
20	77.9	138.7	132.9	133.8	112.2	89.4	98.3	112.3	95.7	141.1
50	76.4	125.6	132.2	110.8	88.0	96.7	110.7	94.5	94.5	142.4
85	76.8	124.2	137.1	126.8	99.9	78.9	-	97.9	100.0	130.5
238	88.4	163.9	156.8	156.1	127.9	101.0	108.9	127.2	108.9	150.8

ETBE, Site 3, sterilized control

Day	0 ft		2 ft		4 ft		7 ft		10 ft	
	microcosm		microcosm		microcosm		microcosm		microcosm	
	1	2	1	2	1	2	1	2	1	2
1	133.4	146.0	119.3	111.2	103.4	98.0	87.7	115.9	95.7	89.7
22	134.6	134.6	112.1	110.7	91.9	92.2	82.8	111.8	89.2	83.9
36	121.3	133.0	114.8	110.7	93.3	94.1	84.5	109.8	92.0	85.0
85	121.5	84.9	72.4	80.8	93.0	-	79.3	97.9	77.2	82.1
236	138.1	145.8	124.8	120.4	99.7	100.1	88.9	121.8	100.5	100.5

TBA, unamended, Site 1(A)

Day	2ft			5 ft			10 ft		
	microcoam								
	1	2	3	1	2	3	1	2	3
wt.(g)	4.0	4.3	4.0	3.6	3.6	3.6	5.1	5.0	
1	69.3	67.0	67.5	61.5	64.0	66.7	72.8	65.7	
25	65.6	64.0	65.5	59.6	60.3	60.1	65.3	64.8	
40	64.6	64.2	68.4	61.5	58.6	60.5	62.8	64.7	
65	60.1	61.1	58.7	56.8	58.3	56.9	61.2	61.2	
110	52.2	53.5	50.2	49.8	51.3	45.3	56.4	55.1	
156	49.7	55.9	46.3	49.3	49.2	44.9	60.6	60.1	
188	40.9	51.9	39.9	39.1	40.7	40.5	55.9	58.2	
207	37.0	51.0	35.7	38.6	38.4	40.3	55.9	55.9	
248	29.0	48.2	28.9	34.2	34.1	37.1	51.8	56.2	
289	21.5	47.1	16.7	34.5	35.4	34.8	51.2	54.4	

TBA, unamended, Site 1(B)

Day	2'			5'			10'			14'		
	microcoam											
	1	2	3	1	2	3	1	2	3	1	2	3
wt.(g)	4.1	4.4	4.2	5.0	5.0	4.7	3.3	3.8	3.9	3.2	3.8	4.2
1	110.5	110.7	114.4	102.9	104.4	106.6	100.7	97.8	101.9	108.3	103.7	102.0
35	102.1	100.3	104.1	99.1	110.8	102.2	111.1	105.1	111.6	100.0	97.9	107.5
84	91.4	96.5	97.5	99.3	102.7	109.5	95.8	92.7	90.3	85.0	79.2	73.3
127	80.2	79.5	78.6	97.1	102.3	101.1	87.1	84.2	86.9	71.4	68.1	66.6
160	68.9	61.7	66.2	86.9	98.5	96.2	83.5	75.8	81.9	69.5	62.0	59.1
207	55.8	54.2	47.8	80.2	91.2	88.2	77.6	78.2	69.7	60.1	55.2	52.5
260	40.7	37.8	30.0	73.7	75.5	75.7	73.6	76.5	66.6	50.5	41.7	39.0
288	32.4	36.2	22.8	67.0	78.4	78.3	74.1	67.8	65.5	53.3	48.0	40.8

TBA, Site 2, unamended

Day	0 ft			5 ft			10 ft			13 ft		
	microcoam											
	1	2	3	1	2	3	1	2	3	1	2	3
wt.(g)	5.6	5.9	6.1	6.8	5.3	4.9	5.4	5.5	5.4	5.7	5.9	
1	110.8	110.7	110.1	106.5	116.1	112.4	102.7	119.2	98.4	98.7	95.2	
39	105.2	98.4	100.3	80.1	97.5	100.7	97.9	96.2	92.1	88.6	90.6	
80	103.9	97.9	101.6	31.6	61.9	91.7	86.7	84.3	77.2	79.0	75.8	
104	99.6	96.7	101.1	4.6	45.9	97.0	80.2	83.4	66.9	75.1	62.2	
151	103.8	94.9	100.3	0.4	0	92.2	72.9	36.3	13.6	30.1	7.2	
240	100.5	98.3	98.1	0	0	8.0	2.8	0	0	0	0	
600	109.4	98.5	103.0									

HTBE, unamended, Site 1(A)

Day	2ft		5 ft		10 ft		
	microcosm	microcosm	microcosm	microcosm	microcosm	microcosm	
1	2	3	1	2	1	2	
1	2	3	1	2	1	2	
wt.(g)	3.5	3.4	3.5	2.8	2.9	3.9	3.4
1	104.9	107.1	106.4	94.8	94.1	96.3	91.7
28	100.6	101.6	98.9	87.9	85.8	85.4	85.6
68	93.0	94.8	91.3	82.5	79.3	80.1	80.1
112	89.7	91.5	90.3	81.7	73.6	77.2	76.5
158	90.5	89.0	92.1	80.6	74.3	78.4	75.8
188	86.9	87.3	85.7	76.0	73.9	74.6	74.6
224	84.8	87.5	86.1	73.8	73.8	74.9	74.1
240	87.2	88.0	82.6	78.9	71.4	75.3	76.7
276	80.0	79.0	80.0	75.2	71.6	68.8	70.5
pH	5.0	5.0	5.0	5.5	5.3	6.1	6.1

HTBE, Site 1(B), unamended

Day	2 ft		5 ft		10 ft		14 ft		
	microcosm								
1	2	3	1	2	1	2	1	2	
1	2	3	1	2	1	2	1	2	
wt.(g)	3.8	3.9	3.9	4.2	4.9	3.3	3.6	4.8	4.2
1	112.4	105.3	108.8	111.4	104.5	109.5	97.1	83.6	95.9
43	109.5	107.5	107.3	106.2	110.5	108.7	101.4	94.7	93.2
90	115.3	116.4	114.1	115.1	118.1	116.2	113.0	95.4	99.9
128	112.3	112.5	114.8	113.5	115.2	110.9	111.0	-	98.6
167	114.1	112.6	115.1	106.5	116.4	115.4	116.7	83.1	97.3
208	116.5	111.5	114.4	-	112.2	117.4	113.1	83.7	105.3
281	119.8	-	109.9	-	104.7	114.0	115.2	81.1	101.6
322	115.9	-	119.0	-	111.8	114.0	74.7	-	107.8
pH	4.0	4.0	4.0	4.0	5.2	5.8	4.5	4.7	

HTBE, site 2, unamended

Day	0 ft		5 ft		10 ft		13 ft			
	microcosm									
1	2	3	1	2	3	1	2	3		
1	2	3	1	2	3	1	2	3		
wt.(g)	4.8	4.3	4.3	6.7	4.8	5.5	3.9	4.8	5.5	5.5
1	94.5	113.1	107.8	105.8	87.6	75.7	93.8	87.2	110.0	80.4
25	91.4	108.4	107.5	70.2	82.8	68.1	89.5	89.9	89.9	82.0
77	90.7	107.4	91.7	60.9	90.1	74.4	90.5	92.2	106.8	91.8
110	94.5	106.7	107.9	66.7	95.2	73.5	90.1	87.8	103.6	80.8
141	92.7	104.8	110.2	70.8	85.0	73.1	90.8	86.8	106.7	87.2
201	101.2	109.8	109.6	72.8	94.0	76.5	95.6	92.6	105.9	93.9
262	91.9	107.5	101.0	89.7	-	-	-	87.1	108.5	93.6

ETBE, unamended, Site 1(A)

Day	2ft			5 ft			10 ft			
	microcosm									
1	2	3	1	2	3	1	2	3	1	
ETBE	ETBE	ETBE	ETBE	TBA	ETBE	TBA	ETBE	TBA	ETBE	TBA
Mt.(g)	4.2	4.4	4.3	3.5	3.3	3.2	3.2	4.8		
1	84.7	81.9	81.1	80.5	81.3	81.2	81.2	69.3		
9	81.6	81.9	81.1	75.6	75.3	75.9	75.9	71.5		
25	75.5	74.1	76.7	69.3	71.2	72.7	68.7	68.7		
40	74.1	72.2	75.3	68.5	69.1	71.6	69.3	69.3		
65	66.5	68.6	69.6	63.5	64.6	66.6	65.6	65.6		
110	55.0	64.0	64.6	56.2	0	59.3	0	55.5	0	58.5
156	48.9	58.2	61.9	16.3	21.9	48.0	3.4	15.0	22.9	52.2
188	42.6	42.8	53.9	0	36.0	41.2	4.8	8.6	32.2	44.0
209	37.9	51.1	53.8	0	35.9	38.0	5.3	0	35.0	45.5
226	32.7	50.2	42.7		34.8	5.5	33.2	40.8	9.5	
248	34.0	45.7	48.9		28.6	5.1	35.1	41.5	10.6	
275	46.1	50.1	50.1		23.7	16.7	36.7	40.1	11.2	
pH	5.0	4.4	5.0	5.3	5.6	5.8	5.8	6.0		

ETBE, Site 1(B), unamended

Day	2 ft			5 ft			10 ft			14 ft		
	microcosm											
1	2	3	1	2	3	1	2	3	1	2	3	
Mt.(g)	4.5	4.3	4.3	4.5	4.6	4.6	3.8	3.4	3.6	5.1		
1	104.1	108.4	116.1	119.5	115.2	132.8	116.9	127.7	110.3	136.9		
47	109.9	110.9	110.6	121.6	113.5	121.8	112.8	118.2	113.0	115.4		
93	104.1	108.3	104.7	129.7	119.8	120.1	124.7	118.1	119.1	-		
131	95.6	-	99.9	121.8	108.9	-	111.3	116.3	110.4	107.0		
166	109.3	89.6	94.8	108.2	109.8	103.9	117.1	106.1	110.1	83.7		
207	93.2	83.4	82.5	93.6	89.2	89.0	102.9	107.7	102.1	40.8		
281	90.1	78.6	79.0	95.7	87.1	81.1	112.4	94.9	105.7	10.5		
pH	4.4	4.7	4.5	4.7	4.7	4.7	5.0	4.8	4.8	5.8		

ETBE, Site 2, unamended

Day	0 ft			5 ft			10 ft			13 ft		
	microcosm											
1	2	3	1	2	3	1	2	3	1	2	3	
Mt.(g)	4.7	3.9	3.9	5.6	5.7	5.2	5.2	5.1	6.9	6.9		
1	107.7	99.1	105.3	70.2	82.3	68.5	67.0	69.4	62.7	69.2		
30	98.2	96.1	100.9	66.2	76.2	63.2	63.8	65.6	68.3	66.0		
66	104.1	98.5	101.0	64.5	75.6	62.4	65.8	65.5	70.9	64.0		
112	106.9	95.2	102.8	63.2	69.5	63.5	64.0	77.7	75.2	71.3		
142	96.9	88.5	101.6	65.7	69.9	63.3	62.2	64.3	62.3	61.4		
224	100.9	93.0	99.7	61.2	70.2	61.9	62.3	62.1	68.1	66.5		
274	102.4	92.6	99.0	60.5	71.2	71.4	76.4	78.9	66.7	67.3		

TBA, Site 3, unamended

Day	0 ft microcosm			2 ft microcosm			4 ft microcosm			7 ft microcosm			10 ft microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	7.8	7.6	7.7	6.7	7.2	6.7	5.9	6.1	5.5	5.8	5.0	5.6	3.4	3.2	3.5
1	104.8	100.9	100.1	96.5	104.9	108.0	109.4	106.9	107.6	145.8	136.2	120.4	148.5	137.1	131.8
10	110.4	112.5	113.7	108.0	108.8	109.0	108.3	101.7	104.1	134.9	138.2	125.5	129.3	129.1	120.5
18	103.6	109.2	106.0	101.7	101.0	103.2	94.1	92.4	98.6	127.2	127.8	121.9	115.4	118.3	118.9
25	99.1	106.0	102.6	102.1	100.4	103.3	92.6	90.4	94.2	122.3	124.8	117.4	111.4	117.3	115.5
39	89.3	97.1	94.7	91.4	91.1	90.9	85.8	78.9	88.5	112.8	122.8	102.5	97.6	109.8	104.3
52	81.9	94.3	85.8	89.4	82.7	89.1	82.8	72.4	82.3	96.4	112.8	80.8	101.8	103.1	99.3
67	71.0	89.5	78.1	77.2	80.2	78.3	65.2	59.5	74.6	78.7	96.8	56.5	96.5	103.8	101.6
103	61.6	64.7	63.9	68.2	60.6	65.0	64.7	45.0	60.0	64.0	67.3	17.4	93.0	98.0	92.3
142	43.1	64.6	50.7	48.0	55.8	54.0	51.8	37.2	48.8	44.2	50.0	0.4	91.9	88.9	92.4
184	9.4	32.0	31.8	50.7	43.4	40.8	14.0	17.4	34.2	25.0	29.1		79.0	86.0	75.5
224	0	33.6	18.3	39.0	33.8	30.4	0	6.8	24.1	9.9	6.2		71.3	75.5	73.8
262		23.1	5.9	30.3	23.9	14.7		0	13.1	0	0		62.1	65.9	62.1
293		10.2	0	14.2	14.2	0			0				48.5	53.5	53.1

NTBE, Site 3, unamended

Day	0 ft microcosm			2 ft microcosm			4 ft microcosm			7 ft microcosm		10 ft microcosm		
	1	2	3	1	2	3	1	2	3	1	2	1	2	3
Wt.(g)	7.0	6.3	7.0	6.7	6.9	6.9	5.8	5.3	5.6	6.1	5.9	3.1	3.3	3.6
1	86.5	105.5	93.0	91.1	90.7	96.5	97.3	98.8	97.8	109.8	100.1	118.0	118.7	99.7
24	106.2	107.3	97.8	96.7	101.5	100.5	92.7	93.4	94.5	95.8	88.7	92.1	95.0	-
76	96.6	103.4	95.8	108.5	95.6	94.7	94.7	90.6	85.5	95.5	94.2	73.6	84.8	87.3
150	96.3	99.4	96.9	99.5	96.6	96.6	98.3	93.4	94.4	89.1	96.8	90.3	84.2	89.6
229	91.1	94.0	-	92.7	96.4	-	95.4	95.7	-	93.0	96.3	89.3	92.5	-
265	95.3	96.2	99.2	93.7	95.1	99.3	95.3	94.9	93.1	95.8	99.2	91.3	93.8	92.5

ETBE, Site 3, unamended

Day	0 ft microcosm		2 ft microcosm		4 ft microcosm		7 ft microcosm			10 ft microcosm			
	1	1	2	3	1	2	3	1	2	3	1	2	3
Wt.	7.4	6.5	7.3	6.9	5.8	5.8	6.1	6.3	6.2	7.0	4.3	3.8	3.5
1	94.9	92.9	90.1	90.6	88.4	86.8	87.1	107.7	105.6	106.7	98.9	94.0	92.5
37	93.5	86.6	69.9	83.4	78.6	76.0	75.6	102.4	98.4	82.6	57.7	65.0	65.2
64	97.0	83.7	51.1	81.6	77.7	73.7	72.7	110.2	90.8	82.0	55.7	62.5	67.3
145	88.0	78.4	21.4	77.9	66.7	73.1	73.2	98.0	92.6	62.5	49.3	61.9	60.8
175	90.8	84.4	12.1	83.6	79.0	73.7	71.2	100.8	89.2	65.7	58.3	63.7	68.9
223	96.2	88.5	1.5	89.4	65.8	77.4	73.9	105.5	83.8	66.5	54.9	62.8	68.3
296	97.2	86.8	0	87.9	62.5	73.9	76.7	106.2	74.0	68.5	55.3	65.9	63.9

