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**AEROBIC BIODEGRADATION OF FUEL  
OXYGENATES**

THESIS

Adam M. Gutshall, Captain, USMC

AFIT/GES/ENV/07-J1

**DEPARTMENT OF THE AIR FORCE  
AIR UNIVERSITY  
AIR FORCE INSTITUTE OF TECHNOLOGY**

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**Wright-Patterson Air Force Base, Ohio**

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AFIT/GES/ENV/07-J1

AEROBIC BIODEGRADATION OF FUEL OXYGENATES

THESIS

Presented to the Faculty

Department of Systems and Engineering Management

Graduate School of Engineering and Management

Air Force Institute of Technology

Air University

Air Education and Training Command

In Partial Fulfillment of the Requirements for the  
Degree of Master of Science in Engineering and Environmental Management

Adam M. Gutshall, BS

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June 2007

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AEROBIC BIODEGRADATION OF FUEL OXYGENATES

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### **Abstract**

Methyl tert-Butyl Ether (MTBE) is the most commonly used fuel oxygenate in the world. Its recalcitrant nature as well as its chemical properties have led to widespread groundwater contamination. Questions regarding its toxicity have spurred a search for viable oxygenate alternatives. Since biodegradability is a key indicator of a chemical's environmental impact, this research used three different well-known methods, BOD<sub>5</sub>, respirometry, and GC analysis, to examine the extent and rates of aerobic biodegradation of MTBE along with tert-butyl alcohol (TBA). The common fuel component toluene was added to some of the samples to determine if the presence of a co-contaminant would effect aerobic microbial degradation of TBA or MTBE.

This group of experiments used an acclimatized microbial consortium to enhance degradation of the oxygenates. BOD<sub>5</sub> experiments were performed separately from the GC and respirometric analyses. The respirometry used 250ml microcosms containing a mix of microbial seed, BOD buffer, and varying concentrations of the oxygenates or oxygenate/toluene mixtures. The respirometer also maintained the microcosms in aerobic conditions for the duration of each experiment. For GC analysis, samples were drawn from the respirometer microcosms at predetermined intervals and first order degradation rate constants were calculated from established calibration curves.

The oxygenates degraded much slower than toluene in all experiments. This degradation characteristic made BOD<sub>5</sub> analysis impractical for MTBE or TBA. BOD<sub>5</sub> did provide valid results for toluene. The respirometer data was not as good as gas chromatography to provide specific measurements of degradation. To facilitate comparison of degradation across experiments with differing seed, oxygenate degradation was compared to toluene. MTBE was effectively degraded under these experimental conditions and degraded at 13.94% the rate of toluene. TBA was more recalcitrant and only degraded at 1.37% of toluene.

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## I. Introduction

### Background

The use of oxygenates in fuels is not a new phenomenon. Research into the use of fuel additives began as early as the 1920s (Moyer, 2003). Oxygenates work by increasing the octane index and improving the combustion efficiency of gasoline. This, in turn, enhances engine performance and decreases the release of unwanted pollutants, primarily carbon monoxide. Due to its blending characteristics, low cost, and ease of production, Methyl *tert*-butyl ether (MTBE) has become the leading oxygenate used in the United States (Squillace et al., 1998). MTBE use in the United States has increased drastically over the last 20 years and in 1999 over 200,000 barrels were produced daily (U.S. EPA, 2006a).

MTBE first was used in the late 1970s as a replacement for *tetra*-ethyl lead. Lead has been used as a fuel additive since the 1920s because it effectively increases octane ratings and reduces engine knock. But by 1924, fifteen workers had died from lead exposure during manufacture and shipping of *tetra*-ethyl lead (Kovarik, 1994). At very low concentrations, lead was also discovered to cause nervous system damage and slow growth in children (U.S. EPA, 2007a). In spite of the early warnings, use of leaded gasoline continued in the United States until the EPA issued a reduction standard in 1973 and then completely eliminated the sale of leaded fuel for on-road automobiles in 1996. Because lead acts to protect vital engine parts under heavy loads, small amounts of leaded fuels are still permitted for use in off-road vehicles (farm/construction machinery, race cars, boats, and aircraft) (U.S. EPA, 2007a).

MTBE again saw an increase in its use with the passage of the 1990 Clean Air Act Amendments (CAA). It should be noted that the CAA do not specify which oxygenate is used to meet the fuel oxygen requirements. The CAA uses a two-pronged approach to achieve better air quality: the Winter Oxyfuel Program and the Year-round Reformulated Gasoline Program. The

winter oxyfuel program was implemented in 1992. It requires gasoline in cities that do not meet the National Air Quality Standards for carbon monoxide to be 2.7% oxygen by weight during winter months. The Year-round Reformulated Gasoline (RFG) Program, implemented in 1995, requires gasoline to be a minimum of 2 percent oxygen by weight in cities with the worst ground level ozone (U.S. EPA, 1998). Currently 30 percent of the gasoline produced in the United States gasoline is RFG, and, of that 30 percent, approximately 87 percent contains MTBE (U.S. EPA, 2006b).

### **Problem**

Oxygenates have been very successful at reducing carbon monoxide emissions. The White House Office of Science and Technology Policy reported in 1997 an approximate 10 percent reduction of ambient carbon monoxide measurements in cities affected by the winter oxygenated fuel programs (OSTP, 1997). In spite of its successes at reducing atmospheric pollutants, the increase in use of MTBE has lead to widespread releases by auto emissions; evaporation; storage tank and pipeline leaks; accidental spills and refinery releases (Ahmed, 2001). Fuel underground storage tank releases have had the most significant impact on MTBE occurrence in the environment, and in 2005 the EPA confirmed that there have been over 447,000 fuel releases from leaking underground storage tanks (LUST) (U.S. EPA, 2007b). The extent of the problem was further identified with the release of the 2001 Toxic Release Inventory. It estimated releases of 3,289,087 pounds of MTBE into the atmosphere, 63,575 pounds to surface water and 4,255 pounds to the soil (U.S. EPA, 2003).

Once released into the environment, MTBE is able to spread due to its high solubility in water; low octanol water coefficient ( $K_{ow}$ ); and its ability to resist microbial attack and degradation (Deeb *et al.*, 2000). MTBE is significantly more soluble and has a much lower  $K_{ow}$

than other constituents of gasoline (Zanardini *et al.*, 2002). So while other fuel components adsorb to soil particles and are retarded, MTBE plumes continue to move with subsurface waters creating plumes that can out distance the fuel plume by greater than 4,000 feet (Johnson and Miller, 2003). Further complicating the problem is MTBE's recalcitrant nature. Its chemical structure of an ether bond (C-O-C) and its tertiary carbon structure enable it to resist degradation and remain in detectable quantities after other fuel components are degraded (Fayolle *et al.*, 2001). This in turn results in widespread MTBE contamination of surface and ground water drinking supplies.

In an attempt to understand the MTBE problem on a national scale, the U.S. Geological Survey (USGS) collected and compiled local, state and federal water sampling data from 1985 through 1995 and found that up to 7 percent of the 2948 wells sampled were contaminated with MTBE (U.S. EPA, 1999). Currently only portions of 17 states and the District of Columbia require RFG use, but it has been detected in water supplies of 35 states 20 percent of the times they have sampled for it (Stephenson, 2002).

MTBE has been widely studied, but much of its potential for causing disease is unknown. Currently the EPA considers MTBE only a taste and odor concern and has designated a drinking water advisory based on odor and taste thresholds of 20 to 40 micrograms per liter (Squillace *et al.*, 1998). Gasoline exhaust containing MTBE has been suggested to cause headaches, dizziness, nausea, sore eyes and respiratory irritation, but no definitive scientific studies can support MTBE's role in these symptoms (McCarthy and Tiemann, 2003). The most pressing question has been centered on MTBE's carcinogenic properties. Laboratory studies have shown inhalation of MTBE at high concentrations causes cancer in laboratory animals (U.S. EPA, 2006a). As a result, the EPA has named MTBE as a potential carcinogen (U.S. EPA, 1997), but

due to the difficulties linking animal and human carcinogenic potential and insufficient toxicology studies the EPA has not issued an enforceable drinking water standard for MTBE (Zogorski *et al*, 2001). Due to the same gaps in scientific evidence, in 1998 the International Agency for Research on Cancer (IARC), The US National Toxicology Program, and California's Carcinogen Identification Committee all declined to list MTBE as a human carcinogen (McCarthy and Tiemann, 2003). In 2000, due to all of the questions surrounding MTBE, the EPA announced its intention to restrict or prohibit MTBE's use as a gasoline oxygenate under Section 6 of the Toxic Substances Control Act (U.S. EPA, 2000).

### **Research Objectives**

The growing concern over MTBE pollution in public drinking water, and its potential harmful effects, have resulted in the search for viable oxygenate alternatives. One of the key issues to selecting the most appropriate alternative will be its potential for biodegradation. This research will look at the aerobic biodegradation potential and rate of MTBE, *tert*-butyl alcohol (TBA) and toluene. These will then be compared with a previous study (Dietz, 2007), which determined the degradation rates of ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME) and ethyl alcohol (ethanol). Toluene served as a representative of other gasoline components as well as link between the two experiments.

### **Research Questions**

1. Will these oxygenates aerobically biodegrade and, if so, at what rate?
2. Can the aerobic biodegradation of these oxygenates be directly and accurately measured using GC?
3. Can O<sub>2</sub>/CO<sub>2</sub> consumption from BOD<sub>5</sub> and respirometry be correlated to a biodegradation rate of selected oxygenates?

4. Does the addition of the common fuel component, toluene, effect the degradation of the selected oxygenates?
5. Can this study be combined with previous studies to support selecting a replacement for MTBE based on aerobic biodegradation rates?

### **Research Methodology**

The primary measure of aerobic biodegradation will be gas chromatography (GC). The GC will measure concentrations over time by comparison of peak areas with a known concentration curve. In addition, the GC will be combined with respirometry measurements to determine the microbial consumption of oxygen and production of carbon dioxide. A comparison of respirometry and GC measurements will be performed to analyze microbial activity relative to oxygenate degradation. Separate analysis of these chemicals will be performed using a five-day biochemical oxygen demand (BOD<sub>5</sub>) test.

### **Scope of Research**

This research will primarily determine if selected oxygenates can be aerobically biodegraded and, if so, can it be measured directly using the gas chromatograph. It will also determine if this direct measure of oxygenate concentration, combined with respirometry and BOD<sub>5</sub>, will correlate the reductions in chemical concentration to aerobic microbial activity. This research will be limited to aerobic degradation and should only be considered a start for the comparison of microbial aerobic degradation of selected oxygenates.

All experiments will be performed under controlled laboratory conditions and will not attempt to replicate the varying degrees of O<sub>2</sub> levels, differences in microbial consortia, presence of other chemicals, or other environmental factors that would potentially be found in different release sites. Carbon losses to biomass and conversion into other chemicals during multi-step



degradation pathways will be beyond the scope of this research. While carbon losses are important this research will focus on the direct measure of reduction of starting material over time.

## II. Literature Review

### Overview

This chapter will review the history of fuel oxygenate use up to its current status. It looks at the problems associated with the production and use of oxygenates, including a discussion of fuel oxygenates' common fate and transportation in the environment, as well as their biodegradation characteristics. This chapter reviews the fuel oxygenates relative toxicity and what is being done to address the problem. To develop a complete understanding of the oxygenate problem, it is appropriate to include a discussion of the fate, transport, biodegradation and toxicity of the other most mobile gasoline components, BTEX. A discussion of the methods used in this experiment, to include: utilizing GC to measure specific loss, respirometry uses and possible drawbacks, and the measure of biodegradability using Biochemical Oxygen Demand ( $BOD_5$ ), ends the chapter.

### Oxygenate History

Oxygenates serve to improve engine performance by reducing incomplete combustion and engine knock. More recently, oxygenate additives have gained attention due to their success at reducing harmful exhaust emissions. The search for ways to boost gasoline engine performance with ether additives dates back to the 1920s when oil company research began. The timetable for the use of oxygenates is outlined in Table 2.1 (Drogos, 2000).

Initially, *tetra*-ethyl lead was the primary oxygenate used, but due to concerns of lead's environmental and health impacts the EPA eliminated the use of *tetra*-ethyl lead in automotive gasoline. During the phase out of leaded gasoline, MTBE became the additive of choice due to its superior blending characteristics and ease of production.

MTBE was initially added to gasoline at 1-8 percent by volume. That number has steadily increased to the current 11 – 15 percent oxygen by volume required in order to meet the requirements of the 1992 Clean Air Act Amendments. In the United States, MTBE accounts for greater than 80 percent of oxygenates used while ethyl alcohol (ethanol) makes up 15 percent and the remaining 5 percent is made up of various other oxygenates (U.S. EPA, 1998).

Table 2.1 Chronology of Use of Oxygenates in U.S. Gasoline (Drogos, 2000)

1920s	Oil company research on ether additives to boost octane
1930s	Alcohols added to gasoline to boost octane
1930/40s	Ethanol-blended gasoline sold in Midwest U.S.
1950s	American Petroleum Institute literature speaks of the applicability of using MTBE in gasoline
1969	TBA was blended into gasoline
1973	The first commercial use of MTBE in Italy
mid-1970s	MTBE and other ethers were added to gasoline to enhance octane and as extenders during the Arab oil embargo
1978	Gasohol program began, adding 10% ethanol by volume in gasoline
1979	MTBE added to gasoline in order to boost octane as a replacement for lead, typically at <1% by volume in regular and 2-8% in premium
1980s	Ether use increases as lead continues to be phased out
1988	Denver, CO implements winter oxygenated fuel program
1989	Southwestern U.S. implements winter oxygenated fuel program
1990	U.S. Clean Air Act Amendments require use of oxygenates in reformulated gasoline
1992	U.S. implements winter oxygenated fuel program, requiring 2.7% oxygen by weight (equivalent to 15% MTBE or 7.3% ethanol by volume) in 40 U.S. metropolitan areas
1995	U.S. implements Reformulated Gasoline Phase I, requiring 2.0% oxygen by weight (equivalent to 11% MTBE or 5.4% ethanol by volume) year-round in 28 U.S. metropolitan areas
1996	California implements California Air Resources Board Phase 2, requiring 2.0% oxygen by weight state-wide and year-round
2000	U.S. implements Reformulated Gasoline Phase II still requiring 2.0% oxygen by weight

### Oxygenate Production and Transportation

This steady increase in usage has led to a marked increase in production of MTBE. In 1993 the United States production of MTBE reached a total of 20 to 24 billion pounds, which made it the second highest produced organic chemical nationally (Reisch, 1994). In spite of

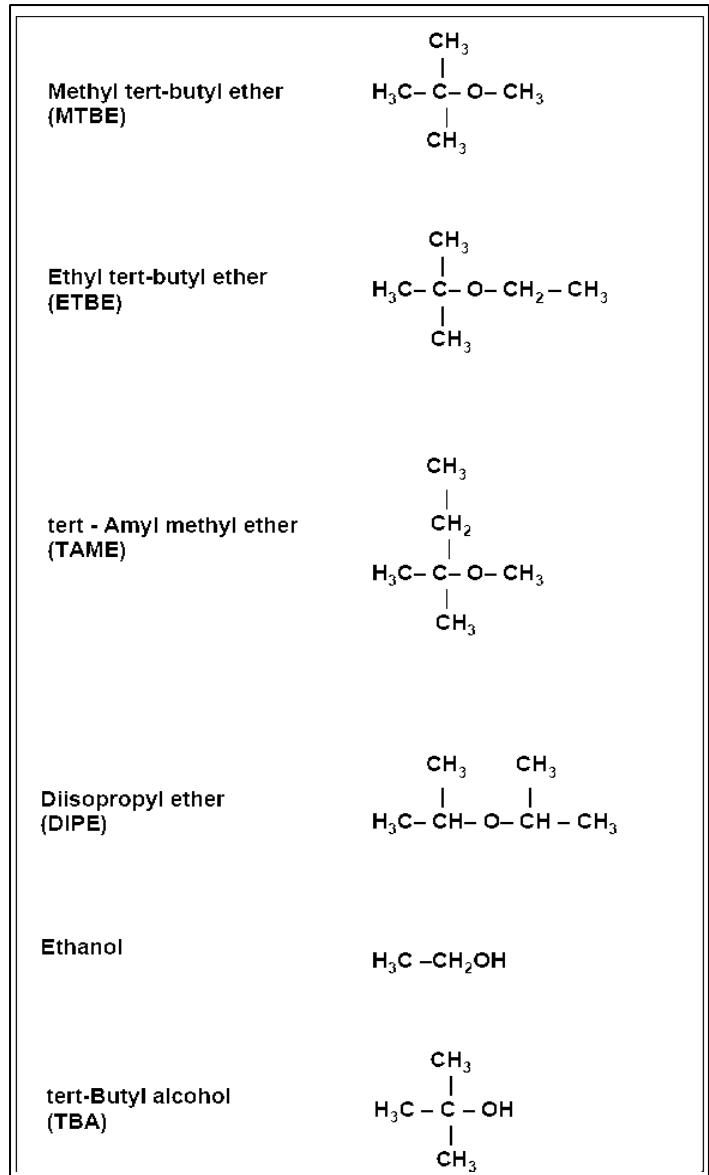
government subsidies, cost of production and transportation continue to favor MTBE over the second leading oxygenate, ethanol. MTBE's superior blending characteristics allow it to be stored and shipped along with gasoline. Ethanol blended gasoline is more unstable and the ethanol must be blended with gasoline

just prior to use. If stored for an extended amount of time, ethanol will begin to separate out of the gasoline and will draw moisture into the fuel, rendering it unusable (U.S. EPA, 1998). The other ether oxygenates, ethyl *tert*-butyl ether, *tert*-amyl methyl ether, diisopropyl ether, all have the same favorable gasoline blending characteristics as MTBE.