## **Appendix B**

- . Degradation in Microcosms with Autoclaved Soils
- . Degradation in Microcosms with Non-autoclaved Soils
- . Aerobic Biodegradation of TBA
- . Aerobic Biodegradation of ETBE

Site 3-7, Sterilization effects, 1 time

Microcosm	MTBE									ETBE								
	1			2			3			1			2			3		
	Day	A	TBA	MTBE	A	TBA	MTBE	A	TBA	MTBE	A	TBA	ETBE	A	TBA	ETBE	A	TBA
0	0	0	86.2	0	0	87.5	0	0	88.1	0	0	122.9	0	0	107.2	0	0	121.8
15	6.2	10.4	52.6	7.1	12.1	50.4	6.6	11.7	60.8	6.1	22.8	60.0	6.7	22.5	65.7	5.3	21.6	69.1
22	7.4	10.9	49.8	7.6	13.4	48.8	-	-	-	7.6	23.3	58.7	4.5	18.8	61.7	5.7	18.4	64.3
28	5.9	10.8	34.7	2.7	12.8	40.9	1.0	9.7	54.2	5.4	22.9	47.6	6.2	25.3	60.1	5.7	23.6	59.9
41	9.3	16.4	27.1	4.3	17.4	33.6	3.7	15.9	44.9	4.7	27.3	30.5	8.9	31.6	41.7	7.8	29.9	42.2
55	9.3	17.1	16.4	5.4	16.8	21.6	5.0	16.8	29.3	9.2	35.6	21.0	13.7	36.8	24.7	7.3	33.6	26.5

Site 3-7, Sterilization effects, 3 times

Microcosm	MTBE									ETBE								
	1			2			3			1			2			3		
	Day	A	TBA	MTBE	A	TBA	MTBE	A	TBA	MTBE	A	TBA	ETBE	A	TBA	ETBE	A	TBA
0	0	0	92.6	0	0	98.2	0	0	89.3	0	0	116.8	0	0	117.3	0	0	108.9
12	6.8	14.0	53.8	6.8	14.0	53.8	6.7	12.9	52.8	7.0	30.6	59.9	5.5	24.3	68.3	5.7	25.3	66.8
19	7.3	12.7	56.5	9.7	17.1	55.5	3.5	10.5	49.1	7.7	31.8	52.0	6.8	27.7	66.5	6.1	26.1	65.8
25	5.4	11.5	40.7	7.2	15.6	39.8	6.8	14.2	38.4	6.6	30.3	37.1	5.1	24.6	49.9	4.6	22.4	43.2
38	7.9	14.1	26.3	11.1	20.4	27.7	10.2	17.6	25.4	10.2	39.2	30.1	8.0	30.5	37.7	3.7	12.6	34.7
52	12.0	17.5	22.2	13.8	18.7	14.8	13.9	16.9	13.0	12.9	40.3	16.7	12.9	35.8	25.6	12.9	36.8	23.4

Site 3-7, Sterilization effects, 5 times

Microcosm	MTBE									ETBE								
	1			2			3			1			2			3		
	Day	A	TBA	MTBE	A	TBA	MTBE	A	TBA	MTBE	A	TBA	ETBE	A	TBA	ETBE	A	TBA
0	0	0	84.3	0	0	80.0	0	0	87.1	0	0	102.1	0	0	91.7	0	0	110.9
9	4.6	7.1	57.5	4.0	6.2	55.6	4.1	8.2	59.1	2.2	10.9	59.6	2.1	10.6	56.3	2.9	15.6	59.1
16	4.5	7.6	46.1	7.9	12.5	44.6	3.2	8.6	46.1	5.6	16.8	41.7	4.9	17.6	39.9	7.3	23.4	33.1
22	6.3	11.6	33.5	3.4	11.8	34.9	5.3	9.8	37.7	5.8	23.7	34.8	5.4	20.5	33.7	5.3	19.8	21.1
35	6.4	13.2	23.0	4.1	17.6	23.9	7.2	11.9	24.0	8.0	32.0	24.0	8.7	23.8	19.3	9.7	23.4	15.1
49	8.1	15.5	13.5	4.8	18.3	15.8	7.5	14.1	17.5	11.0	24.4	8.9	6.2	25.0	11.4	10.6	19.0	3.8

TBA, autoclaved, H<sub>2</sub>O<sub>2</sub>, site 1(B)

Day	2'			5'			10'			14'						
	microcosm	1	2	microcosm	1	2	3	microcosm	1	2	3	microcosm	1	2	3	
A	TBA	A	TBA	A	TBA	A	TBA	A	TBA	A	TBA	A	TBA	A	TBA	
Ut.(g)	4.3	4.8	5.1	4.5	4.8	4.7	4.7	3.9	3.8	3.7	4.1	4.2	5.1			
0	113.2	0	106.1	0	115.4	0	107.6	0	90.9	0	100.2	0	98.9	0	114.1	0
13	5.5	81.6	6.1	74.3	6.8	68.7	8.1	84.0	7.6	78.6	7.5	77.4	8.3	84.2	7.6	71.9
19	11.8	67.6	12.1	61.2	14.0	59.4	12.0	71.0	12.4	69.2	13.4	67.9	3.9	78.8	3.3	70.9
28	6.8	68.1	6.8	58.7	8.0	56.3	9.3	62.9	10.6	59.1	8.7	56.5	5.3	75.5	4.5	67.7
41	8.7	49.2	10.2	50.1	12.7	44.5	3.8	59.9	14.4	50.8	11.3	50.6	6.9	74.2	6.2	68.3
54	13.1	47.0	12.8	45.9	15.0	38.1	15.2	53.1	17.3	45.1	14.1	49.3	6.2	68.8	5.4	59.8
68	13.7	40.4	13.1	39.9	8.5	28.4	13.4	40.6	15.1	30.8	15.0	42.5	7.4	62.8	7.5	59.1
86	15.8	32.4	14.5	30.6	15.9	22.8	14.7	32.6	20.2	28.0	16.1	31.3				

TBA, site 3, autoclaved soil+H<sub>2</sub>O<sub>2</sub>

Day	0 ft		2 ft		4 ft		7 ft		10 ft		
	microcosm	1	2	microcosm	1	2	microcosm	1	2	microcosm	
TBA	A	TBA	A	TBA	A	TBA	A	TBA	A	TBA	A
Ut.(g)	9.1	8.2	8.7	8.1	5.6	5.9	5.2	4.4	4.7		
1	179.9	0	145.5	0	143.4	0	152.3	0	113.2	0	111.9
10	158.2	5.5	152.0	6.0	148.1	6.8	142.1	5.1	106.8	6.9	99.1
18	143.2	0.9	138.5	1.3	139.4	2.1	136.4	0	97.1	8.9	89.3
25	144.9	0	141.3	0	145.2	0.9	136.7	0.3	95.2	8.6	85.7
31	142.8	135.6	135.6	140.5	0	136.4	0	91.9	7.6	81.1	0.6
59	135.8	132.2	131.1	119.8	0	81.8	2.4	73.9	67.5	6.0	100.4
86	124.3	121.8	118.0	104.6	56.5	5.0	64.3	64.2	0.2	96.7	4.0

MTBE, autoclaved, H<sub>2</sub>O<sub>2</sub>, site 1(B)

Day	2'			5'			10'			14'																								
	microcosm	1	2																															
1	A TBA MTBE																																	
5.1	5.4	5.7	7.5	6.8	5.5	5.5	5.5	5.5	5.5	5.1	5.1	5.7																						
0	0	94.4	0	105.7	0	91.4	1.0	102.6	0.8	94.9	0.4	0	94.4	0.4	0	90.1	0.5	0	109.1															
1	5.0	6.1	68.2	5.0	7.5	67.2	2.0	6.2	68.4	2.8	5.5	74.3	4.6	4.8	75.2	4.7	5.2	73.6	4.4	3.1	91.7	0	96.4	0	0.8	88.8	0	0	88.8	-	-	101.3		
13	6.3	10.3	41.7	6.9	12.8	47.2	6.0	11.1	48.8	7.8	10.7	45.2	7.6	9.3	46.6	9.4	13.0	50.6	5.0	4.8	68.5	5.4	4.1	77.6	0	1.6	84.5	0	1.6	79.9	0	1.6	86.4	
19	13.4	14.8	29.6	13.5	17.9	39.4	12.0	14.9	39.1	14.6	17.2	41.7	13.6	14.8	40.3	13.9	16.0	43.1	0	7.7	62.2	0	6.4	67.8	0	2.3	89.1	0	3.9	80.2	-	-	92.9	
28	3.9	10.4	27.4	4.3	13.3	38.7	3.5	13.2	38.1	5.1	11.8	27.8	5.8	11.3	35.3	5.7	12.5	35.2	2.7	6.1	56.6	2.1	4.5	64.5	0.7	1.3	83.3	0.9	1.2	80.9	0.6	1.4	88.1	
41	6.5	13.8	18.0	6.3	16.2	24.7	6.5	17.2	25.4	9.0	16.1	21.5	7.3	13.4	22.3	7.9	15.5	23.7	3.6	7.9	50.9	3.1	6.8	61.0	1.2	2.9	72.2	1.2	-	79.9	1.4	2.2	84.2	
54	8.2	15.0	15.4	7.3	16.7	18.8	8.0	20.8	22.1	9.8	15.3	15.4	-	-	-	9.8	16.4	17.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
68	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

pH

MTBE, site 3, auto-claved soils+ H<sub>2</sub>O<sub>2</sub>

Day	0 ft		2 ft		4 ft		7 ft		10 ft																
	microcosm	1	2	1	2	1	2	1	2	1	2														
1	0	140.5	0	119.4	0	143.1	0	137.9	0	104.2	0	57.2	0	87.5	0	48.0	0	0.9	105.3						
9	1.2	120.6	1.1	96.5	2.8	125.8	0	1.4	109.9	8.0	15.7	71.7	1.7	9.0	23.9	2.7	15.7	41.4	0	0.8	36.1	0	1.4	94.2	
19	1.0	114.4	0.9	94.3	1.5	113.7	0	1.5	101.7	8.9	21.6	54.4	0.6	10.2	20.7	2.7	15.7	35.5	-2.0	2.0	36.2	0	1.1	92.4	
25	1.0	119.1	-	-	1.1	117.2	1.4	2.5	101.8	9.4	23.2	44.5	0	11.2	19.4	0	17.8	34.9	1.4	1.9	37.4	1.4	1.9	92.0	
40	1.0	118.9	0.9	85.7	1.9	115.0	1.6	2.7	102.6	10.0	26.3	45.7	1.0	12.3	7.7	3.3	22.2	21.1	1.5	2.0	35.4	5.8	2.5	77.2	
75	0	111.4	0	81.8	2.7	102.8	4.3	95.2	18.2	30.2	25.1	-	-	-	-	-	-	-	-	-	-	-	-	-	
pH	7.0	7.0	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7

ETBE, autoclaved, H<sub>2</sub>O<sub>2</sub>, Site 1(B)

Day	2'			5'			10'			14'		
	microcosm	1	2									
A TBA ETBE												
5.1	5.6	5.6	6.2	7.5	7.3	5.7	5.7	5.5	4.8	5.6		
1	4.8	11.0	71.7	4.6	11.8	62.4	5.3	10.2	66.7	5.0	9.5	85.1
13	9.6	26.8	49.4	6.4	24.3	38.0	6.3	24.8	40.5	8.0	16.9	41.8
19	12.3	25.6	28.0	12.3	30.7	28.3	13.1	31.1	27.4	10.0	21.7	37.7
28	4.1	21.4	24.9	3.7	25.6	23.3	3.8	24.7	27.7	5.8	23.1	28.9
41	6.3	24.4	10.6	6.8	30.6	16.3	5.9	29.3	19.1	8.2	25.5	16.4
60	9.7	28.2	8.2	8.2	29.6	8.9	7.4	29.5	10.3	11.0	26.5	10.1
71												

13.2 25.7 8.5 14.7 19.8 3.1 13.0 23.5 5.8

pH

ETBE, Site 3, autoclaved, soltes H<sub>2</sub>O<sub>2</sub>

Day	0 ft		2 ft		4 ft		7 ft		10 ft	
	microcosm	1	microcosm	2	microcosm	1	microcosm	2	microcosm	1
ETBE	ETBE	TBA ETBE	A TBA ETBE							
8.6	7.3	7.4	5.6	5.7	6.2	5.3	3.2	5.5		
1	157.5	96.0	0	141.9	0	106.7	0	0	101.4	0
10	146.7	93.4	1.2	131.4	1.7	22.2	67.6	1.8	20.8	64.5
18	135.7	91.5	1.9	128.0	2.0	27.5	49.1	2.5	30.6	52.1
25	129.8	91.8	1.6	127.5	4.9	34.0	40.9	4.7	29.8	46.0
40	134.2	95.1	-	135.6	6.2	35.1	42.6	5.6	32.6	39.5
75	128.5	3.3	131.6	6.3	46.3	26.8	15.4	44.7	18.4	7.8

110.4 89.3 58.7 78.3 84.9 54.8 63.1 84.5 52.4 52.4 80.2 53.1 56.1 89.1 54.2 29.8 4.8 42.6 28.8 75.4 4.0 4.0 4.0 4.0 5.7 5.7

TBA, regular soils, H<sub>2</sub>O<sub>2</sub> ONLY, Site 1(B)

Day	2'			5'			10'			14'																		
	microcosm																											
1	2	3	1	2	3	1	2	3	1	2	3	1	2	3														
3	116.2	111.6	112.7	109.7	115.9	113.8	98.3	107.3	110.1	106.4	106.4	105.7	1	106.7	104.8	110.6	100.2	103.9	103.4	96.4	99.2	101.6	124.8	130.0	129.2	122.7	124.6	126.3
15	108.0	112.1	103.1	113.1	120.0	116.0	104.3	99.0	118.4	90.6	109.4	106.5	10	112.8	110.5	113.4	105.0	107.2	105.3	102.6	101.0	100.8	125.4	124.1	130.6	113.5	117.5	98.0
27	102.7	107.5	93.6	108.2	99.6	114.5	92.3	87.8	106.1	92.7	100.2	96.6	18	107.4	101.1	106.8	100.7	101.4	102.9	93.6	94.6	92.0	117.7	113.1	117.5	103.9	107.8	70.7
37	115.0	112.6	113.9	114.3	110.5	113.3	98.2	99.6	105.6	101.4	104.3	99.4	25	97.1	95.6	106.4	97.8	99.7	101.4	86.7	91.1	85.1	110.9	103.4	108.9	95.9	105.1	53.8
47	111.3	107.5	110.5	112.4	110.0	109.2	94.8	100.7	105.5	98.0	96.9	92.1	32	90.1	85.1	99.0	95.3	93.6	95.4	79.4	83.7	82.8	105.6	90.9	99.7	99.2	104.2	55.2
60	106.4	108.8	107.3	107.0	110.0	94.0	97.8	97.4	106.4	100.3	90.3	90.9	39	82.4	74.7	89.9	93.5	89.3	88.2	74.7	79.4	77.4	98.0	77.9	87.8	98.4	103.5	33.4
75	80.9	97.7	66.6	76.3	80.2	47.1	78.2	99.0	99.5	67.4	97.5	70.3	52	66.0	52.0	69.5	87.1	69.4	68.3	64.0	60.0	44.3	87.4	20.4	41.1	95.1	97.6	17.8
92	-	61.8	29.5	50.6	53.1	24.9	62.5	88.6	76.4	68.1	42.4	67	54.2	34.0	48.8	76.8	52.1	52.9	55.2	31.3	11.8	79.5	1.5	2.8	92.4	89.4	19.1	
112	-	29.5	1.0	31.9	14.7	2.7	47.0	6.8	47.0	6.8	47.0	6.8	82	37.3	21.8	28.5	60.5	30.4	30.0	42.8	2.3	0	69.5	0	84.7	84.7	18.1	
													94	29.0	3.5	6.6	54.7	11.4	9.3	37.0	0	61.4						10.8

TBA, Site 3, H<sub>2</sub>O<sub>2</sub>

Day	0 ft			2 ft			4 ft			7 ft			10 ft		
	microcosm														
1	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	7.1	6.7	6.2	6.5	6.4	6.5	5.7	6.2	5.8	5.1	5.9	5.4	3.3	3.3	3.5

TBA, regular soils, H<sub>2</sub>O<sub>2</sub>+nutrients, Site 1(B)

Day	2'			5'			10'			14'																		
	microcosm																											
1	2	3	1	2	3	1	2	3	1	2	3	1	2	3														
3	130.1	127.9	125.2	130.1	130.1	132.4	128.2	117.0	118.9	117.3	121.2	132.4	10	105.7	104.7	109.0	105.3	105.0	104.7	92.5	92.1	87.5	113.6	119.9	114.2	97.6	107.5	105.5
15	115.6	134.7	119.9	126.1	137.5	127.0	114.8	121.7	122.8	115.1	112.7	110.8	18	95.6	97.0	96.4	99.9	100.8	100.9	78.5	84.4	76.3	97.9	107.6	107.3	75.4	97.0	93.4
27	116.4	121.3	116.9	116.5	129.1	119.4	113.9	118.0	105.1	99.0	102.3	119.0	25	86.7	94.7	92.9	97.0	99.0	99.9	71.2	77.3	70.5	87.9	99.0	102.6	59.3	89.9	83.7
37	127.4	121.1	133.7	130.4	126.3	129.0	118.4	117.3	118.5	114.4	114.5	123.4	32	90.6	91.0	88.3	96.5	96.7	95.9	71.4	75.7	69.2	88.0	100.1	101.6	66.2	90.0	86.1
47	121.1	117.9	121.8	130.4	126.3	129.0	113.3	111.9	110.9	113.3	110.3	113.7	39	87.1	85.0	80.0	91.8	93.3	88.4	71.3	66.6	60.8	84.1	96.2	95.8	65.2	78.0	80.3
60	116.2	81.9	99.9	129.4	126.7	119.3	118.5	112.6	113.5	103.8	81.8	87.4	52	53.0	71.0	62.5	87.3	78.9	65.6	66.3	56.8	44.1	76.4	75.1	87.2	67.1	62.7	69.2
78	76.2	79.9	14.7	106.1	115.2	76.6	114.3	109.8	109.6	74.6	59.3	64.1	67	16.2	35.2	37.9	74.4	61.6	20.6	55.2	41.1	35.4	43.1	60.2	60.4	65.2	58.1	63.3
92	20.8	2.0	0.6	65.7	85.9	61.2	111.8	96.6	107.8	34.8	44.9	46.9	82	5.3	8.0	11.5	29.5	58.1	21.6	41.8	24.8	8.2	15.5	28.3	13.7	65.7	49.9	55.3
112				4.1	47.2	35.6	112.4	75.3	97.1	20.1	19.8	12.8		1.3	3.8	5.7	16.0	22.9	10.6	33.2	9.2	3.2	0	2.8	0	68.0	41.5	50.8

TBA, Site 3, H<sub>2</sub>O<sub>2</sub>+Nutrients

Day	0 ft			2 ft			4 ft			7 ft			10 ft		
	microcosm														
1	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
1	7.1	6.7	6.6	6.6	6.6	6.8	6.6	6.6	6.3	6.3	6.6	7.0	6.0	5.9	4.4



MTBE, Site 3, H<sub>2</sub>O<sub>2</sub> + nutrients

Day	0 ft			2 ft			4 ft			7 ft			10 ft		
	microcosm														
TBA MTBE TBA MTBE TBA MTBE															
1	7.6	7.0	7.0	6.7	7.0	6.4	5.8	5.0	6.0	6.4	5.5	3.5	3.7	3.5	
10	106.9	0	107.4	0	101.1	0	91.3	0	98.0	0	106.4	0	105.6	0	
10	3.5	82.7	4.8	106.3	3.5	110.1	2.2	108.9	1.7	108.2	1.5	107.2	4.5	97.7	
18	6.3	77.4	6.4	99.9	7.3	99.7	3.2	105.8	2.9	103.8	2.6	103.7	7.7	89.4	
24	7.2	72.3	7.2	96.4	9.4	96.2	3.7	102.0	3.8	102.7	3.3	102.8	8.8	85.0	
33	7.5	75.0	7.8	99.3	10.3	93.4	3.4	106.1	3.6	100.3	3.9	101.7	5.1	86.2	
41	7.8	75.2	8.7	93.6	12.7	87.7	3.3	105.3	4.1	100.2	4.5	98.9	7.3	86.1	
53	2.2	72.6	9.4	88.0	14.7	76.4	2.1	107.8	3.7	98.3	7.2	94.6	10.4	82.7	
68	0	87.0	5.6	93.1	7.3	63.5	0	105.6	1.9	95.9	6.2	90.2	5.5	84.1	
76	81.0	5.3	92.8	-	100.9	-	7.5	94.0	3.9	84.1	15.2	56.9	16.0	55.0	
90	82.7	2.9	90.9	-	100.9	-	7.5	94.7	1.3	89.7	8.2	53.8	16.6	57.7	

pH

6.7	6.7	6.7	6.5	6.5	6.5	5.5	5.5	5.7	5.5	5.3	5.3	6.5	6.5	6.5
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ETBE, Site 3, H<sub>2</sub>O<sub>2</sub> + nutrients

Day	0 ft			2 ft			4 ft			7 ft			10 ft		
	microcosm														
TBA ETBE TBA ETBE TBA ETBE															
1	8.1	7.0	8.1	6.7	7.0	6.1	6.6	6.2	5.8	5.2	5.6	3.5	3.8	3.8	
1	109.8	1.0	110.7	1.0	96.6	1.0	100.4	1.0	101.6	6.3	80.3	5.6	74.9	5.7	
9	0	105.4	1.7	105.7	1.5	92.5	1.5	94.4	1.5	96.8	2.5	89.1	1.1	92.7	
17	8.3	90.6	8.7	90.3	2.1	88.2	2.2	90.5	2.3	91.5	9.9	75.6	9.0	75.7	
23	15.7	80.2	15.3	81.3	3.3	85.4	4.0	88.9	4.4	88.1	14.6	68.2	15.4	64.2	
30	15.8	83.9	15.2	81.8	3.1	85.4	3.9	88.9	4.3	90.2	15.8	68.9	16.1	69.8	
37	15.4	81.5	21.4	72.1	1.1	92.0	5.3	89.9	6.0	88.9	16.2	69.6	23.3	59.1	
57	11.0	85.7	28.0	58.9	95.0	6.6	81.1	8.2	80.7	16.6	68.0	32.9	39.9	40.1	
64	2.1	84.2	17.8	42.8	90.1	2.4	81.3	5.8	74.2	12.1	69.8	26.5	32.9	33.9	
71	81.2	16.5	44.9	89.7	2.8	72.5	6.8	67.1	9.9	61.2	29.1	26.9	35.6	12.7	
85	82.6	6.2	37.9	91.0	3.7	66.8	6.5	67.9	23.8	13.0	17.3	6.4	2.1	55.1	

pH

7.0	7.0	6.3	6.3	6.1	5.5	5.5	5.5	4.7	4.7	4.7	4.7	6.7	6.7	6.7
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ETBE, regular soils, H<sub>2</sub>O<sub>2</sub> ONLY, Site 1(B)

Day	2'			5'		10'		14'	
	microcosm			microcosm		microcosm		microcosm	
	1	2	3	1	1	1	2	1	2
	ETBE	ETBE	ETBE	ETBE	ETBE	TBA	ETBE	TBA	ETBE
Wt.(g)	4.1	4.3	4.3	4.0	4.5	4.6	4.7		
3	84.5	85.1	73.1	96.5	95.1	0.5	104.5	0.3	97.1
15	82.8	56.5	75.3	88.5	94.6	1.4	91.4	1.3	87.2
27	86.4	58.7	75.7	74.4	81.2	3.0	87.0	4.0	88.9
37	75.4	60.6	66.8	74.5	76.5	4.1	75.5	3.8	76.3
47	84.8	63.2	70.7	88.8	79.5	4.0	73.0	4.0	78.3
60	71.0	61.7	73.3	82.6	46.2	5.7	66.5	5.2	63.1
75	73.5	58.8	-	73.7	49.1	5.1	57.4	6.4	65.9
92	68.2	50.5	65.5	64.2		6.1	53.7	6.2	61.8
112	63.1	47.2	61.2	68.2		2.6	59.9		

ETBE, regular soils, H<sub>2</sub>O<sub>2</sub>+nutrients, Site 1(B)

Day	2'			5'			10'		14'			
	microcosm			microcosm			microcosm		microcosm			
	1	2	3	1	2	3	1	2	1	2		
	ETBE	ETBE	ETBE	ETBE	ETBE	ETBE	ETBE	ETBE	TBA	ETBE	TBA	ETBE
Wt.(g)	4.3	4.7	4.5	4.0	4.3	4.7	4.2	4.1	4.8	4.6		
3	95.1	94.3	94.3	92.2	90.3	82.5	99.3	87.0	0.5	98.8	0.5	95.5
15	82.0	91.5	93.5	98.5	94.4	84.4	92.6	93.5	1.4	88.3	0.4	90.5
27	72.7	84.6	88.4	82.0	86.5	79.7	70.7	83.2	5.5	81.0	1.0	82.9
37	78.8	85.8	85.7	83.4	80.6	71.2	65.9	83.2	3.2	72.1	2.8	78.7
47	72.4	84.4	86.0	74.1	79.3	72.3	67.5	86.2	3.6	84.5	0.2	79.8
60	73.1	80.4	86.2	-	72.7	74.0	-	75.6	4.8	77.3	4.3	77.2
75	73.5	-	81.8	73.3	-	-	67.5	72.9	7.6	70.0	5.7	74.8
92	71.1	66.2	80.6	72.6	74.0	78.4	64.6	86.5	7.7	66.3	5.6	71.0
112	82.4	70.7	65.3	68.0	70.0	81.2						

Site 3-3, aerobic degradation, sacrificial microcosms with autoclaved soils

Day	TBA				MTBE				ETBE								
	1		2		1		2		1		2						
	A	TBA	A	TBA	Day	A	TBA	MTBE	A	TBA	MTBE	A	TBA	ETBE	A	TBA	ETBE
0	0.1	145.0	0.6	146.0	0	0	0	84.7	0	0	101.3	0	0	80.5	0	0	64.9
3	1.0	129.6	0.8	141.9	3	0.3	0.4	80.5	0.4	0.6	76.3	0.4	1.6	60.3	0.3	1.2	45.9
14	0.3	131.4	0.1	114.3	10	0.5	0.6	85.2	0.7	1.5	64.8	0.2	4.1	60.2	0.4	2.1	56.5
23	0	131.7	0.2	134.4	29	0.8	1.7	87.0	0.2	2.5	78.9	0.2	5.8	64.6	0.4	4.7	57.9
35	0	135.3	0	128.2	41	0.2	7.1	78.6	0.1	3.5	81.2	0.3	4.5	58.5	0.1	3.3	64.3
72	0	132.9	0	130.8	61	0	3.4	79.5	0	5.4	77.8	0	0.1	55.0	0	0.1	55.1
					73	0	5.4	76.2	1.3	3.7	78.5	0	0	60.4			
					110	0.8	6.3	77.5	-	-	0	0	0	62.4			

Site 3-3, aerobic degradation, nutrient-amended sacrificial microcosms with non-autoclaved soils

Day	TBA				MTBE				ETBE								
	1		2		1		2		1		2						
	A	TBA	A	TBA	Day	A	TBA	MTBE	A	TBA	MTBE	A	TBA	ETBE	A	TBA	ETBE
0	0.3	119.1	0.3	124.5	0	0.4	0.7	141.9	0.3	0.5	94.6	0.3	1.3	76.2	0.4	1.1	67.6
3	0.4	113.5	0.3	115.8	3	0.6	2.1	131.2	0.6	2.0	79.5	0.4	2.6	52.1	0.6	2.8	58.9
14	0.1	114.3	0.1	105.7	10	0.2	2.4	131.1	0.2	1.8	88.9	0.3	5.9	57.8	0.2	2.6	56.6
23	0	110.8	0	106.3	29	0	3.3	123.8	0.4	3.3	90.0	0.4	5.3	54.9	0.2	5.0	54.2
35		107.7	0	95.5	41	0.3	4.1	120.0	0.4	2.3	88.7	0.5	3.2	61.2	0.3	3.1	56.5
72		96.9	0	80.2	61	0	3.8	130.4	0	0.1	90.6	0	0	59.1	0	0	50.1
					73	0	0.3	141.8	0	0	93.4			56.6			58.3
					110			133.5			99.7			56.8			57.9

Site 3-3, MTBE aerobic degradation, nutrient-amended, pre-oxidized Site 3-3 soils