The ease of transport of MTBE through existing gasoline distribution pipelines and trucks has led to inadvertent releases throughout the United States. While oxygenate use, primarily MTBE, is only required in selected metropolitan areas of 17 states

and the District of Columbia, it has been detected in 35 states 20% of the time it was sampled for and of those, 24 states found detectable levels in 60 percent of their samples (Stephenson, 2002).

Figure 2.1 molecular Structure of Common Fuel Oxygenates ( U.S. EPA, 2004)



## **Classes of Oxygenates**

The current oxygenates can be separated into two broad classes, ethers and alcohols. Their chemical structures are shown in Figure 2.1 (U.S. EPA, 2004). In order to make comparisons, the general chemical properties of the most common gasoline oxygenates will be depicted in the figures and tables, but for the purpose of this study MTBE, TBA and the gasoline aromatics (BTEX) will be covered in more detail.

### **Ethers**

Ethers are organic molecules characterized by their C-O-C bonds and the presence of tertiary or quaternary carbon structures. Both of these attributes contribute to the difficulty of microorganism's ability to attack and biodegrade these compounds (Kinner, 2001). Ethers in general tend to migrate farther and faster than other fuel components in subsurface environments because they generally do not absorb well onto organic soil particles and are highly water soluble (Fayolle and Monot, 2005). The most common ether oxygenates used include: methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME) and diisopropyl ether (DIPE). The chemical properties of the ether oxygenates are very similar and are listed in Table 2.1.

### **MTBE**

Of all the fuel oxygenates, MTBE is the most widely used and studied. As seen in Figure 2.1, MTBE has a relatively complex chemical structure that serves to provide some resistance to microbial attack. MTBE is a colorless flammable liquid with a distinct taste and odor (ATSDR, 1997). MTBE's very low taste and odor threshold, at approximately 50ppb, may convey some protection from ingestion of contaminated water (HEI, 2001). MTBE does have some limited alternate uses apart from its role as a fuel oxygenate. It has been used medically to digest ulcers,

as a laboratory extractant in analytical laboratories, and for some chemical synthesis, but its alternate uses are extremely small in comparison to its use as an oxygenate (Moyer, 2003).

### **Alcohols**

Alcohol-based oxygenates chemical structure is characterized by the addition of a hydroxyl (O-H) group bonded to an alkyl group. This removal of a hydrogen and addition of the hydroxyl group can be done naturally, as the fermentation products of certain carbohydrates, or synthetically as in the production of TBA (U.S. EPA, 2004). Of the alcohol oxygenates used today, ethanol is the most prevalent, and in 1999 56 billion gallons of ethanol blended gasoline were sold in the United States alone (Powers *et al.*, 2001). Due to its recalcitrant nature, harmful human side effects, and its occurrence with MTBE at contaminated sites, *tert*-butyl alcohol (TBA) has been the focus of many studies.

### ***tert*-Butyl Alcohol**

As seen in Figure 2.1, TBA is an alcohol molecule with three methyl groups attached to a tertiary carbon. It can be manufactured by either catalytic hydration of isobutylene or reduction of *tert*-butyl hydroperoxide (Clark, 2002). At normal room temperature it is a colorless clear crystalline solid with a camphor like odor (OSHA, 1996). Once TBA reaches its melting point of 25.6° C (78.1° F) it forms a flammable, volatile, clear liquid. The presence of TBA in many gasoline release sites can be attributed to several causes: 1. TBA can be used in fuel directly as a gasoline oxygenate; 2. unreacted TBA may be present due to the use of TBA and methanol during the manufacture of MTBE; 3. TBA is a possible intermediate byproduct of MTBE biodegradation (Moyer, 2003). In addition to its use as a gasoline oxygenate, TBA is utilized in many manufacturing process such as the production of plastics, polymers, paint removers,

insecticides, and pharmaceuticals (Zhuang *et al.*, 2005). This creates the added potential for additional TBA environmental releases separate from those associated with gasoline.

Table 2.2 Chemical Properties of Common Fuel Oxygenates (Howard, 1997)

Oxygenate	Pure Phase Solubility (mg/L)	log Kow (log l/kg)	Log Koc (log l/kg)	Vapor Pressure (25C, mmHg)	Henry's Law Constant (Dimensionless)
Methanol	Miscible	-0.75	0.44	121.58	0.0001
Ethanol	Miscible	-0.16	0.2	49	0.00025
TBA	Miscible	0.35	1.57	40	0.00048
MTBE	43000	1.2	1	245	0.024
DIPE	2039	1.52	1.46	149	0.052
ETBE	26000	1.74	1	152	0.108
TAME	20000	1.6	1.3	68.3	0.052

## BTEX

BTEX is an acronym for common fuel components benzene, toluene, ethyl benzene, and the three forms of xylene (m-, o-, and p-xylene) (Adam *et al.*, 2002). Once released into the environment, these volatile aromatic hydrocarbons react very differently from MTBE and the other oxygenates. A comparison of the BTEX properties in Table 2.2, with the oxygenate properties in Table 2.1 shows distinct differences in solubility and carbon partition coefficients. These chemical properties create unique issues when oxygenate containing gasoline is released into the environment. BTEX compounds are readily biodegradable in most subsurface environments. This can often create unique clean up problems since most likely BTEX and oxygenate initial release sites are going to be collocated. Once the contaminants enter groundwater, they move at distinctly different rates, creating plumes of varying sizes and concentrations. Further complicating the issue, BTEX compounds tend to degrade much more readily than oxygenates and require the use of different treatment options.

Table 2.3 BTEX Properties (Zanardini *et al.*, 2002)

Physical and chemical Properties	Water solubility (mg/L)	Dimensionless Henry's law constant	Log $K_{oc}$	Log $K_{ow}$	Vapor Pressure (mmHg at 25°C)
<b>Benzene</b>	1730	0.23	1.18-2.16	2.36	76.95
<b>Toluene</b>	534	0.272	1.56-2.25	2.73	28.4
<b>Ethylbenzene</b>	161	0.336	1.98-3.04	3.24	9.53
<b><i>o</i>-Xylene</b>	175	0.212	1.68-1.83	3.10	6.6

### Environmental Fate and Transport

The fate and transport of gasoline components can be loosely predicted by two main chemical properties, the water solubility and the organic carbon partition coefficient ( $K_{oc}$ ).

#### Oxygenates

Oxygenates in general, are able to quickly and effectively migrate in groundwater. This ability can be attributed mainly to their low affinity for organic carbon ( $K_{oc}$ ) and relatively high solubilities in water. In general, oxygenates have a much lower  $K_{oc}$  than that of the other gasoline components. This characteristic allows oxygenates to more freely move along with subsurface waters, without adhering to the organic material in the soil (Wilson, 2003). The relative solubility in water of most oxygenates is much higher than that of the BTEX components. These two characteristics combine to create oxygenate plumes that can far out distance the BTEX pollutants (Wilson, 2003). To further demonstrate the MTBE and TBA problem, MTBE's  $K_{oc}$  and solubility are orders of magnitude higher than that of the BTEX chemicals and TBA's  $K_{oc}$  and solubility also are dramatically much higher.

#### BTEX

As previously stated, when water solubility and  $K_{oc}$  of the gasoline BTEX components are compared to the oxygenates it is easy to predict that the aromatic BTEX components of



gasoline will move slower in the environment than oxygenates. BTEX compounds have been found to be difficult to dissolve into ground water and preferentially volatilized into the interstitial spaces or absorbed onto the soil particles (Jean *et al.*, 2002). When these characteristics are combined with their ability to quickly biodegrade, BTEX plume migration will stabilize much sooner than oxygenates. In Stocking's review of Bioremediation strategies, he found that once released into the environment BTEX plumes generally stabilize at less than 260 feet from the release site, and that when gasoline contains MTBE, the BTEX plumes can reach lengths of 300 feet (Stocking *et al.*, 2000). The release of a gasoline from a service station at the Naval Base in Ventura County, CA is a good illustration of the different fate and transport characteristics of BTEX and oxygenates. In this case, the MTBE plume out distanced the dissolved BTEX plume by more than 4,000 feet (Johnson *et al.*, 2003).

### **BTEX and Oxygenate Combinations**

When oxygenate and BTEX chemicals are combined they may affect the plume size and characteristics of each. For all the oxygenates discussed, with the exception of ethanol, preferential degradation of BTEX components of gasoline deplete available oxygen and inhibit oxygenate degradation which results in long oxygenate plumes (Sedran *et al.*, 2002). Ethanol is the exception to this rule. It is degraded more readily than the other gasoline components, so it retards the degradation of BTEX (Wilson, 2003). An increase in the length of BTEX plumes is also caused by the oxygenates ability to lessen the absorptive capacity of subsurface soils (Adam *et al.*, 2002).

## **Biodegradation**

### **Oxygenate**

The gasoline oxygenates, such as MTBE and TBA, have historically been difficult to biologically degrade. Both have relatively complex chemical structures, which make them resistant biodegradation. It was previously believed that MTBE was recalcitrant, but many recent studies have demonstrated the ability to degrade MTBE and TBA (Deeb *et al.*, 2000). The ether oxygenates, specifically, MTBE, has an ether bond and a tertiary carbon structure which is resistant to microbial attack (Fayolle *et al.*, 2001). The complex chemical structure of TBA resists microbial degradation. The TBA molecule, as seen in Figure 1, consists of three methyl groups attached to a tertiary carbon (Zhuang *et al.*, 2005). TBA is an intermediate by-product of MTBE biodegradation. TBA's possible accumulation as an intermediate by product from the break down of MTBE must be taken into consideration when attempting to calculate accurate degradation rates for TBA.

More favorable degradation rates for TBA can be achieved under aerobic conditions because more energy is available to the microorganism utilizing oxygen as the final electron acceptor. A site in Port Hueneme, California, was able to show TBA degraded aerobically and demonstrated a marked increase in aerobic biodegradation with the addition of bioaugmentation (Salanitro *et al.*, 2000). Another site in CA saw increases in TBA degradation with the injection of oxygen into the ground water plume (Mackay *et al.*, 2001). Propane oxidizing bacteria have also shown the ability to degrade TBA under aerobic conditions (Steffan *et al.*, 1997).

During a study into utilizing propane-oxidizing bacteria to degrade MTBE, Steffan *et al.*, were able to examine degradation of TBA (Steffan *et al.*, 1997). During the degradation of MTBE, concentrations of TBA increased. Then, once the application of propane was stopped,

degradation of MTBE slowed and TBA concentrations declined rapidly. In some instances they were able to achieve complete mineralization of TBA, but at a much slower rate than the degradation of MTBE.

The large majority of biodegradation studies starts with degradation of MTBE and, as a consequence, looks at TBA degradation. Day and Gulliver (2003) were able to specifically examine TBA degradation by studying a TBA plume from a Texas chemical manufacturing plant (Day and Gulliver, 2003). This site was unique in that the plume contained no MTBE. This enabled them to examine the degradation characteristics of TBA without the potential for more being generated from MTBE degradation byproducts. They found that biodegradation was occurring both aerobically and anaerobically. Natural streambed organisms have also been successful in degrading TBA aerobically (Bradley *et al.*, 1999).

### **BTEX**

BTEX compounds are readily biodegradable under varying conditions. Complex interactions occur between each of the compounds. Respirometry results of BTEX mixtures have shown increased rates of biodegradation for benzene, toluene, and p-xylene when they were combined (Goudar and Strevett, 1998). A good example of the complexity of BTEX chemical interaction is the determination that toluene alone can act as an inhibitory agent, slowing the degradation of benzene (Goudar and Strevett, 1998). Davis and Madsen (1996) found that toluene was capable of being completely degraded within 190 hours under varying conditions (Davis and Madsen, 1996).

### **BTEX / Oxygenate Combination**

BTEX compounds are more readily biodegradable than all of the oxygenates except ethanol. Current studies indicate that the addition of MTBE and TBA will not affect BTEX

degradation, but BTEX compounds appear to inhibit oxygenate biodegradation by competing for available nutrients and electron acceptors (Deeb *et al.*, 2000). When looked at individually, toluene slowed the rate of MTBE degradation while benzene, ethylbenzene, and the xylenes completely inhibited MTBE biodegradation (Deeb *et al.*, 2000). The rapid degradability of ethanol results in a very different effect on gasoline mixtures. Addition of ethanol to gasoline tends to inhibit BTEX compound biodegradation by exerting a large oxygen demand and out competing BTEX for the available oxygen and nutrients (Lovanh *et al.*, 2002).

### **Oxygenate Toxicity**

The toxicity of MTBE has been the most widely studied of all the oxygenates and due to its occurrence as a break down product of MTBE, TBA has received much attention as well.

#### **MTBE**

In spite of the fact that MTBE may be one of the most studied chemicals on earth, not enough evidence exists to definitively understand all of the potential side effects from MTBE exposure (Ellis, 2001). The majority of the human data is related to inhalational rather than ingestional exposures (Davis, 2002).

Short-term inhalational exposure to MTBE has been determined to cause nose and throat irritation in humans (ATSDR, 1997). In short-term rat studies both ingestional and inhalational exposures produced similar effects, primarily affecting the central nervous system, kidneys and liver (Ahmed, 2001). A conclusive link between the animal and human acute effects cannot be established, since the concentrations used during these tests were much higher than the concentrations that would be encountered by the general public (HEI, 2001). Also human side effects to inhalational MTBE exposure could not be separated from the possible side effects of other chemicals gasoline engine emissions. No human data exists for long-term effects of

MTBE exposure, but animal studies have pointed to the possibility that MTBE is a possible carcinogen causing kidney and liver cancer (ATSDR, 1997). Because of the uncertainty surrounding MTBE exposure the EPA has labeled MTBE a possible carcinogen (U.S. EPA, 1997) and established drinking water advisories based on taste and odor concerns of 20 to 40 Micrograms per liter (Squillace *et al.*, 1998).

### **TBA**

TBA toxicity in many ways resembles that of MTBE. TBA is a major metabolite of MTBE. Dealkylation into TBA and formaldehyde is the first step in the metabolization of MTBE (HEI, 2001). In rats, the urinary tract has been identified as TBA's target of toxicity with males being more susceptible than females (Lindamood *et al.*, 1992). TBA was also shown to be capable of producing kidney tumors in rats (63 Cirvello *et al.*, 1995). Both of these metabolite products, TBA and formaldehyde, are identified as probable carcinogens by the EPA (U.S. EPA, 1999).

### **BTEX Toxicity**

Benzene, toluene, ethyl benzene, and the xylenes all have individually been determined to cause some adverse health effects in humans. Benzene is the most toxic and the only one determined to be a human carcinogen (ATSDR, 2005). It has been shown to cause leukemia, a cancer of the blood forming organs, through long term inhalational exposure. The major site of noncancerous action is also related to the blood, it affects bone marrow, resulting in increased risk of infection and anemia. The Agency for Toxic Substances and Disease Registry (2005) also reports that benzene can cause tremors, confusion, and unconsciousness. Toluene preferentially affects the nervous system and can cause tiredness, confusion, nausea, as well as, memory and hearing loss (ATSDR, 2001). While very limited information is available for

ethylbenzene it has been shown capable of causing dizziness and some animal studies have shown nervous system, liver and kidney effects (ATSDR, 1999). The forms of xylene, normally found in BTEX, only affect humans at doses that are much higher than what would be experienced in daily background exposures. At high doses xylene can cause dizziness and confusion (ATSDR, 2005). No present studies are available to assess the human health effects of combinations of BTEX components. But, the U. S. Department of Health and Human Services suggests that Pharmaceutical based P kinetic models point to an additive joint action on the nervous system from exposure to BTEX (ATSDR, 2004).

### **Substance-specific Primary Degradation**

Pagga (1997) defined primary degradation as the loss of material identity. Simply, it identifies the transformation of a certain chemical from its original form and may not take into account other degradation products, losses due to the accumulation of biomass, or release into the atmosphere governed by Henry's constant and vapor pressure (Pagga, 1997). If specific degradation by-products are known, then calculations allow insight into relative times that a chemical spends in certain steps along its degradation pathway. The EPA approved method for the measure of specific loss of MTBE and BTEX compounds is method 8015 GC/FID (U.S. EPA, 2004). This method requires the use of a Gas Chromatograph with a flame ionizing detector. Oxygenates, with their high solubility, lend themselves to this method of analysis since they would tend to stay dissolved in the water, leading to more accurate results (Pagga, 1997). This method, when combined with some form of biochemical oxygen demand or carbon dioxide production, can lead to a more in-depth understanding of biodegradation.