Day	w/o nutrients microcosm									w/ nutrients microcosm										
	1			2			1			2			3							
	A	TBA	ETBE	A	TBA	ETBE	A	TBA	ETBE	A	TBA	ETBE	A	TBA	ETBE					
1-b	0.1	0.3	66.1	0.1	0.3	50.3	0.3	0.8	84.1	0.4	0.5	83.4	0.2	0.5	62.4	0.1	0.4	75.4		
1-a	-	-	-	-	-	-	-	-	-	0.7	0.6	72.1	0.7	0.6	57.4	0.7	0.4	68.1		
4	0.2	0.6	56.3	0.3	1.2	39.0	0.9	1.6	68.8	0.7	1.2	63.5	0.7	1.1	49.3	0.2	0.6	62.1		
8	0.4	0.9	61.2	0.4	1.8	41.3	0.6	2.9	71.6	0.6	1.9	67.7	0.3	1.8	52.9	0.3	0.8	63.9		
11-b	0.5	0.8	60.6	0	1.8	41.7	0.7	3.3	69.7	-	-	-	2.2	64.7	2.0	50.6	1.0	62.9		
11-a	-	-	-	-	-	-	-	-	-	0.2	2.3	63.9	0.1	2.1	45.6	-	-	1.1	62.2	
12	0.2	1.0	57.0	0.4	2.2	35.5	0.5	3.8	65.6	0.3	2.5	64.7	0.3	2.2	47.8	0.3	1.1	60.3		
13	0.2	1.0	56.9	0.5	2.3	36.3	0.6	3.8	64.9	0.2	2.6	64.4	0.2	2.3	48.5	0.5	1.2	63.2		
18	0.1	1.1	58.9	0	2.9	37.4	0.1	4.5	66.1	-	-	-	3.1	64.1	0.1	2.6	47.2	0.1	1.4	62.2
20-b	0	1.1	56.4	0	2.9	36.2	0	4.5	63.4	-	-	-	3.2	63.8	2.7	45.9	1.5	60.8		
20-a	-	-	-	-	-	-	-	-	-	3.2	60.8	-	2.9	46.8	-	-	1.7	59.7		
21	0.2	1.2	54.1	0.3	3.1	34.1	0.3	4.7	60.7	4.9	60.0	-	3.1	46.8	-	-	1.6	60.8		
30-b	0.6	1.4	58.2	0	3.8	33.7	-	6.0	61.9	4.0	63.6	-	3.8	44.7	-	-	2.0	60.5		
30-a	-	-	-	-	-	-	-	-	-	3.8	56.8	-	4.0	45.0	-	-	2.2	60.3		
45	-	-	-	-	0.1	8.5	48.0	-	-	5.4	51.2	-	5.6	35.9	-	-	3.4	53.0		
50-b	1.6	47.2	-	5.4	27.9	-	9.1	48.9	-	6.1	53.6	-	6.2	36.7	-	-	3.5	46.5		
50-a	-	-	-	-	-	-	-	-	-	6.1	52.8	-	6.0	36.2	-	-	3.5	48.8		
53	1.6	47.2	-	5.8	25.2	-	9.9	45.4	-	6.7	50.8	-	6.7	34.9	-	-	3.8	45.9		
58-b	4.7	46.1	-	6.3	24.6	-	10.0	40.7	-	6.8	51.2	-	7.0	34.9	-	-	4.0	41.9		
58-a	-	-	-	-	-	-	-	-	-	6.7	51.2	-	6.4	35.0	-	-	3.6	45.2		
59	-	-	-	-	-	-	-	-	-	6.7	50.3	-	6.7	35.8	-	-	3.7	45.8		
62	1.4	46.2	-	5.5	25.2	-	9.8	36.2	-	7.0	50.2	-	6.9	34.2	-	-	3.6	43.6		
67-b	1.5	42.1	-	-	-	-	-	-	-	7.3	47.8	-	7.3	32.9	-	-	3.8	37.3		
67-a	-	-	-	-	-	-	-	-	-	7.3	47.7	-	7.1	32.3	-	-	3.9	38.9		
77-b	0.4	44.4	-	-	-	-	-	-	-	8.3	50.9	-	9.3	39.4	-	-	4.1	34.2		
77-a	-	-	-	-	-	-	-	-	-	7.7	47.2	-	7.9	33.9	-	-	4.4	33.2		
85-b	-	-	-	-	-	-	-	-	-	8.4	42.4	-	8.2	25.7	-	-	4.5	28.7		
85-a	-	-	-	-	-	-	-	-	-	7.8	71.8*	-	6.7	62.7*	-	-	42.2	56.3* <sup>1</sup>		
86	-	-	-	-	-	-	-	-	-	8.0	69.4	-	6.9	57.3	-	-	41.4	53.9		
91	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	41.5	53.5		
119	-	-	-	-	-	-	-	-	-	10.5	61.5	-	-	-	-	-	40.3	50.8		

-: not measured due to sampling difficulty

\*: redosed with MTBE

1: added 1ml active ETBE-degrading slurry

1-b: day 1, before H<sub>2</sub>O<sub>2</sub> addition

1-a: day 1, after H<sub>2</sub>O<sub>2</sub> addition

Site 3-3, ETBE aerobic degradation, nutrient-amended, pre-oxidized Site 3-3 soils

Day	w/o nutrients microcosm						w/ nutrients microcosm								
	1			2			1			2			3		
	A	TBA	ETBE	A	TBA	ETBE	A	TBA	ETBE	A	TBA	ETBE	A	TBA	ETBE
1-b	0.6	2.1	66.4	0.5	0.4	99.2	0.4	0.5	95.6	0.5	0.5	83.4	0.2	0.6	107.2
1-a	-	-	-	-	-	-	0.6	0.7	86.0	0.7	0.7	74.5	0.3	0.8	94.2
4	0.1	3.3	60.2	0.1	1.3	93.9	0.5	1.4	89.4	0.2	1.4	74.0	0.5	2.5	91.5
8	0	3.8	59.4	0	1.5	85.7	0	1.6	86.2	0	1.7	71.4	0	3.1	85.8
11-b	0.2	3.9	57.8	0	1.6	87.5	0	1.7	81.8	0	1.8	70.1	0	3.3	87.7
11-a	-	-	-	-	-	-	2.0	78.2	2.1	67.1	4.1	80.6	-	-	-
12	0.4	5.8	49.8	0.2	2.3	86.1	0.2	2.1	75.9	0.3	2.4	69.1	0.3	4.8	80.8
13	0.8	6.3	51.1	0.5	2.4	86.4	0.3	2.4	81.7	0.5	2.4	69.1	0.3	4.8	80.8
18	0.1	6.9	50.0	0.1	2.6	86.3	0.1	2.6	79.2	0.2	3.2	70.6	0.1	5.9	82.8
20-b	-	7.1	49.6	-	2.6	83.0	-	2.7	75.5	-	3.2	66.7	-	2.7	83.7
20-a	-	-	-	-	-	-	0.3	2.9	72.8	0.6	3.4	64.7	0.8	6.8	73.6
21	0.4	8.2	42.5	0.1	3.2	77.0	-	3.2	72.9	-	3.7	63.0	-	-	-
30-b	-	9.9	43.1	-	3.7	64.6	-	3.8	74.8	-	4.4	63.9	-	9.9	68.1
30-a	-	-	-	-	-	-	-	3.9	71.6	-	4.9	63.2	-	11.2	68.4
45	-	15.9	28.2	-	3.2	40.8	-	6.6	64.5	-	7.4	55.9	-	55.2	11.3
50-b	-	16.3	26.5	-	5.6	41.3	-	6.8	65.1	-	7.4	53.9	-	61.8	1.2
50-a	-	-	-	-	-	-	-	7.0	61.3	-	7.7	54.7	-	56.9	217.7*
53	-	17.5	22.0	-	6.9	40.0	-	7.8	56.5	-	8.5	50.8	-	67.4	206.2
58-b	-	17.9	20.5	-	5.9	38.4	-	7.9	45.0	-	8.1	13.1	-	108.0	142.5
58-a	-	7.0	39.5*	-	5.9	38.4	-	7.3	39.4	-	7.3	31.4*	-	108.3	139.4
59	-	-	-	-	-	-	-	7.1	39.6	-	7.2	31.3	-	117.5	140.4
62	-	18.0	22.8	-	6.2	40.8	-	7.4	40.7	-	7.6	31.1	-	148.2	97.6
67-b	-	18.5	22.5	-	5.8	38.7	-	7.1	37.1	-	7.6	29.4	-	175.3	56.2
67-a	-	-	-	-	-	-	-	7.9	40.7	-	-	-	-	173.7	58.4
77-b	-	20.7	18.8	-	-	-	-	7.9	25.6	-	-	-	-	166.9	0.9
77-a	-	-	-	-	-	-	-	7.7	29.6	-	8.0	22.5	-	212.5	66.3*
85-b	-	20.0	19.5*	-	-	-	-	8.7	26.7	-	8.9	4.6*	-	248.9	3.5
85-a	-	72.3	34.8 <sup>1a</sup>	-	-	-	-	55.5	67.5 <sup>1a</sup>	-	8.1	46.8	-	86.5	50.1 <sup>2</sup>
91	-	84.6	1.6	-	-	-	-	76.7	33.4	-	9.7	40.0	-	126.2	1.3
95	-	-	-	-	-	-	-	90.2	11.0*	-	14.0	35.0	-	127.0	0.04
105	-	-	-	-	-	-	-	91.0	34.6	-	-	-	-	11.9	39.7*
119	-	-	-	-	-	-	-	108.5	0.8	-	38.2	0	-	134.3	0

--: no: measured due to sampling difficulty

\*: redosed with ETBE

1: added 1ml active ETBE-degrading slurry

2: withdrew 3 ml soil slurry, refilled microcosms with nutrient solution.

1-b: day 1, before H<sub>2</sub>O<sub>2</sub> addition

1-a: day 1, after H<sub>2</sub>O<sub>2</sub> addition

## **Appendix C**

### **. Degradation under Denitrifying Conditions**

TBA, nitrate, site 1(A)

TBA, nitrate, site 1(A)

Day	Nitrate only					Nitrate+nutrients					Nitrate+ethanol					Nitrate+ethanol+nutrients									
	2'	5'	10'	2'	5'	10'	Day	2'	5'	10'	Day	2'	5'	10'	Day	2'	5'	10'	Day	2'	5'	10'			
	TBA	ETOH	TBA	ETOH	TBA	ETOH	TBA	ETOH	TBA	ETOH	TBA	ETOH	TBA	ETOH	TBA	ETOH	TBA	ETOH	TBA	ETOH	TBA	ETOH	TBA	ETOH	
wt.(g)	12.4	11.0	14.2	12.6	10.1	15.6		11.1	10.2	14.7		14.1	11.5	14.4											
1	108.9	102.7	111.5	106.3	101.5	110.6	1	108.3	85.2	99.2	85.7	105.9	94.5	89.7	90.5	86.8	89.0	93.8	97.0						
11	106.3	104.4	108.2	102.8	98.3	106.5	11	106.0	9.4	101.5	68.4	107.6	71.5	80.9	0	78.8	0	85.0	0						
24	103.0	96.4	102.4	101.5	93.8	106.3	24	101.5	0	98.2	39.9	102.5	55.7	83.4		77.8		84.0							
59	75.9	76.2	81.4	67.4	86.3	92.7	59	96.6		94.5	13.6	92.8	12.6	76.2		65.0		83.0							
90	77.7	72.5	71.1	3.2	2.2	81.2	90	99.9		96.9	0	81.2	0	56.8		51.3		79.2							
126	58.4	49.6	52.0	0	0	59.7	126	86.2		91.2		89.5		25.1		25.2		77.2							
154	41.4	31.7	33.9			38.8	154	71.4		93.4		93.9		3.4		8.8		79.5							
183	17.9	12.7	13.0			0	183	61.0		90.4		88.3		0		0		78.4							

TBA, Nitrate+nutrients, Site 1(B)

TBA, Nitrate+nutrients+polydate, Site 1(B)

Day	2'			5'			10'			14'			10'			14'									
	microcosm																								
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3							
wt.(g)	4.1	4.2	4.3	4.3	4.4	3.8	3.7	3.3	3.9	3.8	3.8	3.8	4.1	3.5	4.3	4.7	5.2	5.0	3.4	3.3	3.6	4.0	3.7	4.4	
1	108.1	110.8	107.0	107.3	108.3	108.0	104.6	102.1	103.3	105.1	1	102.1	97.1	96.6	95.3	102.0	100.1	97.4	97.0	96.7	98.0	96.7	93.0		
35	112.5	111.9	110.7	110.5	113.5	116.8	102.5	107.1	103.9	110.5	35	108.6	91.3	111.5	112.0	106.9	91.9	106.6	97.5	97.4	108.1	110.4	104.1		
84	86.0	86.0	62.4	79.4	98.2	98.2	80.0	83.6	57.7	78.2	84	78.8	57.5	67.6	72.9	68.7	16.2	80.2	81.0	70.5	66.0	64.9	66.8		
127	31.7	64.3	10.0	14.4	38.6	79.1	59.0	34.4	4.6	29.3	127	51.7	8.7	28.2	37.8	52.7	2.6	38.6	29.7	38.6	13.7	8.3	29.7		
160	7.0	39.0	4.0	3.2	3.2	63.1	46.5	3.8	1.1	2.6	160	26.2	2.1	4.5	8.5	40.3	2.1	10.3	3.4	23.5	1.7	1.2	4.3		
207	0	1.2	0	0	0	44.9	1.6	0	0	0	207	0	0	0	0	21.2	0	0	0	0	0	0	0		

TBA, Site 2, nitrate amended

TBA, Site 2, Nitrate-nutrients

Day	0 ft			5 ft			10 ft			13 ft			0 ft			5 ft			10 ft			13 ft			
	microcosm	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	6.3	5.6	5.6	7.5	7.0	6.0	4.5	5.8	6.0	5.6	5.5	5.5	4.5	4.3	4.8	4.7	5.1	5.2	4.1	4.5	5.2	5.0	5.5	5.6	
1	120.9	120.1	125.5	116.2	103.3	102.8	114.3	119.2	102.9	106.8	107.1	113.1	1	102.8	109.9	107.8	97.0	74.8	90.3	100.4	107.0	101.5	102.0	103.4	102.1
39	107.2	102.3	112.7	72.9	71.8	80.7	84.2	100.0	88.7	95.3	97.9	98.9	30	102.8	109.4	103.7	91.2	69.3	79.7	87.9	95.1	94.4	94.5	93.5	87.5
80	96.5	87.5	106.4	40.7	36.3	52.4	78.3	89.2	77.6	74.6	83.0	78.8	60	85.4	88.5	89.2	80.9	66.7	66.3	75.2	91.6	82.0	85.7	85.5	90.1
104	103.2	92.5	112.0	7.0	0	14.9	66.3	90.1	74.4	60.2	77.4	63.4	96	72.6	86.9	89.3	72.5	48.6	53.2	78.0	78.1	65.8	67.1	72.6	68.7
151	108.7	91.3	106.0	0	0	4.1	31.7	66.0	34.3	8.7	30.9	8.7	120	64.0	89.2	90.0	70.0	35.4	45.9	70.4	72.7	54.2	54.0	59.4	42.1
240	106.7	101.3	106.7	0	0	0	0	0	0	0	0	0	160	59.2	87.1	85.5	52.9	8.9	34.0	51.5	58.1	39.3	35.4	42.4	32.8
600	113.1	97.3	119.9										195	-	-	-	21.3	0.2	36.4	29.0	49.3	27.6	3.9	28.1	7.8

TBA, Site 3, nitrate

Nitrate-nutrients

Day	0 ft			2 ft			4 ft			7 ft			10 ft			0 ft			2 ft			4 ft			7 ft			10 ft		
	microcosm	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3		
Wt.(g)	7.7	8.1	7.4	7.5	5.6	6.2	5.7	5.8	7.4	6.0	3.9	3.8	3.3	7.5	7.8	8.5	7.5	8.0	9.0	6.1	6.4	6.5	5.8	6.1	3.2	3.4	3.0			
1	127.6	127.8	127.9	126.7	119.0	127.4	125.9	123.5	127.7	103.9	100.0	110.9	110.1	123.6	127.7	128.4	129.1	127.8	125.1	124.4	124.6	130.9	123.3	128.9	104.6	106.9	107.3	121.0	118.3	
40	126.0	124.8	126.7	111.7	104.6	122.0	102.9	99.7	101.1	86.9	82.4	83.0	87.8	88.9	96.9	116.9	107.8	122.1	117.9	117.6	115.5	98.7	110.0	82.2	60.5	55.6	69.5	101.2	93.2	98.7
80	126.2	100.9	115.1	97.5	76.9	110.8	102.4	81.8	76.3	67.4	49.8	84.4	86.9	91.5	85.5	90.4	82.7	103.0	91.9	110.6	92.5	107.5	35.6	0.5	1.3	0.1	93.5	77.4	85.4	
119	128.2	103.3	110.1	77.7	50.4	110.1	93.0	95.0	67.3	44.9	9.3	33.4	79.9	69.3	85.4	72.1	77.0	21.6	99.4	58.7	95.9	78.6	94.9	18.4	0	85.7	59.2	65.3		
161	114.6	74.4	90.0	63.6	10.4	106.8	92.6	83.9	43.7	24.9	0.3	0	80.6	55.8	63.0	46.5	58.0	2.7	81.1	31.7	90.8	61.1	81.2	3.4	0	94.3	46.8	45.7		
201	98.5	44.4	70.8	46.4	1.3	97.0	95.9	85.0	25.0	1.9	0	0	72.4	35.8	51.8	19.7	37.7	0	67.0	9.4	83.3	20.5	70.7	0	0	96.5	32.7	32.6		
239	67.9	9.1	55.9	32.6	0	85.7	92.2	81.2	4.3	0	0	0	62.6	13.1	35.8	3.7	13.7	0	46.5	0	66.6	1.0	68.6	0	0	91.9	21.7	31.7		
270	34.7	0	40.0	21.2	0	68.3	71.5	77.0	0	0	0	0	53.5	0	26.6	0	0	28.1	0	44.4	0	60.0	0	0	0	87.0	15.4	23.1		