### **Respirometry**

Biodegradation is a key indicator of a chemical's long-term environmental fate and ecological impact (Miles and Doucette, 2001). Respirometry is capable of accurately measuring biodegradation with minimal experimental and analytical effort. By measuring the oxygen uptake and carbon dioxide production as indicators of biodegradation, chemical biodegradation kinetics can be estimated (Goudar and Strevett, 1998). Respirometry is also valuable in determining degradation of insoluble or poorly water-soluble chemicals that would tend to partition out of the aqueous phase (Pagga, 1997).

### **Utilization of O<sub>2</sub>**

The measure of O<sub>2</sub> consumption using respirometry is a viable and reproducible measure of biodegradation. Since O<sub>2</sub> consumption does not take into account the amount of chemical that is broken down into intermediate by products, it may not reflect the O<sub>2</sub> required for complete mineralization of test chemical. Therefore, O<sub>2</sub> usage values, represented as a %ThOD (percent of theoretical oxygen demand), can produce a useful measure of the catabolism of a specific chemical (Miles and Doucette, 2001). Miles and Doucette were also able to demonstrate that biodegradation based on O<sub>2</sub> usage was comparable to other test methods that measured CO<sub>2</sub> production or a direct loss of chemical.

### **Production of CO<sub>2</sub>**

Measures of CO<sub>2</sub> production are valuable in determining the complete mineralization of the test chemical (Pagga, 1997). However, CO<sub>2</sub> is susceptible to numerous different sources and sinks that make it a sometimes-unreliable measure of microbial respiration and chemical biodegradation (Miles and Doucette, 2001). It does not take into account the carbon that is accumulated as biomass. Reuschenbach *et al.* (2003) reports that, in general, CO<sub>2</sub> production that results in greater than 60% of theoretical CO<sub>2</sub> production is indicative of sufficient

biodegradation (Reuschenbach *et al.*, 2003). Davis and Madsen (1996) found that biodegradation determined by CO<sub>2</sub> production alone might be insufficient to estimate the rate and extent of biodegradation. During their study of the biodegradation of toluene utilizing radio-labeled carbon, only 29 to 56% of the labeled carbon was recovered as CO<sub>2</sub> (Davis and Madsen, 1996).

### **Five-Day Biochemical Oxygen Demand (BOD<sub>5</sub>)**

The standard BOD<sub>5</sub> (biochemical oxygen demand) test is a valuable tool in measuring the amount of organic material that is capable of being biodegraded. It is one of the most important means of evaluating water quality and assessing the environmental impact of wastewater discharges on natural waters (Young *et al.*, 2005). The test procedure is relatively straightforward, and with the exception of the use of a probe to measure dissolved oxygen, the basic procedure has remained relatively unchanged since the late ninetieth century (Min *et al.*, 2004). The test, as outlined in the 21<sup>st</sup> edition of *Standard Methods for the Examination of Water and Wastewater* (2004), involves mixing known ratios of dilution water, microbial seed, and contaminated sample into airtight bottles for five days at 20°C. Initial values for dissolved oxygen are then compared to values at the end of incubation and adjusted for seed uptake and dilution. As with any scientific test, accurate and consistent procedures must be followed. Minor variations in the measure of initial dissolved oxygen can result in errors as high as 6.9% and minor variations in BOD bottle volume can result in errors of -4.8% (Chiang *et al.*, 2006).



### **III. Research Methodology**

#### **Experimental Design**

This experiment examined the microbial biodegradation of MTBE, *tert*-Butyl alcohol and toluene using three different techniques: gas chromatography (GC), respirometry and biochemical oxygen demand (BOD<sub>5</sub>). The BOD<sub>5</sub> was run separately, while the respirometry portion was conducted in combination with gas chromatography.

#### **Microbial Seed**

The microbial seed used for the duration of the experiment originated from a petroleum refinery's industrial wastewater treatment facility. This seed was stored in two one-liter bottles and kept under constant aeration. Double strength BOD buffer (HACH Chemical Co.) was added to provide nutrients, control the accumulation of toxins, and replace water losses due to evaporation. Every four days 0.01ml of toluene and 0.003ml of MTBE and TBA were added to each bottle in order to acclimatize the seed. In an effort to maintain a high number of microorganisms, approximately one gram of beef extract was dissolved into deionized water and added to each bottle weekly.

#### **Respirometry**

The respirometry portion of this experiment was conducted using a Columbus Instruments Micro-Oxymax respirometer. Utilizing an expansion interface the respirometer was capable of monitoring 20 chambers for O<sub>2</sub> utilization and CO<sub>2</sub> production. For each experiment, 19 test bottles were sampled and one channel was connected to a section of silicone tubing which sampled atmospheric gasses. 17 of the experimental sample bottles (microcosms) were filled with 160 ml of deionized water, BOD buffer, 40 ml of acclimatized microbial seed, along with

varying concentrations of test chemical, while two bottles contained no test chemical and were designated as seed controls.

The 250ml microcosms were fitted with caps containing a septum. This allowed drawing aqueous samples without disrupting the collection of O<sub>2</sub> and CO<sub>2</sub> data from the closed system. The samples drawn for GC analysis were less than 5% of the headspace volume, so no additional water was added to maintain a constant headspace volume. In order to protect the sensors from liquid entering the system, cross contamination between the chambers, and bacterial contamination, a PTFE hydrophobic filter was installed in the gas sampling line from each chamber. Two external driers, fitted with hydrophobic filters were installed to prevent water contamination of the sensors and expansion unit.

Due to the lack of calibration gas, the first experiment relied on previous calibrations of the respirometer. Prior to the second experiment the gas sensors were calibrated using calibration gas supplied by the Weiler Welding Company. The calibration gas was a mixture of 20.5% oxygen, 0.74% carbon dioxide and the balance of gas was nitrogen. In combination with calibration, each chamber was automatically measured for headspace volume.

The operation parameters were identical for each experiment and are listed in Appendix A. The settings for each experiment allowed for maximum sensitivity of the oxygen sensors by balancing sampling intervals, headspace size and refresh intervals. The oxygen and carbon dioxide sensors were adjusted to enable ranges of 10 to 21% for oxygen and 0 to 1% for carbon dioxide.

### **Gas Chromatography (GC)**

For the chromatographic analysis portion of this experiment, a HP Series II Gas Chromatograph with an integrated Flame Ionizing Detector (GC-FID) was used. The installed

capillary column was a J&W Scientific DB-624(#123-1334) with a DuraGuard deactivated fused silica column guard (#160-2325-5). The MSD Chemstation software (Build 75, 26 August 2003) controlled the GC-FID, and with the use of its integrator function, peak areas were calculated. Integration parameters are listed in Appendix B.

Auto injection method parameters, established by Dietz (2007), were utilized to maintain continuity between the two experiments and are listed in Appendix B. An identical GC-FID method was used for each fuel oxygenate tested and was capable of producing identifiable and measurable peaks at the determined retention times.

The conversion of peak area to concentration was accomplished by establishing a calibration curve for each chemical. The chemicals were analyzed at known dilutions and plotted against the GC-FID response peak areas. Each of the chemicals was diluted into deionized water using glass pipettes and volumetric flasks, and for all dilutions smaller than 0.001, successive dilutions were made from a stock 0.001 solution. For GC analysis, the solutions were then pipetted into 2ml amber vials and sealed with a PTFE lined caps. The same GC-FID method was then used to establish the peak areas relative to known concentrations. A best fit line and equation was determined by using Microsoft Excel. Calibration curves for each tested chemical are found in Appendix C.

Method detection limits (MDL) were calculated in accordance with the Code of Federal Regulations (CFR) (40 CFR 136, 1993). A summary of the MDLs calculated for each chemical are listed in Table 3.1 and detailed results can be found in Appendix B. The MDL was calculated utilizing the equation below to determine the lowest level of chemical that can be accurately quantified using the specific GC-FID method developed for this experiment.

$$\text{MDL} = \text{SD} \times t_{0.99}$$

$$\text{Where SD} = \left\{ \frac{\sum_{i=1}^n (x_i - X)^2}{(n-1)} \right\}^{1/2}$$

Where MDL = method detection limit (ppm); SD= standard deviation;  $t_{0.99}$ = t-distribution table value for 99% with the degree of freedom (n-1);  $x_i$ = spiking replicates concentration (i=1...n); X= the mean of spiking concentrations.

Table 3.1 Method Detection Limits

Chemical	GC-FID Method Detection Limit (ppm)
Toluene	0.703
Methyl <i>tert</i> -Butyl Ether	0.302
<i>tert</i> -Butyl Alcohol	0.275

One ml samples were taken from the 250ml respirometry bottles at predetermined intervals and analyzed using the GC-FID. The samples were drawn with a 2ml glass syringe and six inch small gauge needle through the septum in the cap. To ensure cross contamination of samples did not occur, the syringe and needle were triple rinsed with deionized water between sampling events. The samples were then placed into 2ml amber vials and capped with the PTFE/rubber lined crimped cap for analysis with the GC-FID.

### Five-Day Biochemical Oxygen Demand (BOD<sub>5</sub>)

The BOD<sub>5</sub> portion of this experiment was conducted in accordance with Standard Methods 21<sup>st</sup> Edition, 5210A. (Greenberg, 2005). In an effort to minimize the oxygen demand of the dilution water blank, deionized water was aerated and incubated at 20°C for 24hours prior to each experiment. HACH BOD nutrient buffer pillows were added to the deionized water and thoroughly mixed according to the instructions. In preparation of the glucose/glutamic acid standard, the glucose and glutamic acid were dried at 103°C for one hour and stored in a

desiccator for a period of no more than 24hours. 150mg of the dried glucose and 150mg of the dried glutamic acid was added to one liter of deionized water.

The BOD<sub>5</sub> was conducted in 29 standard 300ml flared mouth BOD bottles with ground glass stoppers. Three bottles were dedicated to the dilution water blank check and only BOD buffer mixed with deionized water was added. Six bottles served as seed controls and varying amount of seed was added to the dilution water. The glucose/ glutamic acid check was analyzed in three of the bottles with 6ml of the glucose/glutamic acid solution, either 6 or 3ml of seed suspension, and dilution water. The remaining 18 bottles were divided into three sets of six bottles containing varying amounts of each test chemical combined with 3 or 6ml of seed suspension and dilution water. Each bottle was initially filled approximately 2/3 full of dilution water. Test chemical or glucose/glutamic acid solution, and seed suspension were then added. Each bottle was then filled with dilution water so that the insertion of the ground glass stopper displaced all the air.

Prior to insertion of the stopper, initial dissolved oxygen (DO) was measured using a Yellow Springs Instruments Company (YSI) model 5100 Dissolved Oxygen Meter with an YSI 5010 BOD probe. The meter and probe were allowed to warm up for a minimum of 1 hour prior to calibration and use. Calibration was performed, prior to each experiment, using the auto calibration feature on the 5100 DO meter and dilution water. Dissolved oxygen was then measured and recorded for each bottle. To ensure the consistency of the BOD probe measurements, the DO was checked in the initial calibration dilution water between each series of samples. Glass stoppers were placed on each bottle and deionized water was added to the top to ensure an airtight seal. To guard against evaporation of the deionized water around the glass stopper, plastic caps were placed on top of each bottle.

The bottles were placed into a dark incubator for five days. After incubation, the dissolved oxygen was measured and recorded using the same calibration and DO sampling procedures as previously stated. Using the seed blanks, the oxygen uptake per milliliter of seed could be determined using the slope method. Each test was only considered successful if all the test criteria outlined in Standard Methods 5210B. were met. This included: dilution water blanks must have used  $< 0.20$  mg/L of the DO; the glucose/glutamic acid check equal to  $198 \pm 30.5$  mg/L; and each bottle was only considered if the DO used was greater than two mg/L and at least 1.0 mg/L of DO remained.

## IV. Data Analysis

### Introduction

For this section the respirometry and gas chromatography data will be presented and discussed together. The BOD<sub>5</sub> experiments were run independent of the other experiments and their results will be discussed separately.

### Respirometry and Gas Chromatography Results

The respirometry and gas chromatography data was collected in two separate experiments. Each of the experiments consisted of three test chambers dedicated to toluene, four TBA, and four MTBE alone, while TBA and MTBE were mixed with toluene in three test chambers apiece. Data collection started as soon as the test chambers were filled. Initial concentrations were determined at day zero using the gas chromatograph and samples were then drawn at day 0.5, 1, 1.5, 2, 2.5, 5, 10, and day 15. The second experiment ended two days early due to a power outage which interrupted the respirometry data collection.

All gas chromatography samples were run using the GC method outlined in Appendix B, and the resulting chromatographs were then analyzed using the integration function parameters listed in Appendix B as well. Sample chromatographs are presented in Appendix C. The peak areas were converted into concentrations using the established calibration curves. First order decay was assumed for each chemical and the decay constant,  $\lambda$ , was calculated utilizing the equation below.

$$\lambda = -(\ln(C/C_0)) * (1/t)$$

Where: C = concentration at time t  
C<sub>0</sub> = Concentration at time zero  
t = elapsed time

Respirometry data was collected for the entire duration of each experiment and the complete data for each experiment is presented in Appendix C. The respirometry data was collected as  $\mu\text{l}/\text{min}$  and total  $\mu\text{ls}$  of  $\text{CO}_2$  produced and  $\text{O}_2$  consumed. The total theoretical oxygen demand (ThOD) and carbon dioxide (Th $\text{CO}_2$ ) production were then calculated using initial concentrations determined from the GC analysis. These values were used to determine percentages of ThOD and Th $\text{CO}_2$  for each chamber.

Prior to the start of the first experiment the respirometer was not properly calibrated due to lack of calibration gas. The experiment was continued in the hope that it would produce reliable results for each of the chambers relative to the background chambers containing only seed and BOD buffer. This did not happen and, as reflected in the data in Appendix C, produced negative theoretical values for each chamber with wildly fluctuating graphs for usage rates. Each of the graphs do tend to mirror each other relative to the background chamber and some inference of increased oxygen usage and carbon dioxide production can be inferred but no reliable data can be collected. Since no chamber went below 20.5%, the chambers still served the valuable purpose of maintaining an oxygen saturated environment.

A summary of the respirometry and GC data for both experiments is presented below in Table 4.1. The complete set of data from GC analysis is presented in detail in Appendix C.



Table 4.1 Summary of Respirometry and GC Experimental Data

Experiment	Chemical	Duration (days)	Average $\lambda$ (day <sup>-1</sup> )	CO <sub>2</sub> Production (% Theoretical)	O <sub>2</sub> Consumption % Theoretical
1	Toluene	2.5	0.608	-95.23	-45.31
1	TBA	15	0.009	-15.08	-35.42
1	TBA/Toluene	15	0.008	-125.24	-50.68
1	MTBE	15	0.113	-50.46	-18.82
1	MTBE/Toluene	15	0.108	-64.71	-22.98
2	Toluene	2	1.060	87.11	29.87
2	TBA	13	0.013	25.56	7.52
2	TBA/Toluene	13	0.083	211.41	62.92
2	MTBE	13	0.103	44.54	12.09
2	MTBE/Toluene	13	0.107	235.77	68.02

From GC analysis, toluene did not degrade uniformly over both experiments. The reduction in concentration had a dramatic effect on the calculated  $\lambda$ . Experiment #2 resulted in a  $\lambda$  of 1.060 while Experiment #1, with the higher concentration had a much lower  $\lambda$  at 0.608. The average  $\lambda$  for toluene over all experimental runs was 0.834. The varying ranges of toluene's calculated  $\lambda$ 's, create high levels of variance when calculating the overall percentages of oxygenate degradation in relation to toluene degradation. Consistent results for toluene are valuable in creating a base line for comparison to the oxygenates tested by Dietz (2007).

GC analysis revealed that, under this set of experimental conditions, TBA was recalcitrant or had extremely low degradation rates. When TBA was examined alone, the results for Experiment #2 showed better degradation than Experiment #1. As shown in Table 4.1 the average  $\lambda$  for Experiment #2 was 144% that of Experiment #1. Since the starting concentration in Experiment #2 was less than in Experiment #1, this increase in the degradation coefficient may infer microbial toxicity of TBA at increasing concentrations. Even with the improved

degradation in Experiment #2, the best degradation still only resulted in a reduction from 9.9 ppm to 7.7ppm over the course of 13 days.

With the addition of toluene to the TBA, Experiment #1 showed little to no change in the calculated degradation coefficient  $\lambda$  from the TBA alone. But, Experiment #2 demonstrated a remarkable order of magnitude improvement of the degradation coefficient with the addition of toluene to TBA. An examination of the complete GC data in Appendix C reveals that overall, all three TBA/ toluene samples in Experiment #2 showed increased  $\lambda$ , but one sample in particular was unexplainably 4-7 times higher than the others from Experiment #2. If this stray value is ignored, the increase in TBA degradation with the addition of toluene in Experiment #2 is 4 times that of the TBA alone in Experiment #2.

Contrary to TBA, MTBE generated a consistent  $\lambda$  during both experiments. This stayed the same regardless of the addition of toluene throughout both experiments as well. During Experiment #1, TBA began to appear at low concentrations, demonstrating its presence as a breakdown product of MTBE. As seen in the complete Experiment #1 GC data located in Appendix C, the very low concentrations of TBA did eventually degrade to below identifiable concentrations. Experiment #2 did not accumulate measurable amounts of TBA during the MTBE degradation, most likely due to the lower initial concentrations of MTBE.

### **BOD<sub>5</sub> Results**

The BOD<sub>5</sub> portion of the experiment was conducted in four separate BOD<sub>5</sub> runs, and the methodology for each is described in detail in chapter three. The complete data from the BOD<sub>5</sub> experiments is presented in four separate tables in Appendix C. A summary of the data that met all the required experimental controls, as outlined in Standard Methods (Greenberg, 2005), is listed below in Table 4.2.

The first BOD<sub>5</sub> experiment used 3ml of seed for each of the test bottles and the glucose/glutamic acid check. While this produced glucose/glutamic acid checks that were consistent with the published standards of 198+/-30.5, none of the TBA or MTBE samples used the required 2.0 mg/l of oxygen, and only three of the five toluene samples utilized the required oxygen. Taking these results into consideration, the amount of seed used was raised to 6ml in each of the 300ml BOD test bottles for the remaining BOD<sub>5</sub> experiments.

Table 4.2 Summary of BOD<sub>5</sub> Data

Chemical	BOD <sub>5</sub> Experiment Number	Seed Volume Volume ml/300ml	Percent ThOD	Average %ThOD Per experiment	Average % ThOD
Toluene	1	3	24.04	16.63	23.30
Toluene	1	3	15.79		
Toluene	1	3	10.05		
Toluene	3	6	27.71	26.83	
Toluene	3	6	28.35		
Toluene	3	6	26.18		
Toluene	3	6	25.94		
Toluene	3	6	25.97		
Toluene	4	6	25.39	26.44	
Toluene	4	6	25.33		
Toluene	4	6	24.64		
Toluene	4	6	32.22		
Toluene	4	6	26.23		
Toluene	4	6	24.85		

For the second set of BOD bottles, the glucose/glutamic acid check was extremely high, with the BOD<sub>5</sub> ranging from 345 to 347 mg/l. These values far exceed the required 198 +/- 30.5 that is required by the standard methods for the examination of water and wastewater 21<sup>st</sup> edition (Greenberg, 2005). Even though the test was invalid, it provided some insight into the possible degradation of TBA. Under these conditions, TBA produced decreasing BOD<sub>5</sub> values for higher concentrations, possibly indicating some toxicity at the higher concentrations. MTBE did not produce any BOD<sub>5</sub> results that utilized more than the required 2.0mg/l of oxygen.

BOD<sub>5</sub> experiment #3 produced valid results and the experimental controls were all within published ranges. Four of the five toluene samples produced consistent % ThOD values that ranged from 25.94 to 28.35 percent. For this run, the concentrations of both MTBE and TBA were lowered with the largest addition being 0.0035ml added to the 300ml bottle, producing maximum concentrations of  $9.2 \times 10^{-6}$  mg/l for TBA and  $8.6 \times 10^{-6}$  mg/l for MTBE. Still, neither the TBA nor the MTBE produced any results that utilized the required 2.0 mg/l of oxygen with the highest amount used being 0.56 mg/l.

For BOD<sub>5</sub> experiment #4 the concentrations of MTBE and TBA were lowered even more and the amount of seed used per test bottle remained at 6ml/ 300ml bottle. The maximum concentrations used was  $2.6 \times 10^{-6}$  mg/l for TBA, and  $2.5 \times 10^{-6}$  mg/l for MTBE. Again all experimental controls were met, but no single sample containing MTBE or TBA used the required amount of oxygen. The test was successful in producing valid results for all six of the toluene samples that were tested. All the valid toluene results are listed in Table 4.2.

## V. Conclusions and Recommendations

### Summary

The purpose of this group of experiments was to determine if aerobic biodegradation of fuel oxygenates methyl *tert*-butyl ether and *tert*-butyl alcohol would occur, and if so, could it be accurately measured using respirometry, gas chromatography, or BOD<sub>5</sub>. Using the experimentally determined concentrations, the first order degradation coefficient  $\lambda$  was calculated in order to compare degradation rates. In addition to the fuel oxygenates alone, the aerobic biodegradation rates for MTBE and TBA in the presence of the co-contaminant toluene were examined.

### Answers to Specific Research Questions

1. Will these oxygenates aerobically biodegrade and, if so, at what rate?

The data collected indicates that the selected oxygenates will aerobically biodegrade. Of the two, MTBE experienced a faster and more complete aerobic biodegradation, while TBA exhibited a more recalcitrant nature.

MTBE provided a consistent degradation coefficient regardless of amount of seed or the presence of co-contaminant toluene. The average  $\lambda$  for MTBE was 0.1075 across all experiments, with a range of 0.108 (MTBE alone) to 0.107 (MTBE and toluene).

The TBA biodegradation coefficient was not as consistent as MTBE. In Experiment #1, TBA alone, and TBA with the co-contaminant toluene, degraded at relatively the same rates generating a  $\lambda$  of 0.009 for TBA alone and 0.008 for TBA and toluene. These values are greater than an order of magnitude slower than the degradation coefficients calculated for MTBE. During Experiment #2, there was an increase in the calculated  $\lambda$  to 0.013 for TBA alone. Since Experiment #2 used approximately half the concentration of experiment one,

this may indicate some microbial toxicity to higher concentrations of TBA. Experiment #2 also saw an increase in the average  $\lambda$  for TBA with toluene. This increase was much more dramatic and produced an average  $\lambda$  8 to 10 times that of the other calculated TBA  $\lambda$ s. This increase in degradation may demonstrate that TBA's optimal degradation is at lower concentrations in conjunction with a co-contaminant.

2. Can the aerobic biodegradation of these oxygenates be directly and accurately measured using gas chromatography?

Gas chromatography appears to be capable of producing accurate and consistent results. With the development of appropriate GC methods and an accurate concentration curve, GC is an appropriate measure for each of the oxygenates tested and toluene. Statistical analysis of the calculated  $\lambda$  is presented in Appendix C. While all three chemicals were capable of being accurately measured, according to the statistical evaluation of the calculated degradation coefficients, MTBE was the most precise.

It is important to note that GC data does not take into account losses due to transformation of carbon into biomass or the loss of chemical due to volatilization. Steps were taken to minimize volatilization, including minimizing head space and limiting the refresh function on the respirometer. Due to its much higher Henry's constant, toluene would be much more susceptible to losses due to volatilization, while both MTBE and TBA have Henry's constants as much as three orders of magnitude lower. Due to their affinity for the water phase, the losses due to volatilization of MTBE or TBA were minimal in comparison to losses due to microbial activity.

The GC was also capable of identifying known breakdown products if they should accumulate above the MDL for the GC. MTBE in Experiment #1 did just that. It

produced TBA peaks after several days into the first experimental run. The identification of those peaks served several purposes; one, it demonstrated the recalcitrant nature of TBA in comparison to MTBE; two, it can account for some of the lost ThOD and the reason why the ThOD produced by the respirometer does not coincide with GC data; and three, the small concentrations of TBA did readily degrade.

3. Can O<sub>2</sub>/CO<sub>2</sub> consumption from BOD<sub>5</sub> and respirometry be correlated to a biodegradation rate of selected oxygenates?

BOD<sub>5</sub> and respirometry data did not produce consistent results that could be correlated to the degradation rates calculated by GC analysis. The problems experienced in the first respirometry/GC experiment were discussed in Chapter Four and demonstrate the importance of proper calibration of respirometry equipment. Experiment #2 did produce more consistent results, but in several of the test bottles that contained a combination of oxygenate and toluene, the percent theoretical CO<sub>2</sub> production was much greater than 100%.

Despite its apparent limitations, the respirometry data was consistent with relative values for oxygen usage and carbon dioxide production. The more rapidly degrading toluene produced the most CO<sub>2</sub> (87.11% ThCO<sub>2</sub>) while utilizing the most O<sub>2</sub> (29.87% ThOD). In line with its much slower degradation, TBA consumed much less O<sub>2</sub> (7.52 % ThOD) and produced less CO<sub>2</sub> (25.56 % ThCO<sub>2</sub>), while MTBE was in between these two values. The complete respirometry data is listed in Appendix C and a summary of the averages are listed in Table 4.1. Even though the resulting numbers are not an accurate reflection of the degradation, graphical analysis of the rates of O<sub>2</sub> usage and CO<sub>2</sub>

production show peaks characteristic of the rapid degradation of toluene that takes place within the first 2 days.

BOD<sub>5</sub> data was not an appropriate measure for the degradation of TBA or MTBE. Both of the oxygenates degraded too slowly to be measured by BOD analysis and a more appropriate BOD test would be much longer. Since the degradation rate constant for toluene was 10 times that of MTBE and 100 times that of TBA, neither chemical would be a good candidate for a rapid BOD test. In an effort to reach the required 2.0mg/l oxygen consumption required by the Standard Method, the seed concentration was increased, but further increases in seed concentration would result in a glucose/glutamic acid check that would exceed the 198±30.5 mg/l BOD.

4. Does the addition of the common fuel component, toluene, effect the degradation of selected oxygenates?

The addition of toluene to MTBE made no difference to the degradation coefficient calculated for MTBE. MTBE degradation remained remarkably consistent through out each experiment. Statistical analysis in Appendix C shows that each of the degradation rates for MTBE, when compared to MTBE/ toluene combination, fall within the 95% confidence interval.

The second experiment did show a significant difference in the rate of TBA degradation in comparison to TBA/ toluene combination. While Experiment #1 reflected no difference in the degradation for TBA with or without the addition of toluene, in Experiment #2 the addition of toluene to TBA resulted in a degradation rate constant 10 times that of TBA alone. Lower concentrations of toluene and TBA may have allowed for enhanced microbial degradation.



5. Can this study be combined with previous studies to support selecting a replacement for MTBE based on aerobic biodegradation rates?

Differences in microbial seed, experimental controls, and varying chemicals, make it difficult to compare experimental data with already published results. This particular experiment used the same seed and experimental controls as Dietz (2007) in his analysis of fuel oxygenates, ETBE, TAME, and ethanol. As a way to link both experiments, toluene was chosen a common chemical.

Even with all the similarities it was impossible to generate the same degradation coefficients for the common chemical toluene. So, as an alternative, results will be analyzed as a percentage of toluene's degradation coefficient. Dietz found that ethanol degraded much faster than toluene (180.45%) while ETBE was 8.65% of toluene (95% C.I +/- 2.09) and TAME's calculated degradation coefficient was 8.93% of toluene (95% C.I. +/-4.12). For this experiment, as a percentage of the toluene degradation coefficient, MTBE was 14.18% of toluene (95%C.I. +/- 8.66), and TBA was 1.37% of toluene (95%C.I. +/- 0.28). These oxygenates in order of decreasing aerobic biodegradation potential, would be: Ethanol, MTBE, TAME, ETBE, and TBA.

### **Conclusion**

This thesis provided valuable information regarding the degradation of MTBE, TBA and toluene. At the same time, it also provided some insight into selecting an alternative to the currently used fuel oxygenate MTBE, based solely on aerobic biodegradation. Using the well characterized chemical toluene as link between the two separate experiments, allowed for broad comparisons of numerous fuel oxygenates. Understandably the selection of an MTBE

alternative will not be based on aerobic biodegradation alone, but hopefully this can provide an additional resource when making that decision.

### **Limitations**

The lack of proper calibration of the respirometer eliminated any of the first experiment's respirometry data from detailed analysis. The second experiment produced very limited valid results. The selection of respirometry as the sole source of degradation data, would appear to be problematic due degradation lag time and the overall accuracy of the respirometer. BOD<sub>5</sub> produced valid results for toluene but no other chemicals. Quite possibly another test similar to the BOD<sub>5</sub> could be run that would be much longer in duration in hopes of capturing BOD values with in range.

### **Opportunities for Further Research**

1. Repeat many of the same procedures, but add a way to quantify the amount of seed used, possibly through total suspended solids analysis.
2. Attempt to better quantify the losses due to volatilization by sampling the head space in the respirometry bottles.
3. Test the effects of different co-contaminants. Expand the study to look at the effects of other BTEX components on oxygenate degradation.
4. Examine the effect of oxygen concentration on the degradation of each of the oxygenates.

## Appendix A: Respirometry

Table A.1 Micro-Oxymax Respirometer Equipment Settings

<b>Parameter</b>	<b>Value</b>
Start Channel	1
Stop Channel	20
Sample Interval	5 hr
Sample Duration	0
Refresh Interval	0
Refresh Threshold	0.5
Refresh Window	Auto
Auto Volume Measurement	True
Purge Sensor Enabled	True
Switch Drier Enabled	False
Gas Data Units	$\mu\text{L}$
Time Units	MIN
Normalization Units	N.A.
Aux Temp start at Ch	0
Enable Open Flow	False

## Appendix B: Gas Chromatography

Table B.1 GC-FID Method

**6890 Gas Chromatograph**

Serial Number US 10339021

**Oven**

Initial temperature 40 °C  
Maximum temperature 260 °C  
Initial time 3.30 min  
Equilibration time 1.00 min  
Post temperature 150 °C  
Post time 0.50 min  
Run time 8.97 min

Ramp	Rate (°C/min)	Final Temperature	Final Time
1	30.0	120 °C	3.00
2	0.0(off)		

**Rear Inlet (Split/Splitless)**

Mode split  
Initial temperature 175 °C  
Pressure 18.00 psi  
Split ratio 10:1  
Split flow 46.6 ml/min  
Total flow 53.7 ml/min  
Gas saver on  
Gas type helium

**Capillary Column**

Model Number DB-624, Agilent part number 123-1334  
Inside Diameter 0.32 mm  
Length 30 m  
Film Thickness 1.8 µm

Dura-Guard deactivated silica column guard

Inside Diameter 0.32 mm  
Length 5 m

Maximum temperature 260 °C  
Nominal length 30.0 m  
Nominal diameter 320.00 µm

Table B.1 GC-FID Method (Continued)

Nominal film thickness	1.80 $\mu\text{m}$
Mode	constant pressure
Pressure	18.0 psi
Nominal initial flow	4.7 ml/min
Average velocity	61 cm/sec
Inlet	back
Outlet	front detector
Outlet pressure	ambient
<b><u>Flame Ionization Detector</u></b>	
Temperature	250 $^{\circ}\text{C}$
Hydrogen flow	40.0 ml/min
Air flow	450.0 ml/min
Mode	constant makeup+makeup flow
ml/min (on)	50.0 ml/min
Makeup gas type	nitrogen
Flame	on
Electrometer	on
Lit Offset	2.0
Signal 1	
Data rate	50 HZ
Type	front detector
Save data	on
Zero	0.0
Range	0
Fast peaks	off
Attenuation	0
<b><u>Injection Parameters</u></b>	
Injector location	back
Sample washes	2
Sample pumps	2
Injection volume	1 $\mu\text{l}$
Syringe size	10 $\mu\text{l}$
Pre injection solvent A washes	2
Pre injection solvent B washes	2
Post injection solvent A washes	2
Post injection solvent B washes	2
Viscosity delay	0 seconds
Plunger speed	fast

Table B.2 MSD Chemstation Integration Parameters

Integrator Event		
Name	Value	Time
Initial Area Reject	0	Initial
Initial Peak Width	0.015	Initial
Shoulder Detection	OFF	Initial
Initial Threshold	10	Initial