ETBE, nitrate, Site 1(A)

Day	Nitrate only			Nitrate+nutrients		
	2'	5'	10'	2'	5'	10'
wt.(g)	10.6	10.5	14.0	9.6	10.0	13.8
1	80.6	81.8	81.8	81.3	75.4	73.2
24	64.5	69.8	73.0	50.6	57.7	64.7
53	40.5	63.9	62.0	37.1	52.1	59.4
94	25.8	52.1	67.1	17.1	28.1	26.7
130	7.5	3.8	33.2	0	0	0

ETBE, nitrate, Site 1(A)

Day	Nitrate+ethanol						Nitrate+ethanol+nutrients					
	2'		5'		10'		2'		5'		10'	
	MTBE	EtOH	MTBE	EtOH	MTBE	EtOH	MTBE	EtOH	MTBE	EtOH	MTBE	EtOH
wt.(g)	10.7		11.2		14.9		13.4		12.7		16.2	
1	78.4	90.4	76.7	96.7	76.9	96.9	63.5	91.9	73.4	91.6	76.1	98.3
24	69.7	0	67.3	51.6	65.5	24.3	56.4	0	66.3	0	65.8	0
53	62.1	0	-	-	59.0	0	47.1	-	61.7	-	60.6	-
94	60.8		-	-	-		47.7	-	67.4	-	49.7	-
130	55.0		-	-	-		41.1	-	57.3	-	15.0	-
158	50.6		-	-	-		11.1	-	62.8	-	5.2	-
189	51.6		-	-	-		11.0	-	-	-	-	-

MTBE, nitrate, Site 1(A)

Day	Nitrate only			Nitrate+nutrients		
	2'	5'	10'	2'	5'	10'
wt.(g)	12.6	9.2	13.8	9.7	10.7	15.6
1	104.5	96.6	104.3	98.5	97.0	98.7
11	91.1	85.0	97.7	90.1	94.5	91.0
24	54.0	92.8	95.9	92.5	83.2	89.4
59	-	88.3	89.6	90.1	79.6	88.0
90	-	91.9	52.6	87.3	75.5	80.9
126	-	83.2	49.4	83.2	70.5	82.2
154	-	85.5	55.9	82.0	69.9	82.4
183	-	79.2	53.8	77.6	60.0	76.2

MTBE, nitrate, Site 1(A)

Day	Nitrate+ethanol						Nitrate+ethanol+nutrients					
	2'		5'		10'		2'		5'		10'	
	MTBE	EtOH	MTBE	EtOH	MTBE	EtOH	MTBE	EtOH	MTBE	EtOH	MTBE	EtOH
wt.(g)	10.7		10.4		14.4		10.3		11.6		13.2	
1	96.2	90.6	97.8	92.5	98.8	92.6	94.2	91.2	92.0	92.1	96.8	93.1
11	91.9	43.0	84.8	53.7	95.8	64.6	91.3	0	83.6	0	87.4	0
24	90.5	0	81.0	0	93.4	56.3	87.0	-	81.2	-	85.6	-
59	86.9		37.9		87.5	44.7	76.9		80.6		83.3	
90	87.4		38.9		92.2	6.7	64.1		78.5		75.4	
126	83.6		33.9		84.0		60.5		72.5		76.5	
154	84.1		31.5		78.2		67.3		73.2		75.5	
183	79.4		30.1		-		72.4		62.5		73.4	

MTBE, Site 1(B), Nitrate+nutrients

Day	2 ft		5 ft			10 ft			14 ft	
	microcosm		microcosm			microcosm			microcosm	
	1	2	1	2	3	1	2	3	1	2
Wt.(g)	4.3	4.4	5.0	4.7	4.8	2.7	3.1	3.2	4.8	5.0
1	102.5	116.2	91.5	103.0	101.7	109.2	104.2	109.6	82.2	99.1
43	100.8	105.8	108.6	114.4	111.3	116.2	113.5	110.5	100.4	102.6
90	110.3	112.6	112.8	114.5	115.2	112.9	115.7	111.9	105.3	106.1
128	106.1	112.9	105.1	111.7	108.0	-	86.1	110.6	103.7	77.2
167	103.8	-	109.1	112.8	101.8	89.5	86.4	87.7	102.1	76.6
208	116.5	112.6	114.4	124.0	119.8	87.3	97.9	94.8	107.7	77.4
281	115.4	97.0	89.1	112.9	113.4	87.8	75.3	103.5	113.7	71.0

ETBE, Site 1(B), Nitrate+Nutrients

Day	2 ft		5 ft			10 ft			14 ft	
	microcosm		microcosm			microcosm			microcosm	
	1	2	1	2	3	1	2	3	1	2
Wt.(g)	4.3	4.5	4.5	4.6		3.4	3.4		4.6	4.3
1	117.8	106.4	111.7	100.0		99.0	107.1		93.9	95.5
47	113.0	95.6	114.7	102.4		97.1	104.7		96.7	92.3
93	114.6	113.8	109.0	-		116.1	110.2		104.2	94.8
131	-	101.0	116.6	104.7		97.9	98.9		-	96.3
166	115.8	105.0	99.7	116.1		86.5	86.6		101.4	96.2
207	116.7	89.3	101.9	100.1		83.6	89.6		89.5	83.5
281	119.9	86.6	-	108.2		78.4	88.4		91.5	71.0
310	113.4	85.0	102.5	107.6		32.9	92.1		1.7	7.3

MTBE, Site 2, Nitrate

Day	0 ft			5 ft			10 ft			13 ft						
	microcosm	1	2	microcosm	1	2	microcosm	1	2	microcosm	1	2	microcosm	1	2	3
Wt.(g)	4.8	4.8	6.7	6.3	6.7	4.8	5.5	5.4	5.5	5.5	5.5	5.5	5.5	5.5	5.5	5.5
1	87.7	92.6	85.7	113.9	111.0	80.5	113.9	85.6	116.0	104.4	114.8					
25	85.8	83.5	73.4	94.7	60.8	68.7	89.6	61.1	93.2	67.3	102.7					
77	84.5	85.1	71.9	96.7	67.3	65.5	86.2	58.1	75.2	63.9	96.6					
110	88.5	84.3	74.3	95.5	69.1	68.8	86.6	60.1	88.1	69.3	98.1					
141	80.0	83.4	73.4	107.6	72.8	64.6	90.2	64.5	88.8	83.6	93.7					
201	91.0	86.2	71.2	96.9	69.5	66.4	91.0	59.5	90.3	67.2	96.8					
262	92.5	89.4	79.3	103.6	71.6	68.0	92.1	58.9	92.2	65.0	103.8					

MTBE, Site 2, Nitrate+nutrients

Day	0 ft			5 ft			10 ft			13 ft						
	microcosm	1	2	microcosm	1	2	microcosm	1	2	microcosm	1	2	microcosm	1	2	3
Wt.(g)	4.2	4.2	4.1	5.7	4.4	4.9	4.8									
1	121.9	126.5	128.1	90.6	112.6	115.9	112.2									
35	120.6	122.7	124.7	82.4	100.7	103.0	104.2									
75	116.3	122.6	-	80.8	111.9	119.7	113.9									
112	-	-	-	84.6	95.3	98.0	101.1									
144	115.4	122.9	109.3	91.5	104.5	105.6	105.9									
199	110.2	109.6	114.7	89.1	100.5	106.1	110.1									
247	103.2	103.9	110.5	91.3	102.6	102.5	105.4									

ETBE, Site 2, nitrate

Day	0 ft			5 ft			10 ft			13 ft						
	microcosm	1	2	microcosm	1	2	microcosm	1	2	microcosm	1	2	microcosm	1	2	3
Wt.(g)	4.3	4.3	7.2	7.6	5.1	4.4	5.1	5.6								
1	114.9	115.7	110.1	112.6	72.8	83.8	79.4	78.0								
30	105.3	111.5	74.8	73.0	71.0	66.6	69.1	68.5								
66	104.8	107.4	65.2	68.9	64.8	66.5	66.8	65.9								
112	99.8	103.3	67.7	67.2	57.0	65.7	58.7	58.0								
142	90.8	88.0	68.1	65.1	63.2	62.0	66.8	68.8								
224	90.2	104.9	64.5	69.1	72.7	65.4	60.3	56.4								
274	99.6	102.9	68.2	66.6	62.5	65.2	66.5	64.1								

MTBE, Site 3, nitrate

Day	0 ft microcosm		2 ft microcosm			4 ft microcosm			7 ft microcosm		10 ft microcosm		
	1	2	1	2	3	1	2	3	1	2	1	2	3
Wt.(g)	7.5	7.5	7.0	7.0	6.6	6.1	6.7	6.1	6.1	6.1	3.5	3.3	3.3
1	127.6	127.5	130.0	138.4	140.3	140.9	140.0	141.1	120.6	133.9	135.2	138.9	124.5
37	123.8	123.8	125.6	94.8	128.5	134.3	120.9	130.1	105.0	114.5	98.8	109.5	114.2
115	137.8	128.9	137.1	100.3	140.0	138.4	132.6	145.4	127.6	114.4	110.7	117.3	112.8
194	141.2	137.8	132.9	101.8	135.7	140.9	134.6	132.6	120.4	110.5	107.5	122.3	114.3
265	118.3	125.7	125.9	103.5	127.9	133.2	129.3	135.2	126.2	115.8	110.1	116.2	103.2

MTBE, Site 3, nitrate+nutrients

Day	0 ft microcosm		2 ft microcosm			4 ft microcosm			7 ft microcosm			10 ft microcosm			
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Wt.	7.5	7.5	7.5	6.9	7.2	6.9	6.1	5.2	7.6	5.5	6.1	5.5	3.3	3.1	2.9
1	122.2	118.6	114.6	127.6	123.0	118.9	131.4	126.9	126.5	110.0	104.5	105.4	125.6	117.3	129.0
37	127.6	116.7	118.1	119.1	120.3	117.1	119.8	117.6	107.4	110.1	105.0	106.6	98.1	99.5	105.1
115	132.2	129.6	128.5	132.3	124.9	123.3	134.5	130.8	118.7	111.6	105.6	108.3	105.2	108.3	111.2
194	126.4	124.2	123.7	128.9	115.7	140.3	128.7	120.5	115.4	115.4	113.3	105.2	107.6	107.6	113.2
265	119.4	121.9	118.3	124.1	110.5	121.8	101.3	119.4	117.3	110.2	110.1	100.4	104.9	104.2	126.4

ETBE, Site 3, Nitrate+nutrients

Day	0 ft microcosm		2 ft microcosm			4 ft microcosm		7 ft microcosm		10 ft microcosm	
	1	2	1	2	3	1	2	1	2	1	2
Wt.(g)	6.7	7.5	7.5	7.3	7.3	5.7	6.7	6.1	5.7	3.3	3.7
1	85.8	71.1	120.3	114.0	112.3	120.6	118.9	106.3	96.1	113.7	122.2
39	86.9	72.0	113.9	113.0	114.8	116.3	116.2	99.7	91.9	94.9	102.9
84	86.2	71.5	115.2	111.0	111.3	109.8	107.5	105.7	87.1	87.9	85.0
145	87.5	69.9	115.2	110.5	103.7	109.6	108.0	107.6	87.5	92.1	90.0
193	85.5	68.0	112.1	112.5	110.5	107.0	104.7	105.8	99.6	93.6	94.8
266	89.0	66.0	118.1	111.5	112.4	104.4	98.8	105.5	98.2	95.2	92.7

ETBE, Site 3, Nitrate

Day	0 ft microcosm		2 ft microcosm			4 ft microcosm			7 ft microcosm		10 ft microcosm	
	1	2	1	2	3	1	2	3	1	2	1	2
Wt.(g)	7.3	7.5	7.3	7.3	6.9	6.1	6.7	5.7	5.6	6.5	3.0	3.8
1	96.3	81.7	117.7	112.6	109.9	129.0	123.7	118.4	116.1	114.4	125.6	115.5
39	90.3	78.5	101.9	110.3	106.4	118.6	105.9	109.0	104.8	95.3	103.6	95.9
84	71.6	82.7	113.7	108.3	105.1	118.2	110.3	105.4	112.5	94.6	102.2	84.2
145	62.6	79.6	110.8	110.8	101.4	113.4	106.4	109.9	106.9	94.6	97.5	95.5
193	63.5	78.1	110.3	111.1	105.9	112.8	107.1	105.9	108.4	97.7	105.0	100.1
266	60.4	77.1	109.4	113.9	95.9	112.8	102.5	110.1	107.7	98.3	100.9	104.2

## **Appendix D**

. Degradation under Anaerobic Conditions

TBA, Site 1(A)-2, 100 mg/l ethanol every two weeks

Day	ethanol			ethanol+BESA+Mo			Ethanol+SO <sub>4</sub> <sup>-2</sup> +BESA			Ethanol+MoO <sub>4</sub>			Ethanol+SO <sub>4</sub> <sup>-2</sup>		
	microcosm			microcosm			microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
wt.(g)	4.3	4.6	4.3	4.3	4.2	4.2	4.2	4.1	4.6	4.6	4.2	4.3	4.3	4.3	4.2
1	94.6	87.6	90.2	89.9	101.0	93.0	94.2	90.3	90.9	96.2	99.2	99.2	93.8	93.4	91.9
28	89.5	88.6	86.9	93.9	92.7	95.1	92.4	95.7	88.1	95.7	94.1	95.4	91.4	92.6	90.1
41	89.3	87.9	89.2	96.6	90.0	94.8	95.2	92.6	90.6	92.7	95.7	92.5	91.8	84.5	96.8
80	84.5	88.8	80.0	87.8	91.1	92.3	93.0	90.4	87.1	92.8	86.2	92.7	84.6	90.0	87.6
111	84.0	81.6	83.7	92.8	91.9	92.0	89.2	91.2	87.0	87.9	90.2	90.1	87.1	84.0	88.9
138	86.1	83.6	79.4	88.3	88.8	88.2	87.7	86.8	81.3	88.9	93.2	89.6	89.4	85.3	88.3
185	82.5	79.6	82.7	88.4	87.1	88.5	87.1	88.1	80.2	86.7	86.1	83.4	83.6	82.3	83.0
253	81.1	78.4	76.1	83.9	88.0	88.5	85.9	86.1	79.0	80.0	85.4	85.6	85.5	83.5	87.7

TBA, Site 1(A)-5, 100 mg/l ethanol every two weeks

Day	ethanol		ethanol+BESA+Mo			Ethanol+SO <sub>4</sub> <sup>-2</sup> +BESA	Ethanol+MoO <sub>4</sub>			Ethanol+SO <sub>4</sub> <sup>-2</sup>	
	microcosm		microcosm			microcosm	microcosm			microcosm	
	1	2	1	2	3	1	1	2	3	1	2
wt.(g)	3.5	3.3	3.6	3.3	3.6	3.4	3.5	3.5	3.2	3.5	3.5
1	88.8	92.9	93.3	96.5	89.3	91.9	91.3	91.0	95.0	91.5	88.7
28	90.7	87.4	91.7	90.0	92.1	91.5	88.4	88.0	91.6	91.1	87.7
41	92.5	89.8	89.3	90.4	91.8	88.4	84.6	86.8	95.1	87.8	90.1
80	87.2	80.4	85.7	87.6	87.7	81.0	87.9	74.1	87.7	86.6	83.0
111	88.0	81.1	89.0	90.1	88.6	82.5	82.8	75.9	88.1	84.7	81.3
138	90.7	82.3	88.0	90.1	84.8	79.5	86.7	62.4	85.8	86.0	78.3
185	90.5	80.2	78.6	85.8	83.0	77.7	81.3	63.9	-	82.3	-
253	86.8	71.2	86.8	88.9	89.7	81.3	80.3	62.2	87.2	87.7	-