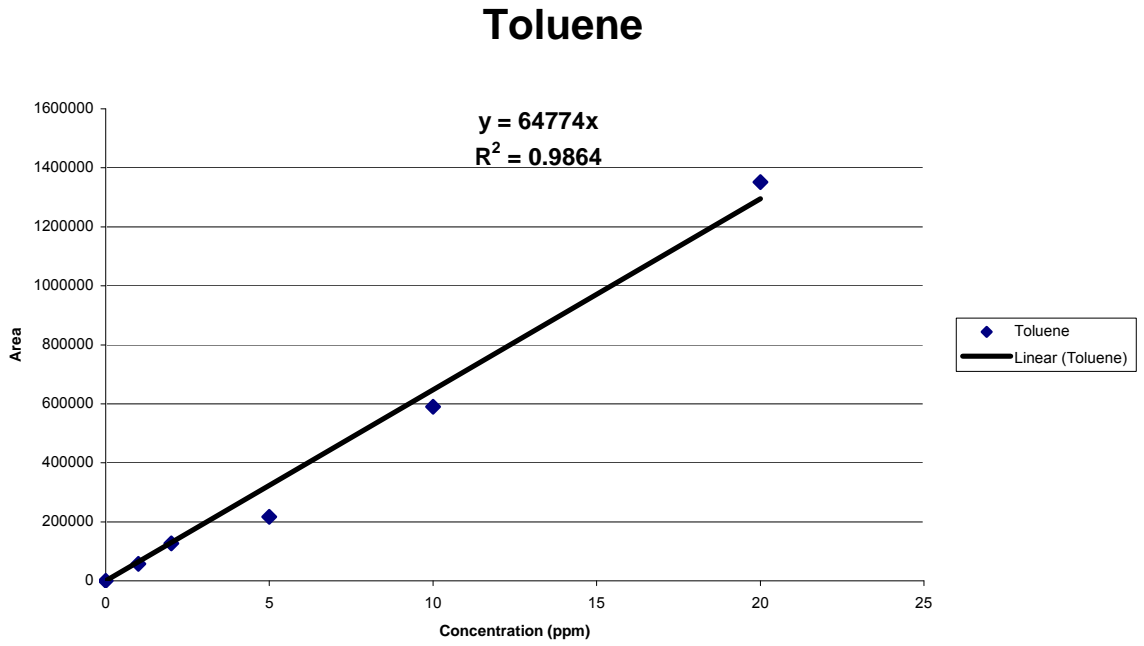


Figure B.1 Toluene Calibration Curve

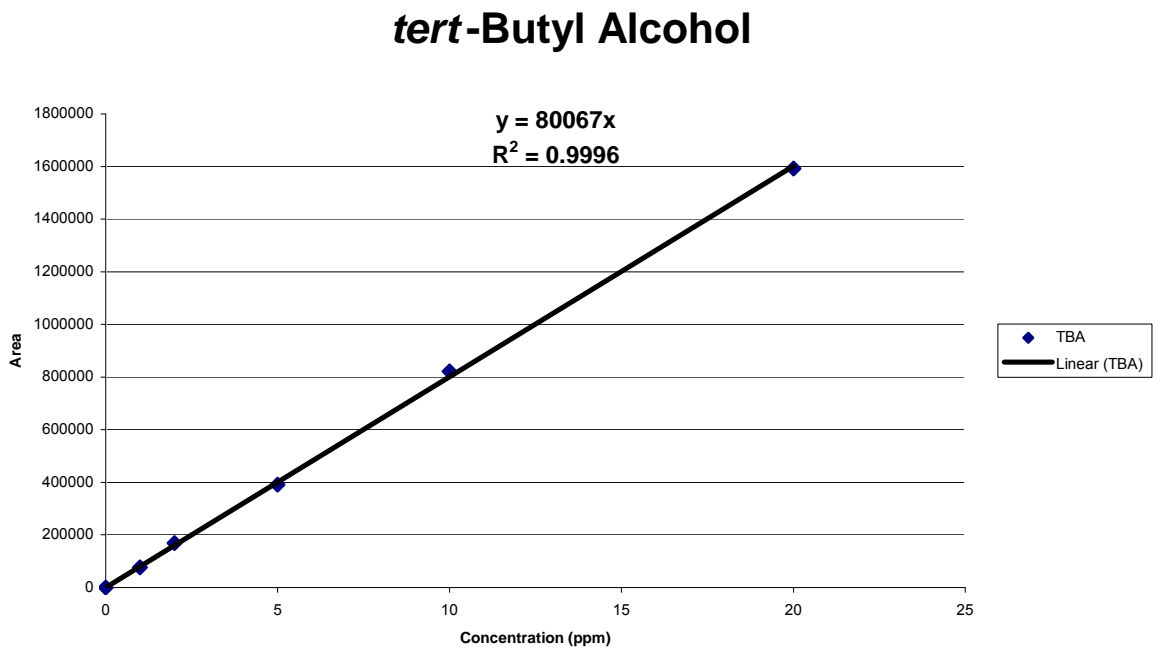


Figure B.2 *tert*-Butyl Alcohol Calibration Curve

## Methyl *tert*-Butyl Ether

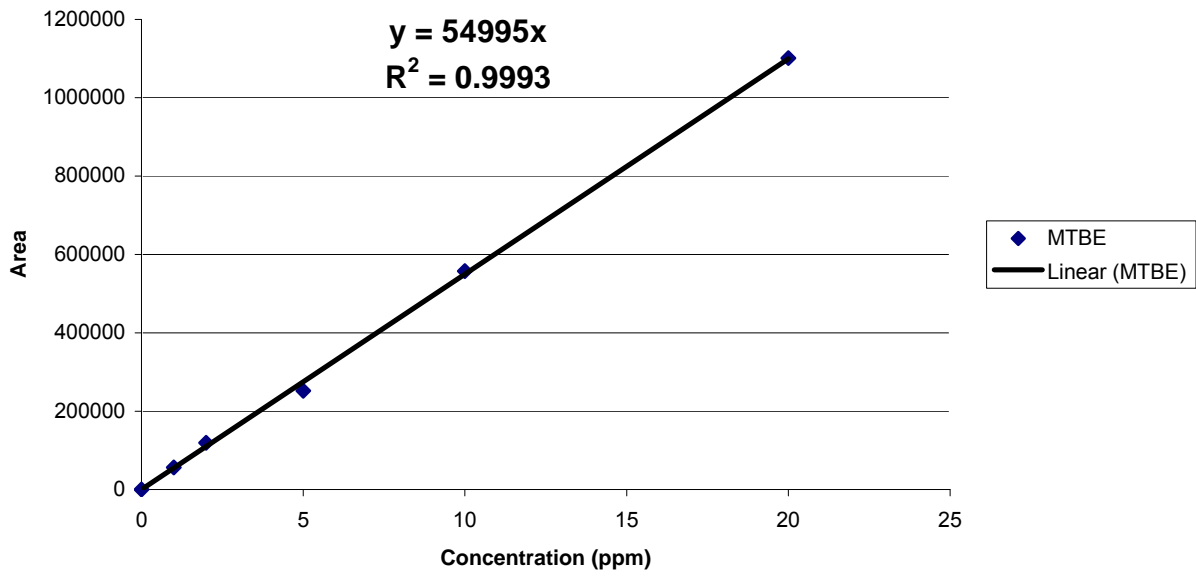


Figure B.3 Methyl *tert*-Butyl Ether Calibration Curve.

Table B.3 GC-FID Toluene MDL Calculations

<b>TOL</b>			
<b>Sample</b>	<b>Peak Area</b>	<b>Ret. Time</b>	<b>Conc.</b>
<b>1</b>	<b>54114</b>	<b>6.45</b>	<b>0.84</b>
<b>2</b>	<b>67365</b>	<b>6.45</b>	<b>1.04</b>
<b>3</b>	<b>52060</b>	<b>6.45</b>	<b>0.80</b>
<b>4</b>	<b>55386</b>	<b>6.45</b>	<b>0.86</b>
<b>5</b>	<b>54824</b>	<b>6.45</b>	<b>0.85</b>
<b>6</b>	<b>89649</b>	<b>6.45</b>	<b>1.38</b>
<b>StdDev</b>	<b>0.22</b>		
<b>t<sub>99</sub></b>	<b>3.14</b>		
<b>MDL</b>	<b>0.70</b>		

Table B.4 GC-FID MTBE MDL Calculations

<b>MTBE</b>			
<b>Sample</b>	<b>Peak Area</b>	<b>Ret. Time</b>	<b>Conc.</b>
<b>1</b>	<b>56848</b>	<b>3.41</b>	<b>1.03</b>
<b>2</b>	<b>48290</b>	<b>3.41</b>	<b>0.88</b>
<b>3</b>	<b>53781</b>	<b>3.40</b>	<b>0.98</b>
<b>4</b>	<b>45762</b>	<b>3.41</b>	<b>0.83</b>
<b>5</b>	<b>58070</b>	<b>3.40</b>	<b>1.06</b>
<b>6</b>	<b>47103</b>	<b>3.41</b>	<b>0.86</b>
<b>StdDev</b>	<b>0.096</b>		
<b>t<sub>99</sub></b>	<b>3.14</b>		
<b>MDL</b>	<b>0.30</b>		

Table B.5 GC-FID TBA MDL Calculations

<b>TBA</b>			
<b>Sample</b>	<b>Peak Area</b>	<b>Ret. Time</b>	<b>Conc.</b>
<b>1</b>	<b>87430</b>	<b>3.28</b>	<b>1.09</b>
<b>2</b>	<b>73842</b>	<b>3.29</b>	<b>0.92</b>
<b>3</b>	<b>89936</b>	<b>3.28</b>	<b>1.12</b>
<b>4</b>	<b>83155</b>	<b>3.29</b>	<b>1.04</b>
<b>5</b>	<b>80314</b>	<b>3.28</b>	<b>1.00</b>
<b>6</b>	<b>72727</b>	<b>3.29</b>	<b>0.91</b>
<b>StdDev</b>	<b>0.088</b>		
<b>t<sub>99</sub></b>	<b>3.14</b>		
<b>MDL</b>	<b>0.28</b>		

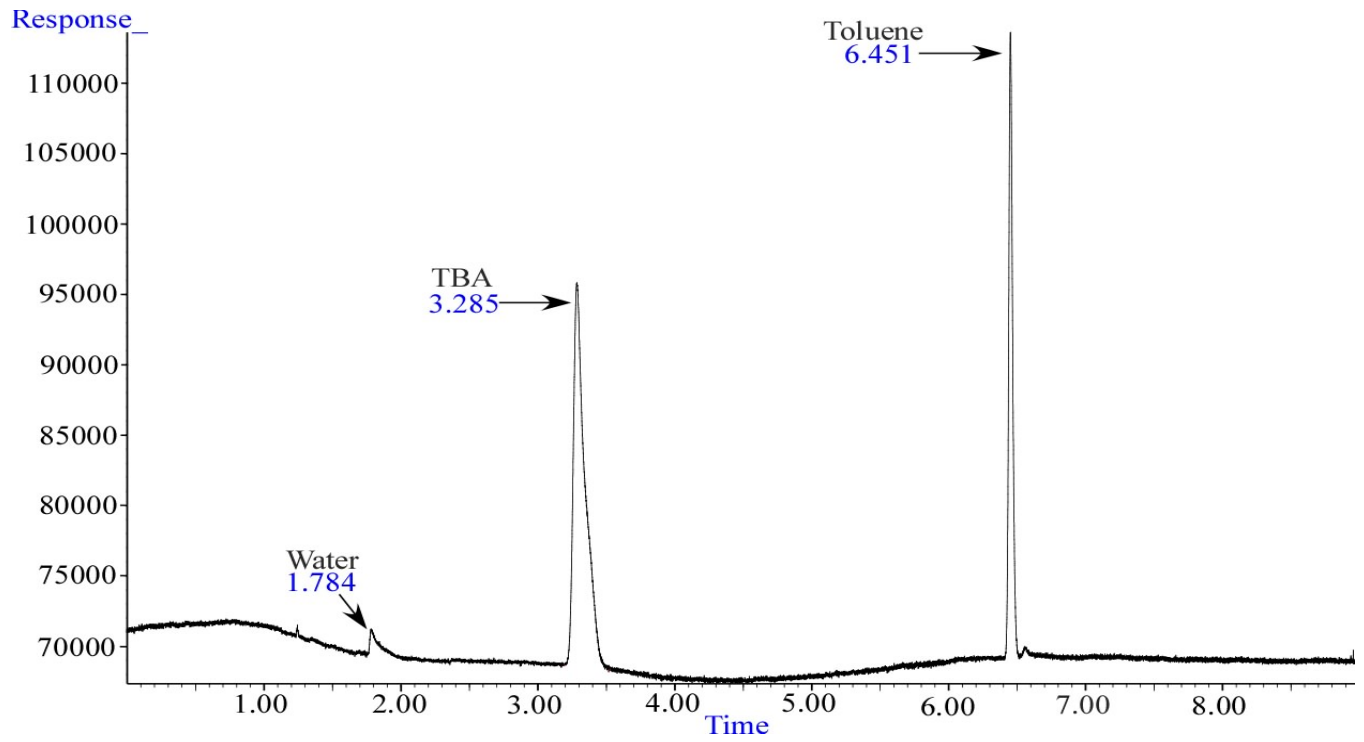


Figure B.4 Sample TBA and Toluene Chromatograph

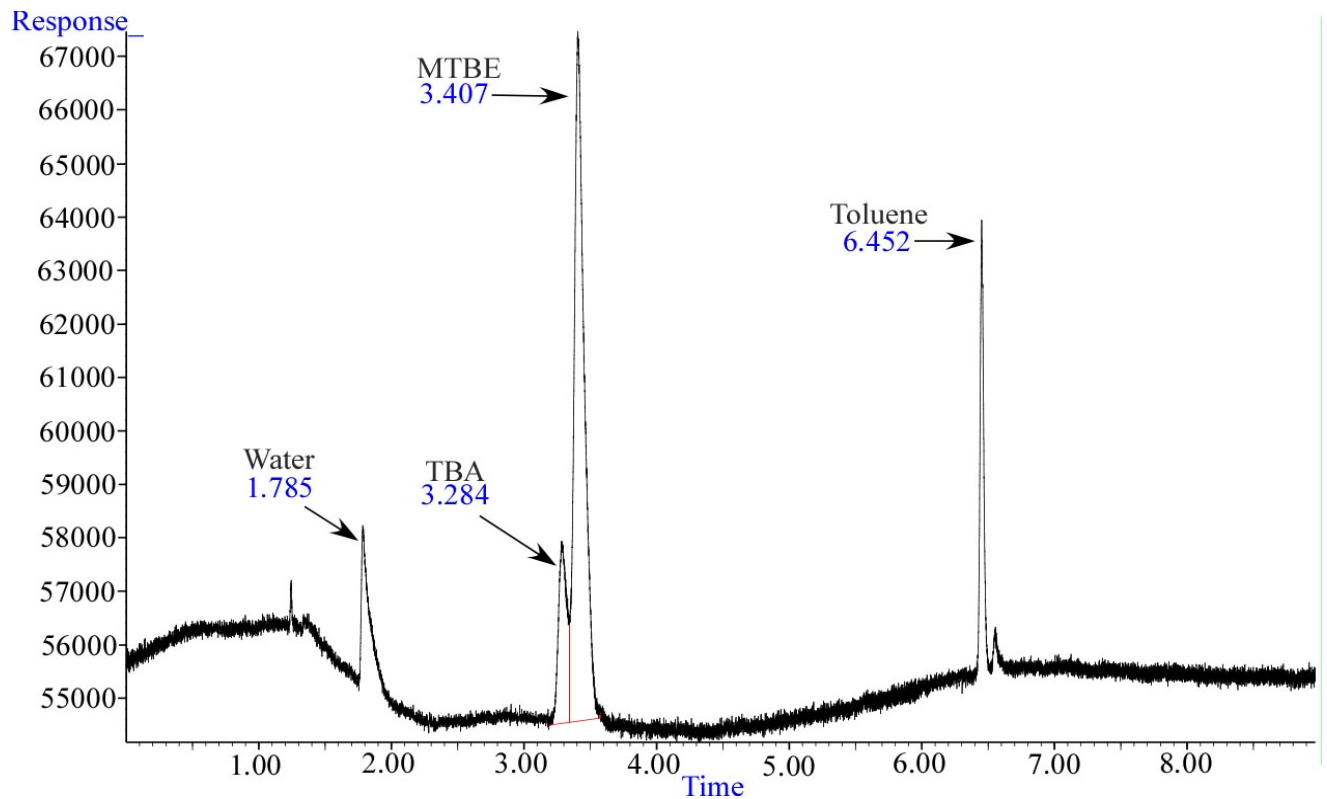


Figure B.5 Sample MTBE and TBA as Degradation By-product Chromatograph

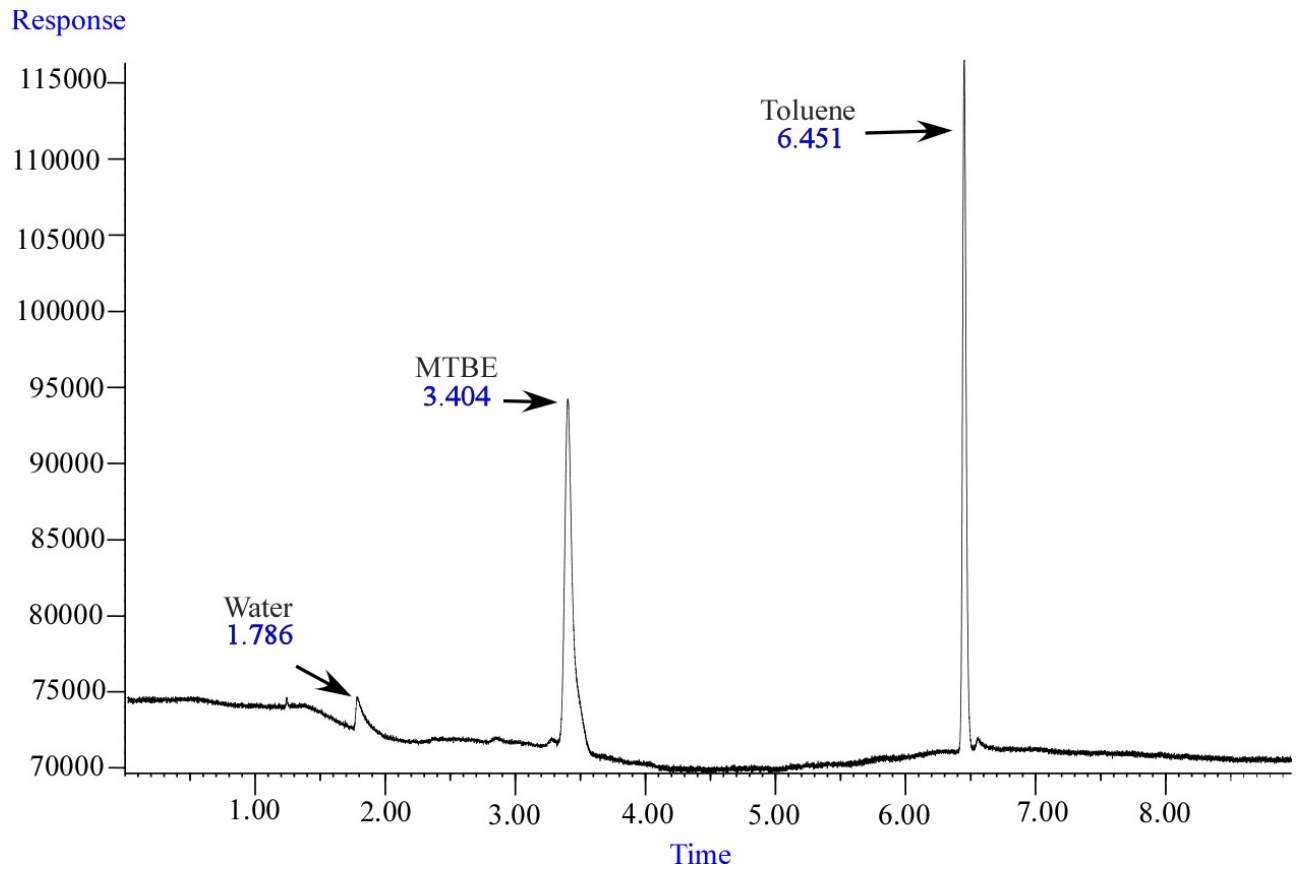


Figure B.6 Sample MTBE and Toluene Chromatograph

## Appendix C: Experimental Data

Table C.1 Complete BOD<sub>5</sub> Experiment #1 Data

Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)				
1	dilution water blank	300	9.25	9.08	0.17				
2	dilution water blank	300	9.23	8.94	0.29				
3	dilution water blank	300	9.26	9.2	0.06				
Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)	s			
4	seed	1	9.28	8.26	1.02	1.02			
5	seed	10	9.18	8.25	0.93	0.093			
6	seed	50	8.68	0.33	8.35	0.167			
7	seed	75	8.41	3.89	4.52	0.06			
8	seed	100	8.06	1.49	6.57	0.066			
s ave						0.063			
Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)	P	BOD mg/L	mg O <sub>2</sub> mg Chem	% ThOD
9	Glucose/Glutamic acid	6	9.14	5.22	3.92	0.02	186.55	0.62	61.86
10	Glucose/Glutamic acid	6	9.06	5.2	3.86	0.02	183.55	0.61	60.87
11	Glucose/Glutamic acid	6	9.07	5.18	3.89	0.02	185.05	0.61	61.36
12	toluene	0.001	9.25	7.65	1.6	3E-06	423315	0.49	15.60
13	toluene	0.0015	9.23	5.78	3.45	5E-06	652210	0.75	24.04
14	toluene	0.002	9.25	7.63	1.62	7E-06	214657.5	0.25	7.911
15	toluene	0.0025	9.26	5.5	3.76	8E-06	428526	0.49	15.79
16	toluene	0.003	9.25	7.86	1.39	1E-05	120105	0.14	4.43
17	toluene	0.0035	9.27	5.9	3.37	1E-05	272661.43	0.31	10.05
18	TBA	0.001	9.27	8.88	0.39	3E-06	60315	0.08	2.95
19	TBA	0.0015	9.25	8.84	0.41	5E-06	44210	0.06	2.16
20	TBA	0.002	9.25	8.14	1.11	7E-06	138157.5	0.18	6.76
21	TBA	0.1	9.24	8.61	0.63	3E-04	1323.15	0.002	0.065
22	TBA	0.5	9.26	7.84	1.42	0.002	738.63	0.0009	0.036
23	TBA	1	9.26	8.29	0.97	0.003	234.32	0.0003	0.01
24	MTBE	0.1	9.21	8.97	0.24	3E-04	153.15	0.0002	0.008
25	MTBE	0.5	9.16	8.81	0.35	0.002	96.63	0.00013	0.005
26	MTBE	1	9.07	8.47	0.6	0.003	123.32	0.0002	0.006
27	MTBE	3	9.02	8.61	0.41	0.01	22.11	2.9E-05	0.001
28	MTBE	5	8.84	8.76	0.08	0.017	-6.537	-8.8E-06	-0.0003
29	MTBE	7	9.14	8.63	0.51	0.023	13.76	1.8E-05	0.0007

Table C.2 Complete BOD<sub>5</sub> Experiment #2 Data

Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)				
1	dilution water blank	300	8.95	8.77	0.18				
2	dilution water blank	300	8.97	8.78	0.19				
3	dilution water blank	300	8.95	8.79	0.16				
Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)	s			
4	seed	1	8.95	8.57	0.38	0.38			
5	seed	10	8.85	6.15	2.7	0.27			
6	seed	50	8.42	0.41	8.01	0.16			
7	seed	75	8.14	0.25	7.89	0.105			
8	seed	100	7.6	0.24	7.36	0.074			
					s ave	0.27			
Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)	P	BOD mg/L	mg O <sub>2</sub> mg Chem	% ThOD
9	Glucose/Glutamic acid	6	8.81	0.29	8.52	0.02	345	1.14	113.86
10	Glucose/Glutamic acid	6	8.8	0.25	8.55	0.02	346.5	1.14	114.36
11	Glucose/Glutamic acid	6	8.8	0.24	8.56	0.02	347	1.15	114.52
12	toluene	0.001	8.85	4.6	4.25	3E-06	789000	0.91	29.08
13	toluene	0.0015	8.81	0.69	8.12	5E-06	1300000	1.50	47.91
14	toluene	0.002	8.8	0.31	8.49	7E-06	1030500	1.19	37.98
15	toluene	0.0025	8.83	0.76	8.07	8E-06	774000	0.89	28.53
16	toluene	0.003	8.86	4.55	4.31	1E-05	269000	0.31	9.91
17	toluene	0.0035	8.85	3.93	4.92	1E-05	282857.14	0.33	10.42
18	TBA	0.001	8.83	7.52	1.31	3E-06	-93000	-0.12	-4.55
19	TBA	0.002	8.85	7.08	1.77	7E-06	22500	0.03	1.10
20	TBA	0.005	8.87	6.23	2.64	2E-05	61200	0.08	2.30
21	TBA	0.1	8.89	6.84	2.05	3E-04	1290	0.002	0.06
22	TBA	0.5	8.84	5.25	3.59	0.002	1182	0.001	0.06
23	TBA	1	8.82	3.46	5.36	0.003	1122	0.001	0.05
24	MTBE	0.001	8.82	6.89	1.93	3E-06	93000	0.13	4.62
25	MTBE	0.002	8.82	6.89	1.93	7E-06	46500	0.06	2.31
26	MTBE	0.005	8.85	7.26	1.59	2E-05	-1800	-0.002	-0.10
27	MTBE	0.1	8.85	7.91	0.94	3E-04	-2040	-0.003	-0.10
28	MTBE	0.5	8.78	7.73	1.05	0.002	-342	-0.0005	-0.02
29	MTBE	1	8.68	7.38	1.3	0.003	-96	-0.0001	-0.005



Table C.3 Complete BOD<sub>5</sub> Experiment #3 Data

Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)
1	dilution water blank	300	9.25	9.12	0.13
2	dilution water blank	300	9.17	9.15	0.02
3	dilution water blank	300	9.26	9.16	0.1

Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)	s
4	Seed	1	9.3	9.17	0.13	0.13
5	Seed	10	9.22	8.61	0.61	0.061
6	Seed	25	9.07	7.52	1.55	0.062
7	Seed	50	8.77	5.36	3.41	0.068
8	Seed	100	8.14	1.84	6.3	0.063
s ave						0.066

Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)	P	BOD mg/L	mg O <sub>2</sub> mg Chem	% ThOD
9	Glucose/Glutamic acid	6	9.23	4.3	4.93	0.02	226.82	0.75	74.96
10	Glucose/Glutamic acid	6	9.24	4.4	4.84	0.02	222.32	0.73	73.47
11	Glucose/Glutamic acid	6	9.23	4.07	5.16	0.02	238.32	0.79	78.76

12	Toluene	0.001	9.25	6.35	2.9	3E-06	751920	0.87	27.71
13	Toluene	0.0015	9.24	5	4.24	5E-06	769280	0.89	28.35
14	Toluene	0.002	9.24	4.11	5.13	7E-06	710460	0.82	26.18
15	Toluene	0.0025	9.24	2.98	6.26	8E-06	703968	0.81	25.94
16	Toluene	0.003	9.24	1.8	7.44	1E-05	704640	0.81	25.97
17	Toluene	0.0035	9.23	0.85	8.38	1E-05	684548.57	0.79	25.23
18	TBA	0.001	9.22	8.66	0.56	3E-06	49920	0.06	2.44
19	TBA	0.0015	9.23	8.74	0.49	5E-06	19280	0.02	0.94
20	TBA	0.002	9.23	8.68	0.55	7E-06	23460	0.03	1.15
21	TBA	0.0025	9.24	8.82	0.42	8E-06	3168	0.004	0.16
22	TBA	0.003	9.24	8.77	0.47	1E-05	7640	0.01	0.37
23	TBA	0.0035	9.15	8.6	0.55	1E-05	13405.71	0.02	0.66
24	MTBE	0.001	9.15	8.72	0.43	3E-06	10920	0.01	0.54
25	MTBE	0.0015	9.16	8.8	0.36	5E-06	-6720	-0.01	-0.33
26	MTBE	0.002	9.13	8.68	0.45	7E-06	8460	0.01	0.42
27	MTBE	0.0025	9.15	8.86	0.29	8E-06	-12432	-0.02	-0.62
28	MTBE	0.003	9.15	8.85	0.3	1E-05	-9360	-0.01	-0.46
29	MTBE	0.0035	9.15	8.87	0.28	1E-05	-9737.14	-0.01	-0.48

Table C.4 Complete BOD<sub>5</sub> Experiment #4 Data

Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)				
1	dilution water blank	300	9.28	9.16	0.12				
2	dilution water blank	300	9.28	9.18	0.1				
3	dilution water blank	300	9.29	9.2	0.09				
Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)	s			
4	seed	25	8.95	7.93	1.02	0.041			
5	seed	50	8.57	6.53	2.04	0.041			
6	seed	75	8.27	4.82	3.45	0.046			
7	seed	100	7.94	2.81	5.13	0.051			
8	seed	125	7.59	2.06	5.53	0.044			
					s ave	0.046			
Bottle#	Sample description	Dilution (mL/300)	init DO	Fin DO	(Doi-Dof)	P	BOD mg/L	mg O2 mg Chem	% ThOD
9	Glucose/Glutamic acid	6	9.2	4.7	4.5	0.02	211.32	0.70	70.04
10	Glucose/Glutamic acid	6	9.21	4.84	4.37	0.02	204.82	0.68	67.89
11	Glucose/Glutamic acid	6	9.21	4.92	4.29	0.02	200.82	0.67	66.56
12	toluene	0.001	9.21	6.64	2.57	3E-06	688947	0.79	25.39
13	toluene	0.0015	9.2	5.49	3.71	5E-06	687298	0.79	25.33
14	toluene	0.002	9.2	4.47	4.73	7E-06	668473.5	0.77	24.64
15	toluene	0.0025	9.2	1.64	7.56	8E-06	874378.8	1.01	32.22
16	toluene	0.003	9.2	1.81	7.39	1E-05	711649	0.82	26.23
17	toluene	0.0035	9.19	1.05	8.14	1E-05	674270.57	0.78	24.85
18	TBA	0.0001	9.18	8.69	0.49	3E-07	649470	0.82	31.79
19	TBA	0.0002	9.19	8.76	0.43	7E-07	234735	0.30	11.49
20	TBA	0.0004	9.18	8.69	0.49	1E-06	162367.5	0.21	7.95
21	TBA	0.0006	9.18	8.68	0.5	2E-06	113245	0.14	5.54
22	TBA	0.0008	9.18	8.68	0.5	3E-06	84933.75	0.11	4.16
23	TBA	0.001	9.19	8.68	0.51	3E-06	70947	0.09	3.47
24	MTBE	0.0001	9.2	8.76	0.44	3E-07	499470	0.67	24.80
25	MTBE	0.0002	9.18	8.68	0.5	7E-07	339735	0.46	16.87
26	MTBE	0.0004	9.18	8.73	0.45	1E-06	132367.5	0.18	6.57
27	MTBE	0.0006	9.18	8.69	0.49	2E-06	108245	0.15	5.37
28	MTBE	0.0008	9.19	8.69	0.5	3E-06	84933.75	0.11	4.22
29	MTBE	0.001	9.19	8.69	0.5	3E-06	67947	0.09	3.37

Table C.5 GC Concentration Data Experiment 1

Day-	0		0.5		1			1.5			2			2.5			5			10			15
Chemicals	ppm	ppm	ppm	ppm	ppm	ppm	TBA ppm	ppm	ppm	TBA ppm	ppm	ppm	TBA ppm	ppm	ppm	TBA ppm	ppm	ppm	TBA ppm	ppm	ppm	TBA ppm	ppm
Toluene	14.95		8.82		7.59			6.01			4.64			3.53			0.86			0.00			0.00
Toluene	14.23		10.64		7.23			5.80			4.08			3.39			0.54			0.00			0.00
Toluene	14.22		8.94		6.57			4.98			5.22			3.67			0.70			0.00			0.00
TBA	19.78		17.38		18.00			18.19			17.98			17.73			17.41			16.92			17.97
TBA	19.75		17.99		18.70			19.89			19.51			18.92			19.36			18.59			17.77
TBA	20.41		19.25		19.60			19.57			19.05			19.64			17.27			18.55			17.80
TBA	19.94		20.01		19.88			19.56			19.25			19.27			19.41			19.04			18.02
MTBE	13.65		11.59		9.75		0.07	9.16		0.09	8.89		0.28	8.77		0.42	7.74		0.52	3.77		0.19	2.48
MTBE	19.09		16.99		15.42		0.32	14.58		0.30	13.07		0.16	12.82		0.35	10.33		0.48	5.92		0.30	3.84
MTBE	18.37		16.37		15.72		0.25	15.07		0.21	14.45		0.37	12.65		0.36	12.20		0.71	6.17		0.00	3.72
MTBE	19.00		18.07		15.59		0.15	15.07		0.33	14.07		0.47	13.09		0.44	9.85		0.55	6.59		0.27	3.80
MTBE/TOL	18.86	13.69	15.55	7.91	15.19	6.26	0.00	14.33	4.28	0.06	12.78	2.75	0.27	12.48	2.55	0.19	9.64	0.00	0.00	5.66	0.00	0.00	3.74
MTBE/TOL	19.81	14.18	16.41	8.86	14.96	5.94	0.24	14.97	4.50	0.30	13.41	3.20	0.37	13.67	2.80	0.56	10.79	0.00	0.24	5.77	0.00	0.00	3.57
MTBE/TOL	15.39	5.73	17.35	8.71	15.86	6.02	0.30	14.71	5.33	0.15	14.19	3.43	0.33	12.34	2.50	1.90	10.45	0.00	1.68	6.17	0.00	0.00	3.90
TBA/TOL	20.31	13.55	18.11	8.06	19.99	5.91		20.29	4.98		19.93	3.54		19.07	3.24		19.94	0.73		19.08	0.00		17.78
TBA/TOL	19.60	1.64	19.56	8.53	20.10	5.54		19.98	4.61		20.00	3.40		19.35	2.92		19.41	1.09		19.07	0.00		17.84
TBA/TOL	20.39	13.53	20.51	8.17	18.44	5.65		20.02	3.99		20.16	3.25		19.40	2.60		18.36	0.43		19.34	1.19		17.47