TBA, Site 1(A)-10, 100 mg/l ethanol every two weeks

Day	ethanol		ethanol+BESA+Mo			Ethanol+SO <sub>4</sub> <sup>-2</sup> +BESA			Ethanol+MoO <sub>4</sub>			Ethanol+SO <sub>4</sub> <sup>-2</sup>
	microcosm		microcosm			microcosm			microcosm			microcosm
	1	2	1	2	3	1	2	3	1	2	3	1
wt.(g)	4.8	4.8	4.6	4.8	4.6	4.5	4.6	4.6	4.2	4.6	4.2	4.6
1	95.3	98.6	102.2	103.2	104.9	100.4	98.6	99.6	106.7	101.2	106.0	98.9
28	93.6	91.3	100.5	96.9	101.6	97.5	96.6	94.3	100.0	97.8	100.5	99.1
41	99.7	99.0	97.1	100.3	97.7	99.8	93.7	95.6	94.4	98.6	98.8	97.4
80	90.1	95.5	91.4	97.7	91.2	95.4	89.0	92.9	93.1	96.5	93.6	94.6
111	94.4	91.8	93.9	92.6	94.6	90.9	92.3	85.5	97.9	90.7	94.1	88.5
138	92.6	88.7	92.8	94.5	97.3	86.8	87.0	85.6	91.4	94.1	95.7	88.9
185	91.2	91.2	93.5	94.5	93.1	90.6	86.7	88.1	90.8	88.7	93.1	84.2
253	93.5	92.1	92.2	94.3	91.5	87.4	84.7	84.0	91.7	88.0	-	81.2

TBA, molybdate, Site 1(A)

Day	2ft		5 ft			10 ft	
	microcosm		microcosm			microcosm	
	1	2	1	2	3	1	2
Wt.(g)	4.0	4.0	3.6	3.6	3.6	5.1	5.0
1	92.0	87.3	90.2	89.1	85.3	84.5	88.1
14	85.6	83.8	84.4	84.2	81.5	85.0	80.6
36	78.4	67.7	79.2	75.6	76.0	89.8	78.8
57	63.6	56.2	74.8	69.6	69.2	84.4	74.0
76	62.9	63.2	77.4	78.6	76.8	91.1	82.1
128	59.9	54.8	75.5	75.8	73.4	86.1	77.0
140	53.6	49.9	73.5	73.7	72.0	87.3	75.0
180	49.0	40.0	62.3	61.7	61.6	88.5	79.6
210	43.0	36.0	64.6	69.1	64.2	82.0	71.7

TBA, Site 2, molybdate

Day	0 ft			5 ft		10 ft			13 ft		
	microcosm			microcosm		microcosm			microcosm		
	1	2	3	1	2	1	2	3	1	2	3
Wt.(g)	4.4	4.6	4.5	6.0	7.2	4.4	4.5	4.6	6.1	5.1	5.6
1	119.6	114.9	111.2	103.8	109.5	107.2	106.8	103.1	114.2	102.5	105.3
39	106.8	116.2	111.7	85.2	86.7	101.2	101.3	94.9	89.4	97.7	95.6
80	94.1	89.3	98.2	52.3	51.4	92.9	93.2	95.0	86.5	91.7	93.0
104	102.2	112.4	108.8	27.4	20.1	101.4	99.6	97.6	82.2	77.5	91.6
151	104.9	94.0	101.3	2.7	0	50.3	49.6	41.7	27.7	41.6	50.5
240	109.7	112.4	110.2	0		1.4	0.5	0.3	0.4	12.4	0.4

TBA, Site 3, molybdate

Day	0 ft			2 ft			4 ft			7 ft			10 ft		
	microcosm			microcosm			microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	6.8	7.1	6.4	6.7	8.1	6.7	6.6	6.3	6.0	6.0	5.6	5.6	4.3	4.2	3.7
1	119.0	115.1	116.0	111.7	117.2	119.5	113.1	116.7	115.3	107.9	104.7	96.6	113.5	110.3	114.0
32	98.7	96.0	106.1	86.4	97.1	87.1	94.9	96.1	99.4	74.2	87.3	78.2	81.4	85.8	80.3
73	68.9	72.5	72.3	55.5	84.1	90.4	55.2	73.0	70.7	0.2	38.1	0.8	78.1	66.0	83.0
105	48.0	53.0	62.6	44.8	68.9	81.2	38.3	64.2	56.3		0	0	62.4	61.7	84.1
146	34.8	44.6	69.7	35.3	67.3	71.1	34.1	48.1	70.0				67.3	50.2	-
186	17.8	27.5	59.6	23.3	59.3	61.7	24.1	35.3	54.4				40.5	40.6	87.9
228	5.6	9.7	40.8	11.3	52.4	46.5	6.5	18.3	45.0				8.1	22.8	87.1
255	0	0	30.6	0	47.9	39.9			40.3				4.0	19.1	83.2

TBA, Starch only Site 1(A)

Day	2ft			5 ft		Day	10 ft	
	microcosm			microcosm			microcosm	
	1	2	3	1	2		1	2
wt.(g)	4.8	4.6	4.3	4.1	4.5		4.3	4.8
1	108.2	113.0	106.0	106.2	106.6	1	95.4	99.6
21	111.0	110.0	110.7	105.9	107.9	32	93.7	89.1
41	104.8	101.3	103.3	99.2	100.2	54	89.9	89.8
74	100.6	99.3	96.5	94.2	97.2	74	79.6	80.2
115	96.0	93.1	92.5	91.4	93.5	108	75.0	76.8
137	95.0	85.8	93.0	85.5	88.2	150	73.6	72.5
						172	71.5	69.3

TBA, Site 2, 200 mg/l starch

Day	0 ft			5 ft		10 ft			13 ft		
	microcosm			microcosm		microcosm			microcosm		
	1	2	3	1	2	1	2	3	1	2	3
Wt.(g)	4.4	4.4	4.5	6.2	6.9	4.9	5.3	5.2	5.0	5.2	5.3
1	116.8	118.3	108.8	93.2	99.4	107.0	105.0	103.5	106.9	112.9	99.8
39	109.6	107.3	110.8	78.8	82.2	94.9	89.6	90.9	100.7	92.2	95.5
80	106.8	96.1	100.9	65.8	59.1	91.9	78.0	79.0	86.7	79.9	84.0
104	104.9	110.0	109.8	51.6	49.5	83.3	71.6	78.4	96.4	82.7	88.7
151	101.7	110.6	89.3	0	30.8	56.1	17.6	31.2	42.1	62.3	46.8
240	107.7	110.0	116.9		0	9.7	0	0	18.8	30.1	36.0

TBA, Nutrients, Site 1(B)

Day	2'			5'			10'			14'		
	microcosm			microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3
wt.(g)	4.0	4.1	3.9	4.2	4.7	4.4	3.9	4.2	3.3	4.0	4.3	4.2
1	122.9	120.9	120.5	123.8	120.4	117.2	119.8	118.9	125.9	113.0	123.6	114.7
28	98.2	114.8	120.6	107.8	122.4	115.8	96.9	94.4	104.9	103.8	112.8	106.2
77	106.4	106.4	104.0	112.5	94.0	105.3	98.0	92.8	98.7	102.9	92.4	95.2
119	98.9	102.3	75.7	98.1	63.4	105.7	81.4	86.8	82.6	81.8	51.8	48.8
155	93.5	93.6	51.5	86.6	37.4	102.7	71.1	80.2	68.2	76.2	2.5	8.8
205	78.9	30.7	0	66.2	3.2	83.8	42.9	67.2	24.2	2.8	0.	0
258	49.7	2.9	0	34.0	0	72.9	3.6	34.1	2.8	0		
288	27.2	0	0	16.8	0	56.9	0	15.3	0			

TBA, Site 2, nutrients

Day	0 ft			5 ft			10 ft			13 ft		
	microcosm			microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	4.6	4.2	4.5	5.6	5.0	5.8	4.6	5.9	4.5	5.5	4.8	4.4
1	103.2	105.3	105.4	93.8	97.8	97.5	100.7	96.7	96.4	95.0	101.7	101.4
30	98.2	100.3	99.5	81.3	84.5	70.9	94.6	80.7	95.5	86.2	90.7	91.5
60	89.5	92.6	90.6	56.6	72.0	50.9	94.6	81.7	93.4	85.6	91.1	89.8
96	78.9	71.6	76.2	26.5	52.5	25.7	84.8	68.9	86.4	80.2	83.8	77.3
120	85.9	76.8	82.1	6.5	36.6	5.3	87.5	53.8	79.8	82.2	73.9	81.8
160	85.4	74.4	82.6	3.0	22.8	1.3	80.4	32.3	61.8	73.2	65.5	42.1
195	-	-	-	0	0.9	0	61.9	3.1	46.1	75.1	42.1	65.9

TBA, Site 3, nutrients

Day	0 ft			2 ft			4 ft			7 ft			10 ft		
	microcosm			microcosm			microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	6.0	7.5	7.1	8.2	6.1	7.6	6.7	5.5	6.1	5.7	5.3	5.7	3.8	3.7	3.9
1	115.4	114.5	113.8	113.8	114.3	112.3	110.1	109.0	114.7	95.8	99.8	95.8	111.1	112.7	111.7
32	107.6	111.4	104.1	104.9	109.5	105.1	88.5	96.6	98.7	95.9	93.2	95.3	-	-	-
73	99.9	91.3	57.1	97.4	109.9	101.1	75.3	80.2	85.8	60.4	77.6	81.4	83.1	79.3	82.9
105	96.6	79.7	28.7	71.8	103.9	71.9	41.9	72.3	57.7	34.4	75.5	64.0	73.9	67.9	80.4
146	80.0	6.7	0	18.2	101.1	28.4	34.8	53.3	57.4	29.3	19.9	3.3	62.1	78.9	74.3
186	13.2	0		3.7	85.1	11.4	2.7	6.3	44.1	0.9	0.5	0	57.8	70.1	71.5
228	2.8				71.6	0.8	0	0	5.8				14.7	62.3	64.0
255					58.9								5.4	9.5	63.8



TBA, Site 2, Starch+nutrients+sulfate

Day	0 ft microcosm			5 ft microcosm			10 ft microcosm			13 ft microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	4.8	4.2	4.6	6.8	5.7	5.7	4.9	5.2	4.9	5.3	5.2	6.1
1	109.2	106.4	122.0	100.5	103.2	120.4	105.8	113.6	117.7	110.6	111.6	103.3
39	110.1	111.1	102.7	87.4	89.6	91.4	97.3	95.1	99.1	103.4	96.6	95.2
80	100.3	102.6	93.0	45.8	36.6	48.7	78.4	81.0	88.6	92.5	85.1	75.4
104	109.1	113.3	101.4	13.7	5.5	11.3	69.1	72.6	84.5	97.5	75.6	58.2
151	113.0	109.3	99.3	0	0	0	23.1	27.4	44.0	55.5	5.1	4.7
240	108.6	108.2	107.5				0	0.9	0.3	43.9	0	0

TBA, Site 3, starch+nutrients+sulfate

Day	0 ft microcosm			2 ft microcosm			4 ft microcosm			7 ft microcosm			10 ft microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	7.7	8.0	7.8	6.4	7.7	7.7	6.3	6.2	6.6	6.8	6.8	6.7	3.8	3.5	3.8
1	103.7	104.3	103.0	102.7	100.5	104.1	103.3	102.2	101.7	98.1	85.9	95.8	97.2	99.6	96.8
32	84.4	95.2	90.0	88.9	91.7	97.1	86.1	85.5	88.0	71.4	79.8	77.0	71.7	83.0	78.3
73	63.1	52.9	11.7	89.2	90.5	87.1	76.5	84.1	80.8	59.7	79.5	75.9	68.4	79.2	77.0
105	9.8	8.1	1.0	84.7	86.5	60.3	67.9	75.7	69.4	0.4	74.8	74.9	66.8	69.7	75.7
146	0	0	0	78.5	82.9	17.3	47.8	77.5	53.1		63.9	67.7	54.9	76.1	-
186				64.6	82.1	8.5	26.7	11.9	12.2		42.3	25.2	52.4	67.9	76.4
228				41.8	15.0	0	1.2	2.4	2.9		13.2	1.4	36.3	54.0	73.7
255				30.4	0								28.9	31.2	73.4

TBA, starch+nutrient, Site 1(A)

Day	2ft			5 ft		10 ft	
	microcosm			microcosm		microcosm	
	1	2	3	1	1	2	2
wt.(g)	4.8	4.6	4.3	4.5	4.3	4.8	
1	94.5	86.6	93.3	87.4	70.8	73.1	
14	95.6	85.4	92.1	87.0	73.3	72.6	
29	90.1	80.8	91.0	87.5	74.9	69.0	
50	86.2	73.2	83.7	82.1	62.2	43.9	
67	79.6	27.3	77.7	84.8	50.8	36.7	
105	58.8	8.9	78.4	80.1	29.4	16.3	
137	38.2	0	48.8	66.5	14.9	5.9	

TBA, Site 2, nutrients + starch

Day	0 ft			5 ft			10 ft			13 ft		
	microcosm			microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3
wt.(g)	4.7	4.2	4.5	4.7	6.0	4.9	4.6	4.3	4.9	5.0	5.8	4.9
1	97.2	97.9	96.1	87.6	83.5	86.6	94.0	92.8	93.6	94.2	91.8	89.6
30	94.5	83.2	88.7	79.4	76.6	76.7	80.5	80.7	95.5	86.2	90.7	91.5
60	79.2	80.4	85.6	66.0	70.8	74.5	80.4	89.8	79.8	84.3	76.0	86.2
96	52.2	69.9	67.3	41.5	68.5	75.3	79.1	78.2	61.2	72.3	67.5	75.1
120	57.2	75.3	70.1	19.6	67.8	63.7	82.6	74.1	51.6	64.7	55.2	70.3
160	60.3	79.4	70.0	8.3	54.9	49.3	81.5	72.9	29.1	47.2	33.7	56.6
195	-	-	-	0	63.7	2.2	75.4	56.2	12.9	30.2	3.3	54.7

TBA, Site 3, Starch+Nutrients

Day	0 ft			2 ft			4 ft			7 ft			10 ft		
	microcosm			microcosm			microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
wt.(g)	7.3	7.2	6.5	6.7	7.0	6.2	6.1	6.0	6.9	5.1	5.6	5.9	3.7	3.2	4.0
1	112.8	111.5	111.8	111.0	111.3	112.9	102.3	114.0	110.0	98.3	95.0	109.4	105.2	107.2	102.2
32	104.9	107.1	103.9	101.4	106.0	105.1	93.4	94.3	96.4	87.3	88.9	90.4	91.6	88.6	75.4
73	71.9	51.1	69.3	78.6	105.6	109.2	72.1	95.4	75.0	87.6	79.1	86.5	84.1	85.7	71.4
105	14.8	8.6	9.6	27.7	101.9	110.8	61.0	91.7	64.4	91.8	61.3	64.8	78.2	81.5	74.0
146	0.5	0	0	3.9	91.9	106.9	41.8	87.6	45.0	84.1	46.6	63.9	84.5	76.5	-
186				0	68.6	99.2	4.7	78.3	7.8	79.0	18.0	7.4	86.3	75.6	75.2
228					13.9	94.0	0	63.9	0	63.2	2.4	0	81.7	17.9	70.0
255						90.0		50.1		41.8			82.5	0	63.0

TBA, competition ,Site 1(B)-2'