Table C.6 GC Concentration Data Experiment 2

Day	0		0.5		1		1.5		2		2.5	5	10	13
Chemical	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Toluene	11.19		7.36		5.61		3.28		2.03		0.74	0.24	0	0
Toluene	9.58		6.76		5.23		3.77		1.28		0.18	0	0	0
Toluene	11.01		6.31		5.12		3.16		0.64		0	0	0	0
TBA	9.91		9.06		9.03		8.87		8.90		9.00	8.77	9.40	7.72
TBA	9.71		9.56		9.50		9.44		9.50		9.50	8.91	8.87	8.19
TBA	9.55		9.71		9.83		9.66		9.46		9.52	8.76	8.34	8.10
TBA	9.96		9.56		9.92		9.76		9.83		9.81	9.38	8.96	7.85
MTBE	8.59		7.07		6.46		6.44		6.07		5.83	3.79	2.68	2.20
MTBE	8.31		6.25		6.76		6.62		6.47		5.61	3.63	3.38	2.19
MTBE	8.38		7.38		6.66		6.71		5.77		6.47	3.48	2.81	1.96
MTBE	8.39		6.98		6.55		5.92		6.38		5.40	4.15	2.97	2.09
MTBE/TOL	7.85	10.59	6.26	5.89	6.52	4.50	6.24	2.98	5.49	0	5.77	3.45	3.40	1.91
MTBE/TOL	7.31	10.37	7.23	6.30	6.11	0	5.92	0	5.82	0	5.59	3.86	2.73	2.01
MTBE/TOL	7.98	10.69	6.95	5.92	6.33	0	6.17	0	5.67	0	5.43	3.14	2.26	1.32
TBA/TOL	9.76	10.24	9.53	6.39	9.78	4.72	9.57	2.38	9.55	0.19	9.65	8.47	6.56	4.45
TBA/TOL	9.60	9.56	9.69	5.38	9.57	4.10	9.71	3.02	9.47	0.73	9.34	8.69	7.76	6.35
TBA/TOL	9.67	9.98	9.47	4.50	9.523	3.85	9.29	1.87	8.83	0.30	8.72	5.30	1.45	0.57

Table C.7 Gas Chromatography Degradation Rate Summary

Chemical	Concentration ppm		$\lambda$ (Day <sup>-1</sup> )	Avg. $\lambda$			
	Day 0	Day 2.5/2					
Toluene	Day 0	Day 2.5/2	$\lambda$ (Day <sup>-1</sup> )	Avg. $\lambda$			
exp #1	14.953	0.864	0.581	0.608			
exp #1	14.233	0.539	0.648	1.060	Avg. $\lambda$ By Chemical		
exp #1	14.222	0.699	0.595				
exp #2	11.186	2.033	0.831		Toluene	0.834	
exp #2	9.584	1.275	1.203				
exp #2	11.007	0.643	1.146				
TBA	Day 0	Day 15/13	$\lambda$ (Day <sup>-1</sup> )		Avg. $\lambda$		
exp #1	19.784	17.974	0.012	0.009			
exp #1	19.750	17.769	0.007	Avg. $\lambda$	Avg. $\lambda$ By Chemical		
exp #1	20.406	17.799	0.012				
exp #1	19.944	18.021	0.006		TBA	0.011	
exp #2	9.907	7.719	0.016		0.013		
exp #2	9.707	8.190	0.011				
exp #2	9.554	8.097	0.012				
exp #2	9.955	7.848	0.014				
TBA/Toluene	Day 0	Day 15/13	$\lambda$ (Day <sup>-1</sup> )	Avg. $\lambda$			
exp #1	20.315	17.779	0.008	0.008			
exp #1	19.596	17.835	0.005	Avg. $\lambda$	Avg. $\lambda$ By Chemical		
exp #1	20.392	17.473	0.010				
exp #2	9.759	4.453	0.046		TBA	0.019	
exp #2	9.600	6.345	0.025		0.035		
exp #2	9.675	0.566	0.179				
MTBE	Day 0	Day 15/13	$\lambda$ (Day <sup>-1</sup> )		Avg. $\lambda$		
exp #1	13.650	2.482	0.122	0.113			
exp #1	19.085	3.839	0.113	Avg. $\lambda$	Avg. $\lambda$ By Chemical		
exp #1	18.365	3.720	0.107				
exp #1	19.000	3.796	0.110		MTBE	0.108	
exp #2	8.593	2.197	0.106		0.103		
exp #2	8.311	2.189	0.097				
exp #2	8.376	1.956	0.108				
exp #2	8.391	2.088	0.103				
MTBE/Toluene	Day 0	Day 15/13	$\lambda$ (Day <sup>-1</sup> )	Avg. $\lambda$			
exp #1	18.860	3.741	0.116	0.108			
exp #1	19.806	3.568	0.120	Avg. $\lambda$	Avg. $\lambda$ By Chemical		
exp #1	15.386	3.900	0.089				
exp #2	7.846	1.919	0.098		MTBE	0.107	
exp #2	7.307	2.006	0.094		0.107		
exp #2	7.978	1.317	0.128				