Day	S+N			S+H+SO <sub>4</sub> <sup>-2</sup>			S+H+Hollydeter+BESA			S+H+BESA+SO <sub>4</sub> <sup>-2</sup>		
	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	
1	2	3	1	2	3	1	2	3	1	2	3	
wt.(g)	4.5	4.4	4.6	4.1	4.6	4.6	4.6	4.7	4.4	4.2	4.1	4.4

TBA, competition ,Site 1(B)-5'

Day	S+N			S+H+SO <sub>4</sub> <sup>-2</sup>			S+H+Hollydeter+BESA			S+H+BESA+SO <sub>4</sub> <sup>-2</sup>		
	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	
1	2	3	1	2	3	1	2	3	1	2	3	
wt.(g)	4.2	4.2	4.6	4.8	4.7	4.3	4.1	4.5	3.9	4.8	4.1	

1	98.0	98.0	94.2	95.9	91.9	91.7	81.7	79.9	79.1	86.0	89.2	87.7
34	91.7	93.0	89.0	88.5	86.8	86.9	76.7	76.7	75.3	83.8	84.9	86.5
90	87.0	95.2	14.0	86.4	81.6	86.5	79.0	70.2	67.5	74.2	13.0	83.7
127	90.9	87.7	1.3	73.6	75.0	80.1	71.1	10.7	56.0	88.9	2.2	82.8
167	82.9	75.9	0	58.5	64.6	36.7	28.5	2.8	37.6	76.5	3.1	71.5
195	65.0	63.3		28.0	45.2	5.1	5.9	0	22.9	82.3	0	69.1
224	47.3	42.0		4.6	16.7	0	0	0	5.8	84.8	64.9	
268	8.8	12.8		0.2	0				0	8.7	60.1	

1	102.4	103.8	101.8	92.5	92.3	82.9	84.5	87.8	89.7	92.3	89.7
34	96.3	98.0	97.4	87.3	89.1	80.0	83.9	83.0	89.9	87.7	88.9
90	107.8	98.0	98.8	62.6	45.9	77.9	24.5	77.9	59.4	59.1	80.8
127	97.7	96.9	96.2	8.5	9.9	80.4	3.3	79.1	36.9	11.1	10.4
167	92.4	91.9	96.3	5.1	0.3	85.2	3.5	77.4	4.7	2.1	6.2
195	83.0	94.1	93.4	0	0	79.0	0	79.2	0	0	0
224	56.9	89.5	87.6			83.6	82.0				
268	4.1	62.1	56.9			78.7	78.1				

TBA, competition ,Site 1(B)-10'

Day	S+N			S+H+SO <sub>4</sub> <sup>-2</sup>			S+H+Hollydeter+BESA			S+H+BESA+SO <sub>4</sub> <sup>-2</sup>		
	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	
1	2	3	1	2	3	1	2	3	1	2	3	
wt.(g)	4.1	4.4	4.2	4.5	4.1	3.9	4.3	4.8	3.6	4.1	3.5	3.7

TBA, competition ,Site 1(B)-14'

Day	S+N			S+H+SO <sub>4</sub> <sup>-2</sup>			S+H+Hollydeter+BESA			S+H+BESA+SO <sub>4</sub> <sup>-2</sup>		
	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	microcoam	
1	2	3	1	2	3	1	2	3	1	2	3	
wt.(g)	4.1	4.4	4.2	4.5	4.1	3.9	4.3	4.8	3.6	4.1	3.5	3.7

1	129.6	112.0	120.7	112.0	112.1	94.2	107.1	105.1	109.0	106.7	98.9	113.6
33	115.8	100.3	106.4	103.5	97.6	101.4	105.8	89.2	104.9	100.1	103.2	107.7
76	109.7	50.1	96.4	96.7	97.1	103.6	97.7	103.5	97.7	87.3	106.2	94.3
132	99.2	0.9	53.6	83.0	74.3	97.5	102.6	75.7	106.3	89.8	82.9	93.4
169	68.4	0.5	27.3	64.7	26.9	75.0	98.3	80.8	99.4	88.0	77.9	96.1
209	27.8	2.1	31.6	2.9	55.6	89.9	78.9	100.4	75.4	75.4	95.2	167
237	2.6	0	0	0	0	1.0	94.3	80.0	102.7	80.9	70.5	195
266							58.9	72.8	106.8	60.4	49.6	224
297							1.7	27.2	94.8	37.2	4.7	268

MTBE, Site 1(A)-2, 100 mg/l ethanol every two weeks

Day	ethanol			ethanol+BESA+Mo			Ethanol+SO <sub>4</sub> <sup>-2</sup> +BESA			Ethanol+MoO <sub>4</sub>			Ethanol+SO <sub>4</sub> <sup>-2</sup>		
	microcosm			microcosm			microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
wt.(g)	4.6	4.6	4.2	4.2	4.6	4.6	3.7	4.2	4.6	4.7	4.7	4.6	4.6	4.6	4.2
1	77.3	58.1	63.1	93.5	93.4	89.5	82.3	83.4	83.8	85.6	89.1	82.9	71.4	71.0	72.0
31	56.8	54.2	57.7	81.9	81.9	84.2	82.4	75.6	80.6	77.1	85.8	78.5	66.4	65.8	69.1
86	55.3	55.4	53.6	82.1	79.4	81.5	78.9	75.8	76.1	79.5	81.5	78.9	65.5	65.6	66.3
107	55.5	53.0	80.7	79.1	83.3	75.7	77.8	76.5	81.1	77.3	-	66.0	64.0	66.3	-
140	54.0	-	57.4	83.5	79.5	80.2	76.0	71.7	72.4	72.6	80.5	76.6	65.6	59.3	61.8
200	50.6	54.3	52.1	77.1	73.3	78.3	78.2	66.9	66.9	68.3	73.7	69.8	56.9	53.5	56.9

MTBE, Site 1(A)-5, 100 mg/l ethanol every two weeks

Day	ethanol			ethanol+BESA+Mo			Ethanol+SO <sub>4</sub> <sup>-2</sup> +BESA		Ethanol+MoO <sub>4</sub>			Ethanol+SO <sub>4</sub> <sup>-2</sup>		
	microcosm			microcosm			microcosm		microcosm			microcosm		
	1	2	3	1	2	3	1	2	1	2	3	1	2	3
wt.(g)	3.4	3.7	3.4	3.6	3.4	3.8	3.7	3.7	3.7	3.6	3.4	3.6	3.7	3.6
1	59.0	55.0	57.0	88.2	84.4	80.0	77.7	76.4	83.3	78.8	86.2	63.5	68.7	63.7
31	57.5	54.9	54.4	87.1	85.1	83.4	71.4	75.6	81.4	79.4	84.0	68.4	63.4	67.3
86	54.1	47.9	55.0	78.0	80.7	76.9	67.8	68.6	75.2	77.1	80.4	62.7	62.2	62.3
107	54.6	50.3	53.2	80.4	81.9	77.1	61.8	71.6	76.7	77.2	80.7	57.5	58.1	61.8
140	51.8	50.3	51.8	75.0	82.0	78.6	-	67.9	68.3	73.6	78.0	57.1	57.1	57.7
200	53.8	52.3	49.5	-	-	72.4	-	65.4	64.3	68.8	73.6	52.8	53.8	54.6

MTBE, Site 1(A)-10, 100 mg/l ethanol every two weeks

Day	ethanol			ethanol+BESA+Mo	Ethanol+SO <sub>4</sub> <sup>-2</sup> +BESA		Ethanol+MoO <sub>4</sub>			Ethanol+SO <sub>4</sub> <sup>-2</sup>
	microcosm			microcosm	microcosm		microcosm			microcosm
	1	2	3	1	1	2	1	2	3	1
wt.(g)	4.4	5.0	4.3	4.3	4.2	4.9	4.9	4.3	4.7	4.7
1	58.5	57.3	60.3	83.6	81.7	79.0	84.3	77.8	82.5	67.3
31	58.9	58.8	57.4	80.9	77.1	80.3	83.4	74.8	79.0	69.8
86	58.4	61.6	54.3	83.1	79.5	74.3	80.8	76.5	78.6	63.5
107	57.9	56.2	55.8	86.4	77.5	75.0	80.4	76.6	79.7	63.7
140	53.0	50.8	52.6	83.7	71.4	65.1	79.1	73.6	73.8	59.2
200	50.8	47.0	45.9	80.6	72.1	65.3	73.8	69.4	64.6	57.2

ETBE, Site 1(A)-2, 100 mg/l ethanol every two weeks

Day	ethanol			ethanol+BESA+Mo			Ethanol+SO <sub>4</sub> <sup>-2</sup> +BESA			Ethanol+MoO <sub>4</sub>			Ethanol+SO <sub>4</sub> <sup>-2</sup>		
	microcosm			microcosm			microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
wt.(g)	3.9	4.2	4.5	4.3	4.3	4.3	4.4	4.1	4.3	3.8	3.9	3.5			
1	57.4	67.9	62.2	63.2	57.9	65.5	64.4	73.9	77.6	76.7	55.8	68.0	54.0		
42	55.1	64.4	63.8	60.1	59.9	62.3	76.0	69.9	74.5	71.9	55.9	51.1	55.2		
78	49.5	58.1	56.4	59.1	55.0	56.8	59.9	66.1	68.7	62.6	51.4	46.2	48.2		
109	48.8	59.2	54.0	59.3	51.0	57.7	57.4	64.5	66.5	65.8	47.7	46.8	-		
137	47.6	61.2	51.5	56.8	49.1	54.1	53.3	66.1	66.2	64.2	46.1	45.0	-		
197	45.8	59.3	52.7	55.3	49.9	50.2	54.1	65.8	64.2	63.9	45.7	46.3	-		

ETBE, Site 1(A)-5, 100 mg/l ethanol every two weeks

Day	ethanol		ethanol+BESA+Mo			Ethanol+SO <sub>4</sub> <sup>-2</sup> +BESA			Ethanol+MoO <sub>4</sub>			Ethanol+SO <sub>4</sub> <sup>-2</sup>	
	microcosm		microcosm			microcosm			microcosm			microcosm	
	1	2	1	2	3	1	2	3	1	2	3	1	2
wt.(g)													
1	53.3	54.9	68.6	67.9	66.0	69.7	65.8	59.9	68.9	69.9	72.8	54.0	52.5
42	50.3	50.8	67.8	67.2	65.5	62.7	63.0	61.1	71.9	67.2	78.3	48.5	55.5
78	51.2	48.1	60.3	64.2	58.6	62.2	55.3	59.8	64.7	62.6	64.7	45.9	47.4
109	46.0	45.3	56.9	61.7	62.0	65.6	55.2	57.3	65.3	65.8	61.9	45.1	50.6
137	47.0	45.9	59.1	65.0	60.4	56.7	54.4	55.5	66.2	59.6	65.9	41.0	47.3
197	48.7	44.6	60.9	67.2	62.1	58.6	53.8	52.9	63.9	64.3	61.2	43.8	44.8

ETBE, Site 1(A)-10, 100 mg/l ethanol every two weeks

Day	ethanol		ethanol+BESA+Mo			Ethanol+SO <sub>4</sub> <sup>-2</sup> +BESA			Ethanol+MoO <sub>4</sub>		Ethanol+SO <sub>4</sub> <sup>-2</sup>		
	microcosm		microcosm			microcosm			microcosm		microcosm		
	1	2	1	2	3	1	2	3	1	2	2	2	
wt.(g)	4.4		5.2	4.2	4.9	4.2	4.3	4.3	5.0	5.1	4.3	4.4	4.0
1	55.4		74.0	72.7	62.1	76.1	68.2	67.2	73.3	66.7	55.1	56.7	55.3
42	51.1		74.9	68.2	71.5	72.7	75.3	63.4	76.0	67.9	56.7	56.6	55.7
78	50.6		64.6	68.4	64.9	72.2	66.2	69.7	68.4	61.8	50.5	46.9	52.6
109	46.1		68.1	64.7	68.5	71.7	69.4	69.4	72.3	63.5	53.8	43.2	51.6
137	47.9		66.3	63.7	61.0	70.9	68.4	67.0	66.6	59.0	49.0	49.7	47.3
197	46.9		65.1	65.9	63.6	72.5	67.5	64.1	64.8	63.0	51.0	47.1	45.3

MTBE, Site 1(B), Nutrients

Day	2 ft			5 ft			10 ft			14 ft		
	microcosm			microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	4.6	4.2	4.1	4.7	4.5	4.6	3.6	4.0		4.1	4.1	4.0
1	107.2	95.2	80.5	91.9	95.4	96.0	96.2	96.0	89.3	99.3	87.5	
43	96.2	88.6	90.7	86.3	94.9	87.8	80.7	81.3	83.3	88.8	86.8	
90	98.4	92.2	90.7	85.6	91.7	89.9	86.5	89.1	84.6	89.1	93.7	
128	96.7	93.7	89.8	83.1	89.4	88.4	72.5	88.7	65.7	89.7	94.8	
167	97.6	90.0	88.9	88.0	87.6	90.4	71.7	85.7	56.9	88.9	94.0	
208	108.0	106.0	99.9	92.5	80.2	95.6	72.5	97.5	54.4	105.3	84.6	
281	112.6	96.9	-	90.1	103.5	-	71.4	96.3	37.6	104.0	95.3	
322	107.9	108.9	-	74.2	98.8	101.4	66.5	96.7	9.7	99.8	-	
pH	4.7	4.7		4.7	4.7	4.7	5.8	5.5	5.5	6.5		

MTBE, Site 1(B), Starch+Nutrients+molybdate

Day	2 ft			5 ft			10 ft			14 ft	
	microcosm			microcosm			microcosm			microcosm	
	1	2	3	1	2	3	1	2	3	1	2
Wt.(g)	3.9	3.8	4.1	4.4	4.4	4.8	3.6	4.0	3.4	4.6	4.5
1	98.7	96.9	93.7	91.3	90.8	89.9	89.6	82.1	88.2	78.2	78.5
43	89.1	96.0	92.5	92.4	89.4	81.6	84.3	81.7	86.7	78.6	79.8
90	94.5	95.8	96.2	89.4	94.7	76.6	90.6	84.6	90.1	80.4	82.7
128	90.9	98.1	95.0	90.8	95.2	83.2	82.3	83.2	88.9	80.2	81.9
167	90.2	90.9	96.5	91.5	97.0	82.8	84.8	89.1	90.2	86.0	83.1
208	102.7	104.3	97.6	104.5	96.3	89.0	95.4	94.2	93.3	82.5	81.6
281	98.9	103.1	-	95.2	93.3	-	91.4	95.4	-	54.7	86.8
pH	4.7	4.7	4.7	4.7	4.7	4.7	5.8	5.4		5.8	5.8

ETBE, Starch, Site 1(A)

Day	2ft		5 ft		10 ft			
	microcosm		microcosm		microcosm			
	1	2	1	2	Day	1	2	
Wt.(g)	4.0	4.5	4.4	4.3	4.3	4.5		
1	103.2	98.6	111.3	117.3	1	99.3	115.4	
21	101.1	94.5	100.5	101.0	32	90.6	112.7	
41	88.9	90.6	86.7	96.3	54	89.5	114.6	
68	93.4	88.8	95.8	92.7	74	88.7	111.3	
115	93.4	91.9	96.4	95.9	108	89.8	107.9	
144	95.8	93.7	94.7	92.5	150	90.0	113.3	
180	83.7	87.3	88.8	85.2	172	92.1	108.0	
208	87.3	81.9	97.8	93.9	186	80.3	106.0	
266	86.4	82.9	100.3	97.8	222	86.1	106.0	
280	90.5	84.9	88.6	89.8	250	74.7	88.4	
					302	67.7	84.1	
					320	69.0	78.8	

ETBE, Starch + molybdate, Site 1(A)

Day	2ft			5 ft			10 ft		
	microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3
Wt.(g)	4.2	4.3	4.4	4.1	4.0	4.3	4.4	4.4	4.4
1	123.9	128.6	124.3	0	111.8	112.4	118.1	1	95.8
21	122.0	118.3	116.3	0	110.1	109.3	97.6	32	110.7
41	105.7	112.8	113.6	0	107.7	97.6	90.9	54	114.7
68	110.9	104.9	111.2	0	97.6	93.5	90.6	74	104.7
115	106.4	101.2	104.6	4.5	104.5	103.1	100.0	108	105.8
144	115.7	102.5	76.5	17.1	104.5	107.0	104.9	150	108.8
180	114.7	85.9	51.1	27.3	103.3	97.0	99.2	172	106.8
208	107.1	86.0	41.8	33.6	101.4	100.2	101.4	186	102.8
266	102.8	79.1	10.0	30.9	89.8	98.1	97.5	222	98.2
280	105.1	82.2			94.1	96.1	97.1	250	93.8
								302	87.9
								320	80.0

ETBE, Starch + molybdate+nutrients. Site 1(A)

Day	2ft			5 ft			10 ft		
	microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3
Wt.(g)	4.6	4.5	4.1	4.9	4.1	4.6	4.1	4.6	4.6
1	103.1	0	109.4	111.3	104.5	0	1	79.1	110.9
21	101.5	0	102.7	106.6	93.4	0	32	61.3	109.2
41	91.0	0	101.4	95.8	77.1	0	54	68.2	111.2
68	89.0	0	93.0	103.8	38.0	17.8	74	59.0	103.8
115	89.9	0	97.3	102.1	15.4	13.5	108	60.0	107.8
144	68.8	1.9	93.9	104.4	0	8.4	150	65.0	110.8
180	24.5	14.3	95.4	98.8	0	0	172	67.8	110.8
208	3.8	3.1	90.7	94.4	0	0	186	66.1	104.4
266	0	0	83.2	94.3			222	60.1	97.2
280	0	0	90.4	95.0			250	53.0	97.4
pH	5.5	5.0	5.0	5.5	5.5	6.1	6.5		

ETBE, Site 1(B),Nutrients

Day	2 ft			5 ft			10 ft			14 ft					
	microcosm			microcosm			microcosm			microcosm					
	1	2	3	1	2	3	1	2	3	1	2	3			
	ETBE	ETBE	ETBE	ETBE	ETBE	ETBE	ETBE	TBA	ETBE	TBA	ETBE	ETBE	TBA	ETBE	TBA
Wt.(g)	3.8	4.3	3.9	4.4	4.5	4.5	3.3		4.2		4.2	4.1			
1	103.8	101.6	118.2	102.7	123.7	114.5	103.0	-	100.2	-	109.7	97.1	-	93.2	-
47	101.8	100.7	100.5	99.9	98.5	95.8	92.8	-	83.8	-	87.0	87.8	-	99.8	-
93	102.0	95.9	104.3	98.9	96.7	92.8	94.1	-	85.4	-	90.8	88.6	-	99.4	-
131	95.7	92.9	-	101.5	-	98.3	91.0	-	86.0	-	87.7	87.1	-	98.0	-
166	99.8	106.1	105.9	104.4	99.1	101.4	70.4	0	86.7	0	89.4	64.6	-	96.1	-
207	95.5	89.6	102.8	102.3	103.2	96.2	57.6	31.2	67.8	20.0	90.5	1.0	34.3	98.9	0
281	92.0	80.4	-	97.5	96.8	99.1	23.2	41.7	34.5	37.8	81.3	0.3	0	81.2	4.2
pH	4.7	4.7	4.7	4.7	4.7	4.7	5.7		5.5		5.0	5.7		5.3	

ETBE, Site 1(B), Starch+Nutrients+molybdate

Day	2 ft			5 ft			10 ft			14 ft					
	microcosm			microcosm			microcosm			microcosm					
	1	2	3	1	2	3	1	2	3	1	2	3			
	ETBE	ETBE	ETBE	ETBE	ETBE	ETBE	TBA	ETBE	ETBE	TBA	ETBE	TBA	ETBE	TBA	ETBE
Wt.(g)	4.3	4.0	4.1	4.4	4.5		4.2	4.3	3.4		3.8		3.9	3.5	
1	113.3	119.9	116.4	110.6	104.1	112.0	-	102.7	112.4	-	102.8	-	96.6	-	96.1
47	96.3	99.3	96.6	94.5	-	86.6	-	95.9	88.3	-	101.8	-	84.9	-	80.8
93	95.4	97.7	96.1	96.0	96.0	88.6	-	90.3	86.2	-	84.7	-	86.7	-	81.9
131	96.2	97.6	-	90.6	-	96.3	-	87.7	-	-	87.7	-	81.7	-	87.1
162	103.4	93.8	99.1	107.3	106.9	93.6	-	92.4	57.9	26.9	99.5	0	96.3	0	81.1
207	101.4	102.5	101.1	95.0	90.6	89.9	0	87.4	44.7	36.1	87.9	5.1	80.7	3.7	87.2
281	96.4	93.9	95.9	86.1	97.5	50.9	23.3	71.7	19.9	47.1	43.0	16.1	66.1	0.2	
pH	4.0	4.0	4.0	4.3	4.3	5.7		5.3	5.5		5.3		5.3		5.3

MTBE, Site 2, Nutrients

Day	0 ft			5 ft		10 ft			13 ft		
	microcosm			microcosm		microcosm			microcosm		
	1	2	3	1	2	1	2	3	1	2	3
Wt.(g)	4.3	4.3	4.4	6.7	6.6	4.8	4.8	5.5	5.5	5.5	5.5
1	109.8	119.4	115.9	107.0	109.6	117.6	103.3	105.8	105.2	100.9	98.3
35	108.3	106.5	116.8	108.3	100.2	101.8	100.1	101.5	82.0	84.3	75.3
75	103.9	113.6	105.6	103.9	102.9	100.3	103.5	101.5	93.2	107.0	80.3
112	109.3	112.9	102.3	105.3	106.2	90.0	106.8	104.5	73.3	87.4	71.5
144	-	-	-	116.8	116.0	90.5	107.9	104.5	86.9	102.5	79.4
199	99.3	103.5	109.3	105.9	110.3	90.4	109.4	107.8	85.9	100.1	80.5
247	104.3	110.8	101.2	102.7	105.8	89.2	100.2	106.5	89.4	99.3	84.2

MTBE, Site 2, Starch+Nutrients

Day	0 ft			5 ft		10 ft			13 ft		
	microcosm			microcosm		microcosm			microcosm		
	1	2	3	1	2	1	2	3	1	2	3
Wt.(g)	4.1	4.1	3.6	4.8	4.4	4.4	4.3	4.8	4.8	4.9	
1	113.8	110.9	101.5	94.1	105.4	107.3	109.0	107.0	110.2	107.4	
35	106.5	103.5	97.9	88.2	103.2	101.9	104.8	107.6	85.4	93.8	
75	117.7	115.1	112.9	86.0	109.5	108.9	110.3	115.7	104.9	105.9	
112	-	-	96.0	88.5	95.0	101.5	100.0	97.1	92.1	82.6	
144	110.1	112.8	102.9	96.0	101.8	101.5	103.5	102.3	101.6	97.0	
199	114.8	108.4	104.3	90.3	99.3	97.9	106.5	104.7	99.5	98.2	
247	105.3	102.3	100.4	89.2	101.4	97.3	100.4	98.9	103.2	101.3	

MTBE, Site 2, Starch+Nutrients+Molybdate

Day	0 ft			5 ft			10 ft			13 ft		
	microcosm			microcosm			microcosm			microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	4.2	4.7	3.8	4.8	4.8	3.6	4.5	4.5	4.4	4.9	5.4	4.6
1	110.8	115.6	113.7	99.7	100.0	102.0	119.9	112.9	108.6	113.3	104.1	103.9
35	109.0	112.7	115.5	86.3	103.9	86.9	89.5	97.3	102.6	90.9	88.1	88.1
75	112.9	119.0	116.1	93.5	90.0	85.3	111.6	109.8	111.6	98.5	94.4	94.6
112	-	-	-	74.8	87.1	89.2	83.1	90.7	94.1	80.4	82.2	82.0
144	108.2	109.2	113.4	72.5	94.2	97.1	97.8	106.1	105.3	89.1	94.4	91.4
199	106.9	103.2	100.6	79.8	99.2	89.9	95.3	92.4	101.8	95.4	91.9	89.2
247	103.2	104.3	109.4	71.8	95.3	95.3	89.2	100.7	104.6	91.3	89.3	85.9

ETBE, Site 3, Nutrients

Day	0 ft			2 ft			4 ft			7 ft			10 ft	
	microcosm			microcosm			microcosm			microcosm			microcosm	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2
Wt.(g)	7.4	7.1	5.8	6.9	6.9	6.9	6.5	5.6	5.9	4.9	5.1	5.0	2.8	2.7
1	91.6	93.0	89.9	99.1	83.1	96.0	100.8	100.3	104.0	88.7	88.2	89.8	82.5	84.7
29	93.4	83.2	86.6	96.0	77.2	87.8	89.3	94.0	103.5	93.9	94.0	94.8	83.8	74.1
75	88.9	78.7	83.7	90.8	70.3	83.7	84.0	97.8	96.9	80.8	80.6	85.2	77.6	69.1
136	89.3	76.1	77.2	87.0	60.9	78.8	77.4	69.6	97.3	90.1	90.2	89.2	71.6	78.2
184	91.3	76.6	80.5	84.3	61.5	77.9	80.8	62.9	95.1	90.9	91.9	89.9	70.5	75.5
266	92.4	66.7	79.3	84.8	61.0	74.3	80.8	56.8	102.4	94.8	93.3	95.4	73.9	77.2

ETBE, Site 3, Starch+Nutrients

Day	0 ft			2 ft			4 ft			7 ft			10 ft	
	microcosm			microcosm			microcosm			microcosm			microcosm	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2
Wt.(g)	7.4	6.9	6.3	5.6	6.7	5.2	6.8	6.0	4.4	5.3	5.0	2.6	2.7	
1	102.3	95.4	93.3	97.8	100.5	109.2	103.6	105.6	87.9	97.2	91.3	78.4	76.0	
29	96.9	97.5	94.6	96.5	107.3	104.3	104.1	92.8	97.4	107.1	94.8	75.6	88.1	
75	94.8	85.7	97.1	99.1	95.7	91.6	89.7	99.3	81.5	83.3	91.7	66.2	73.4	
136	95.6	75.0	92.3	105.1	95.1	102.0	90.7	93.6	90.6	92.6	89.2	75.7	79.0	
184	96.2	68.3	96.2	103.2	100.1	100.9	100.9	99.2	93.4	92.4	94.6	75.7	79.0	
266	100.1	68.0	102.2	104.6	105.1	104.0	98.9	102.5	93.1	96.1	99.2	76.0	81.9	

ETBE, Site 3, Starch+Nutrients+molybdate

Day	0 ft			2 ft			4 ft			7 ft			10 ft	
	microcosm			microcosm			microcosm			microcosm			microcosm	
	1	2	3	1	2	3	1	2	3	1	2	3	1	2
Wt.(g)	6.6	6.9	6.9	6.8	6.5	6.0	6.4	4.7	5.6	5.5	2.8	2.7		
1	99.7	95.6	94.7	95.1	103.4	99.6	98.0	88.2	99.9	85.4	86.4	82.8		
29	94.1	98.0	90.8	100.3	91.7	91.4	94.6	93.3	85.7	86.4	88.4	80.9		
75	96.6	95.4	96.1	90.3	101.7	99.5	92.3	83.5	84.5	81.3	69.5	71.3		
136	97.0	96.8	92.3	95.2	92.8	98.7	93.9	91.0	83.0	86.7	75.4	77.1		
184	98.7	95.3	94.3	95.6	93.2	97.8	99.1	91.7	84.6	83.9	78.1	79.2		
266	103.5	102.7	93.8	92.2	95.9	101.6	98.2	89.4	88.1	87.5	80.4	75.9		

NTBE, Site 3, nutrients

Day	0 ft microcosm			2 ft microcosm			4 ft microcosm			7 ft microcosm			10 ft microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	7.1	7.2	7.2	6.7	7.1	8.4	6.6	6.2	6.2	5.0	5.1	5.3	3.6	3.7	3.6
1	121.4	139.4	118.2	122.6	102.8	115.8	88.7	92.3	92.1	71.8	85.4	83.7	83.1	102.2	118.3
30	106.7	102.9	99.4	96.7	84.8	105.7	86.8	71.9	88.1	68.9	63.6	61.5	78.3	68.5	71.7
101	101.3	98.1	91.2	101.0	81.7	103.7	81.3	78.2	89.9	62.2	59.8	56.4	52.9	61.2	65.7
180	107.7	108.2	93.4	98.0	96.2	99.4	78.8	86.3	84.2	63.8	61.7	59.7	59.0	65.0	70.3
260	110.3	103.2	99.4	98.5	99.2	100.3	82.9	88.3	84.9	70.3	60.2	58.5	55.7	61.8	69.2

NTBE, Site 3, Starch+nutrients

Day	0 ft microcosm			2 ft microcosm			4 ft microcosm			7 ft microcosm			10 ft microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	7.8	7.8	7.8	7.1	6.8	7.5	6.2	6.4	6.6	4.7	5.0	5.1	3.6	3.7	4.3
1	115.2	138.4	144.7	109.6	107.4	98.3	77.8	73.7	95.5	78.9	79.3	73.2	104.6	95.2	87.6
30	91.9	115.4	110.7	97.3	87.8	90.9	64.8	57.2	82.5	57.3	59.9	61.3	68.9	70.0	70.8
101	97.2	104.3	101.4	88.0	93.1	92.1	69.9	70.7	83.8	54.8	47.7	50.6	70.6	65.3	71.4
180	110.3	108.5	103.2	99.1	99.2	89.5	75.9	72.5	79.5	61.6	56.6	53.7	74.9	78.8	72.8
260	92.3	101.4	99.3	101.2	94.3	93.9	70.5	71.8	83.2	65.1	55.3	59.2	70.2	77.2	69.1

NTBE, Site 3, Starch+nutrients+molybdate

Day	0 ft microcosm			2 ft microcosm			4 ft microcosm			7 ft microcosm			10 ft microcosm		
	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
Wt.(g)	7.2	7.8	7.1	6.8	7.5	6.8	6.4	5.8	5.6	4.7	6.2	3.9	4.4	5.5	
1	101.8	102.0	100.7	83.7	111.0	95.8	91.0	82.1	87.4	71.6	64.5	80.1	46.1	99.0	
30	90.5	79.3	98.7	73.1	85.5	84.9	78.7	71.5	62.9	70.3	59.2	72.2	51.5	88.8	
101	86.1	76.1	91.7	75.4	89.0	88.3	85.1	71.5	60.3	50.1	55.0	66.5	47.2	81.0	
180	101.9	98.0	93.6	81.6	100.2	92.6	88.2	86.1	64.9	71.0	62.0	76.4	52.7	83.9	
260	94.3	95.9	91.6	79.3	91.4	85.6	83.7	80.2	61.5	69.2	66.2	71.9	50.2	83.2	



### Vita

Kuei-Jyum (Carol) Yeh was born in Tainan, Taiwan, Republic of China. She received a BS degree in Environmental Engineering from National Chung Hsing University in 1985. She worked as a research associate in the Department of Environmental Engineering at National Chung Hsing University from 1985 to 1986. She then pursued graduate study at VA Tech. She received a MS degree in Environmental Engineering in May 1988 and continued her studies receiving her Ph.D. in Civil engineering in March 1992.

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