### Toluene Exp #1

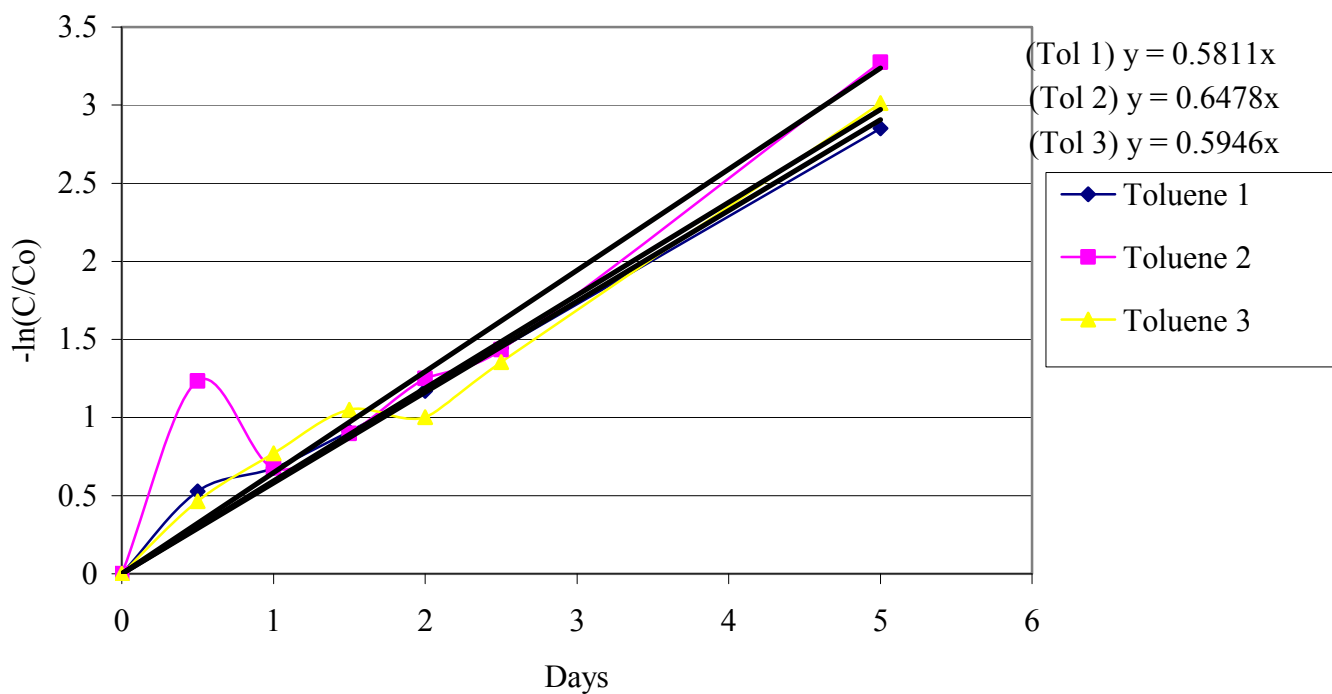


Figure C.1 Toluene Results Experiment #1

### TBA Exp #1

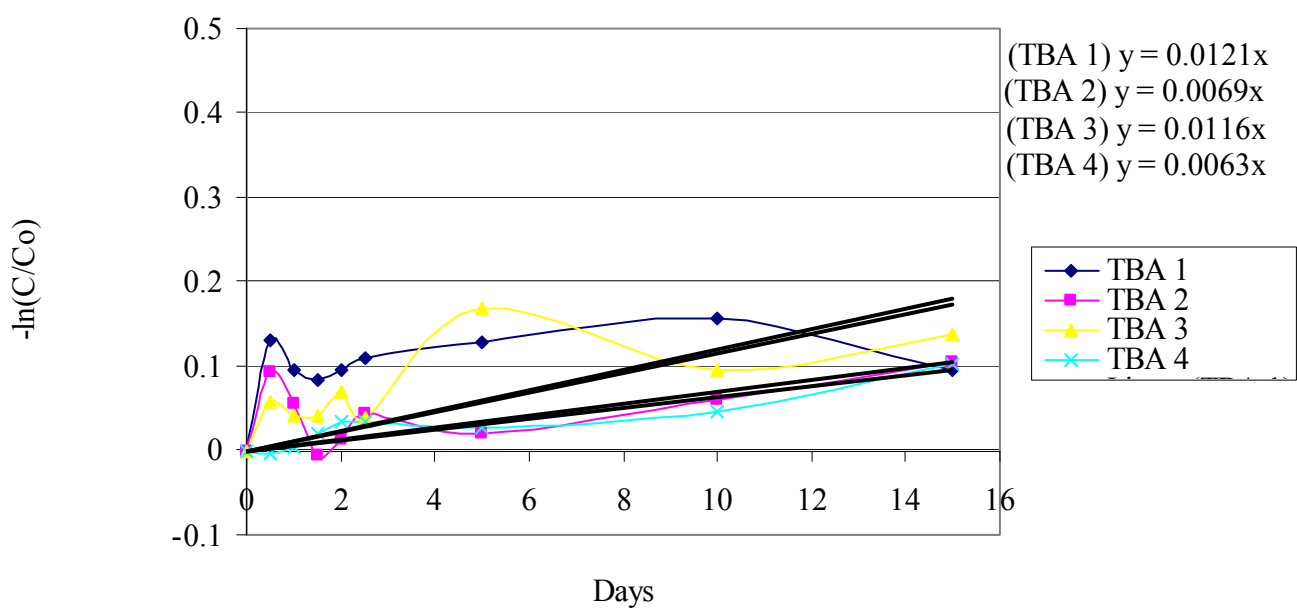


Figure C.2 TBA Results Experiment #1

### MTBE Exp #1

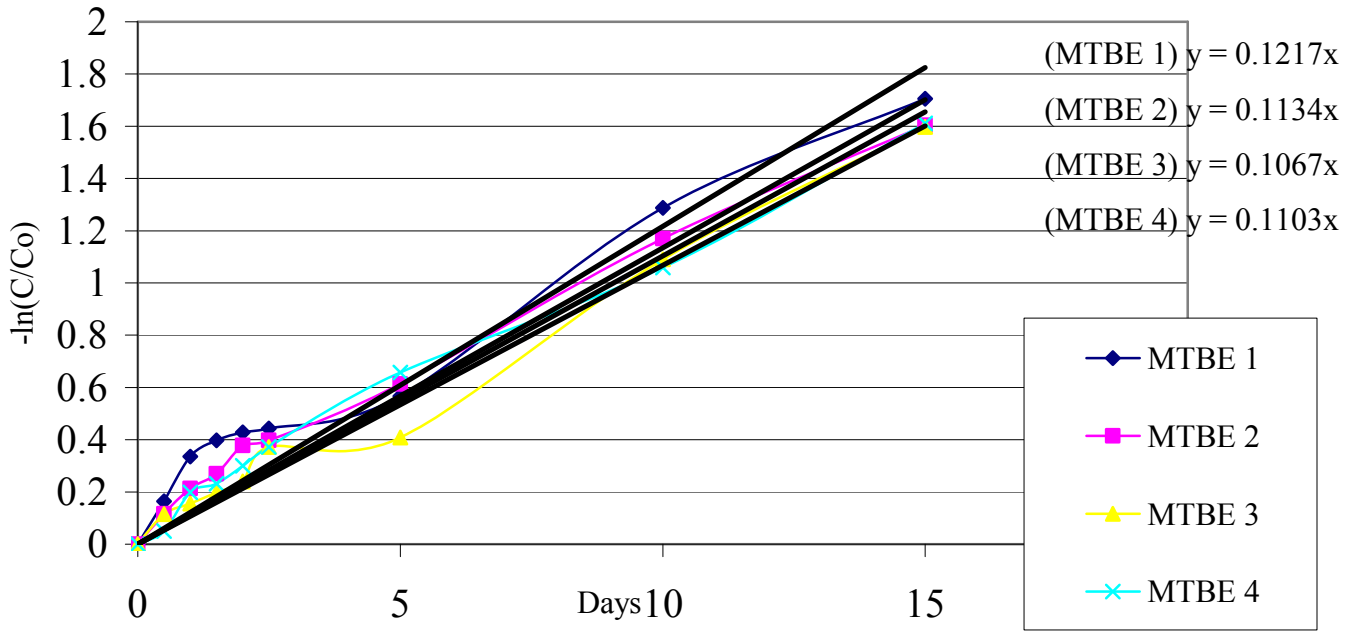


Figure C.3 MTBE Results Experiment #1

### MTBE/Toluene Exp #1

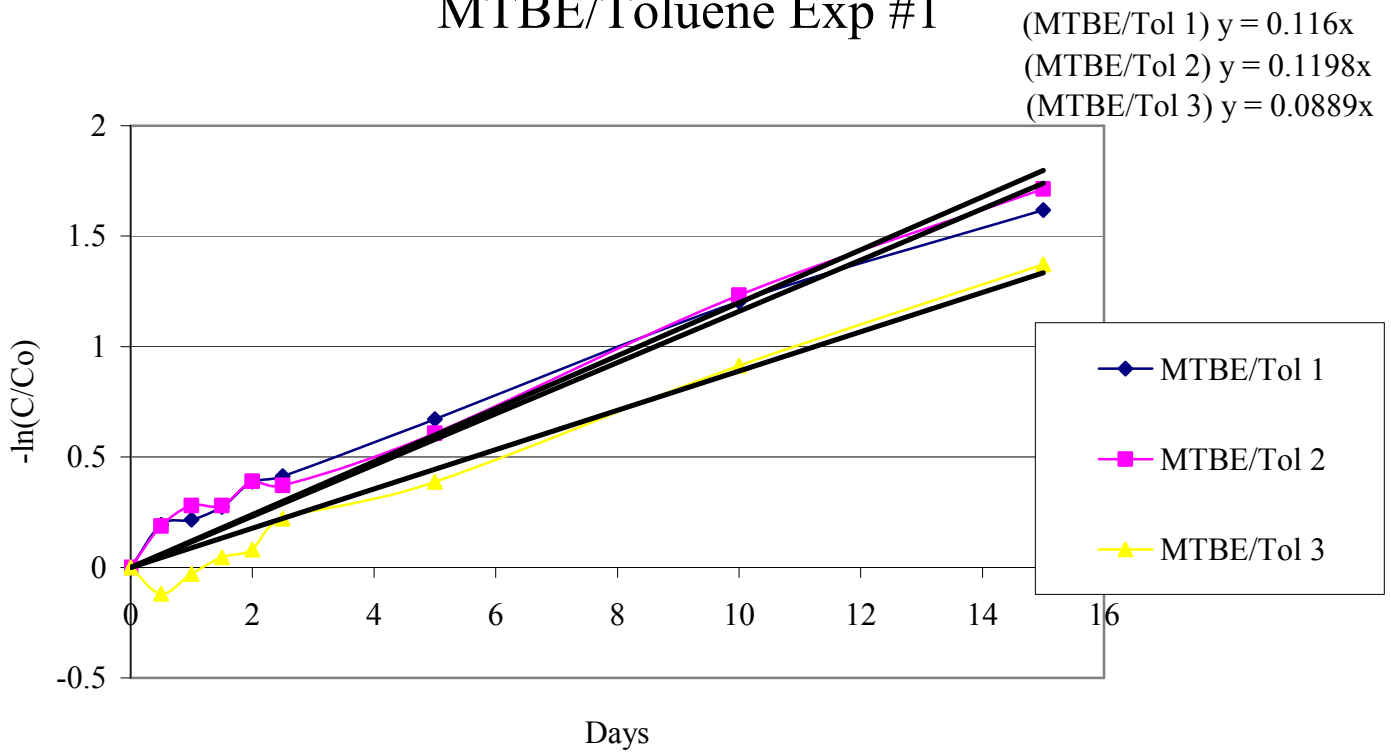


Figure C.4 MTBE/Toluene Results Experiment #1

### TBA/Toluene Exp #1

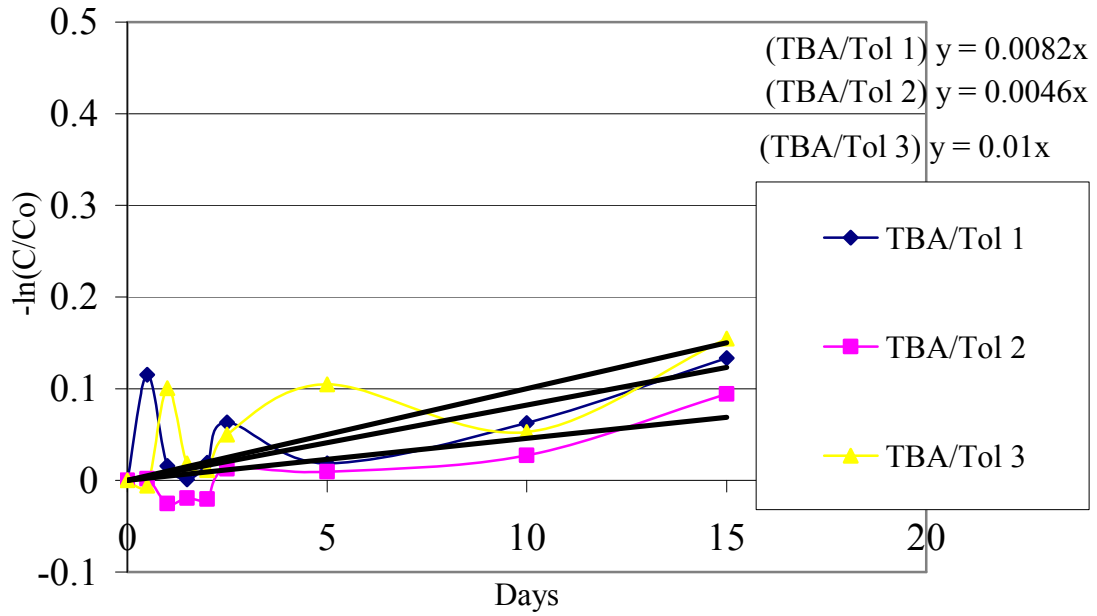


Figure C.5 TBA/Toluene Results Experiment #1

### Toluene Exp #2

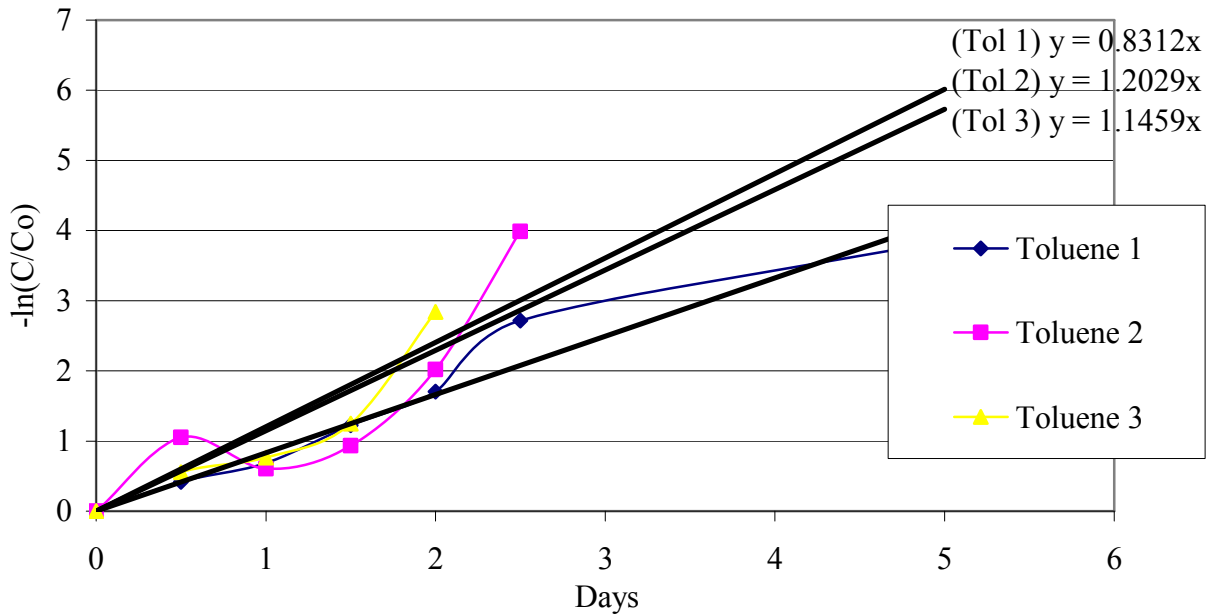


Figure C.6 Toluene Results Experiment #2



## TBA Exp #2

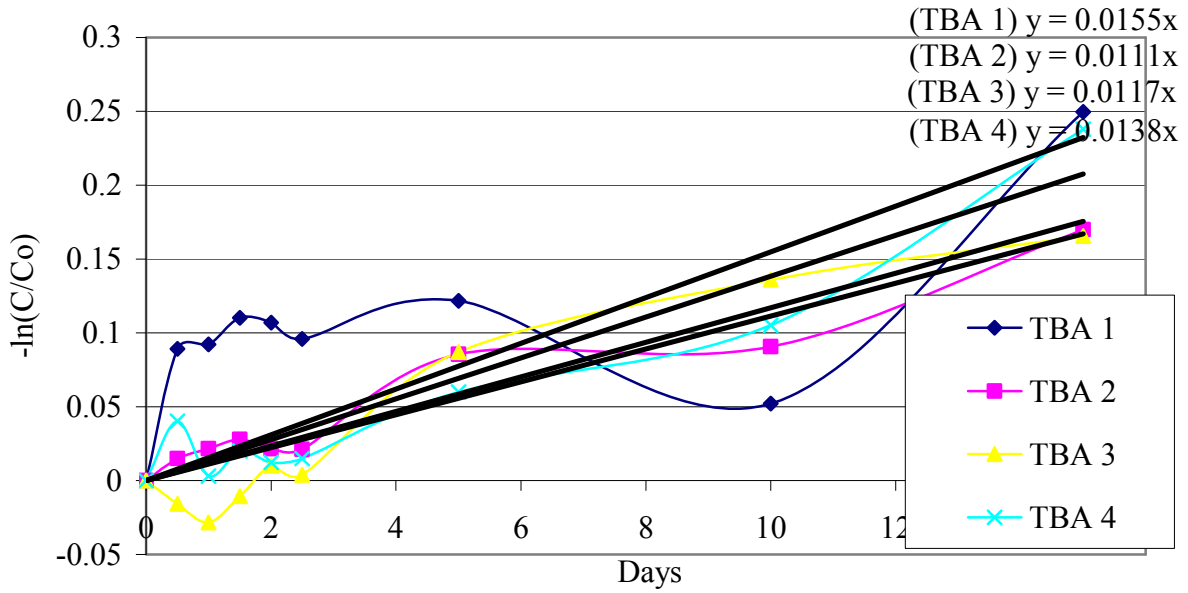


Figure C.7 TBA Results Experiment #2

## MTBE Exp #2

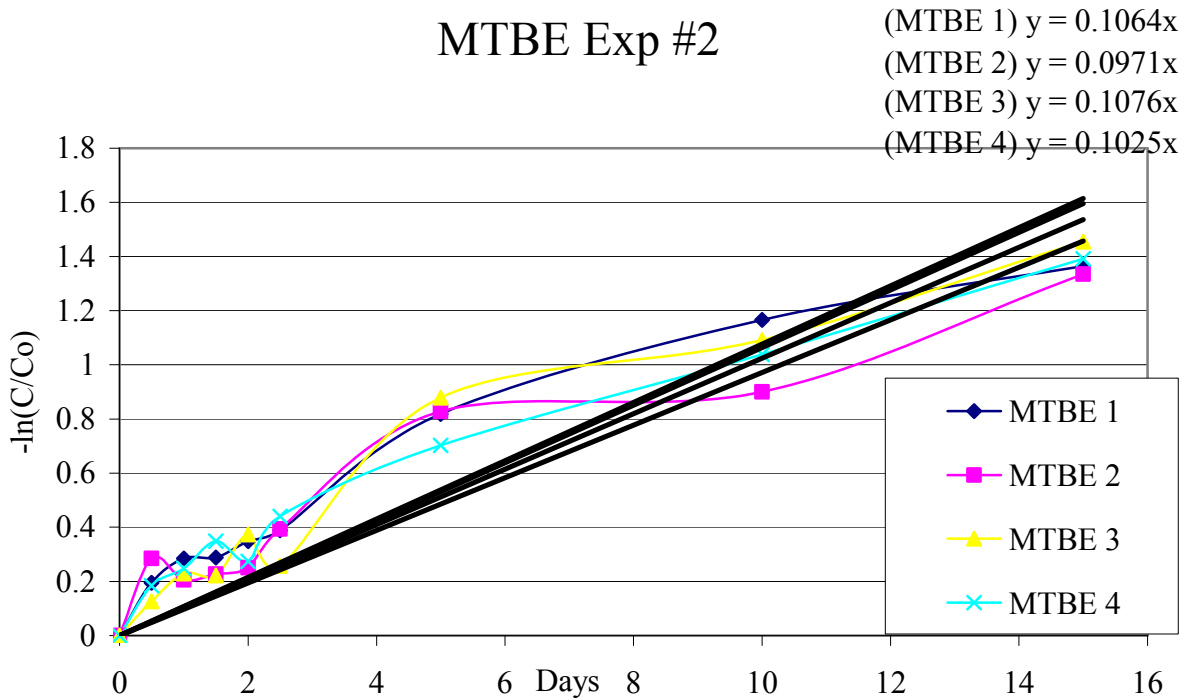


Figure C.8 MTBE Results Experiment #2

## MTBE/Toluene Exp #2

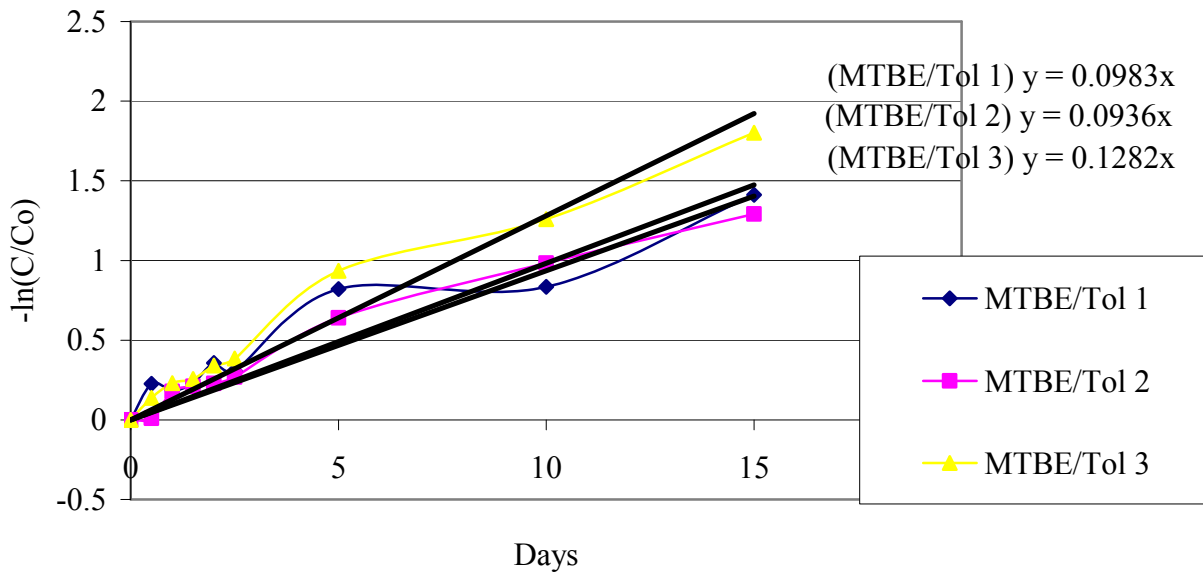


Figure C.9 MTBE/Toluene Results Experiment #2

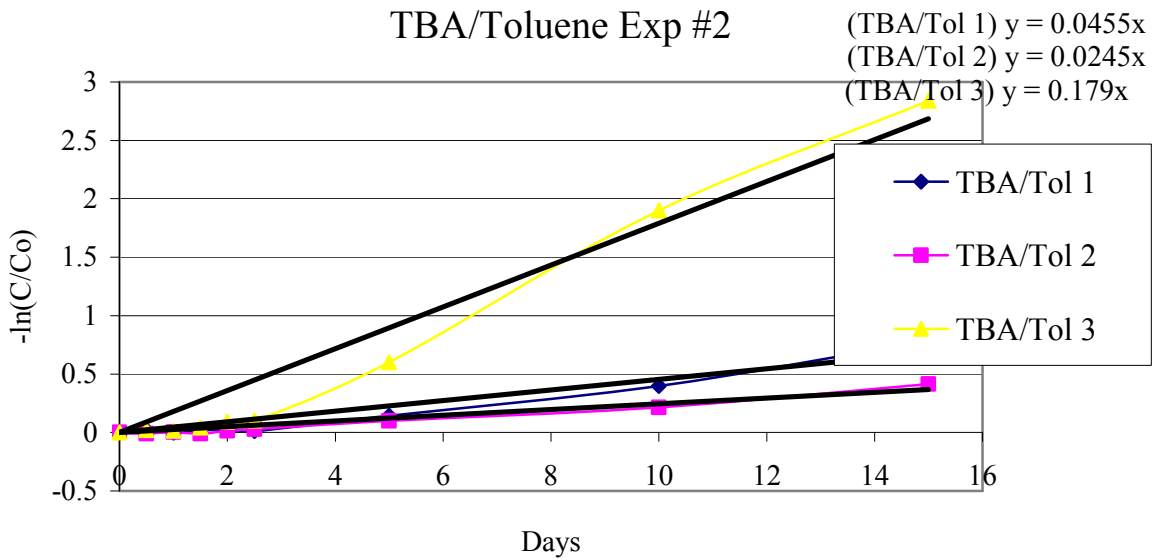


Figure C.10 TBA/Toluene Results Experiment #2

Table C.8 Complete Respirometry Data Summary

Experiment	Chemical	Starting Concentration (ppm)	Total O2 Consumed uL	Percent of Theoretical	Total CO2 Consumed uL	Percent of Theoretical
#1	Toluene	14.95	5359.58	-36.57	9403.51	-77.46
#1	Toluene	14.23	5140.80	-42.11	9053.54	-88.95
#1	Toluene	14.22	4241.98	-57.27	7655.25	-119.28
#2	Toluene	11.19	2055.42	29.72	5715.86	87.21
#2	Toluene	9.58	1861.73	29.85	5259.85	87.15
#2	Toluene	11.01	2048.60	30.05	5656.62	86.98
#1	TBA	19.78	6654.98	-15.87	11625.58	-37.09
#1	TBA	19.75	6668.18	-15.68	11597.11	-37.83
#1	TBA	20.41	6283.09	-21.16	11056.54	-49.22
#1	TBA	19.94	7165.36	-7.62	12431.25	-17.55
#2	TBA	9.91	946.80	8.97	3160.84	29.55
#2	TBA	9.71	961.34	9.63	3209.27	32.53
#2	TBA	9.55	784.66	3.92	2862.23	15.76
#2	TBA	9.96	903.45	7.55	3056.15	24.40
#1	MTBE	13.65	6288.41	-31.94	10863.14	-81.41
#1	MTBE	19.09	7155.30	-8.23	12220.45	-23.91
#1	MTBE	18.37	6849.35	-13.91	11598.82	-41.17
#1	MTBE	19.00	6392.31	-21.19	10986.28	-55.36
#2	MTBE	8.59	943.27	10.35	3218.98	37.78
#2	MTBE	8.31	1007.65	13.19	3371.53	47.92
#2	MTBE	8.38	993.62	12.55	3357.79	46.76
#2	MTBE	8.39	986.56	12.26	3340.68	45.69
#1	MTBE/Tol	18.86 / 13.68	6650.44	-16.94	11307.78	-47.55
#1	MTBE/Tol	19.81 / 14.18	5054.47	-42.04	8859.69	-104.89
#1	MTBE/Tol	15.39 / 5.73	7166.19	-9.98	11836.65	-41.69
#2	MTBE/Tol	7.85 / 10.59	1722.55	43.28	5027.18	152.58
#2	MTBE/Tol	7.31 / 10.37	2318.51	72.71	6384.44	253.46
#2	MTBE/Tol	7.98 / 10.69	2850.78	88.06	7527.34	301.26
#1	TBA/Tol	20.31 / 13.55	4121.56	-55.04	7345.92	-136.43
#1	TBA/Tol	19.60 / 1.64	4239.15	-55.14	7649.39	-134.03
#1	TBA/Tol	20.39 / 13.529	4954.36	-41.87	8659.16	-105.26
#2	TBA/Tol	9.76 / 10.24	2210.25	50.21	6014.83	169.25
#2	TBA/Tol	9.60 / 9.56	1918.47	41.39	5440.34	143.56
#2	TBA/Tol	9.67 / 9.98	3628.28	97.17	9076.85	321.43

### Toluene Average O<sub>2</sub> Consumption

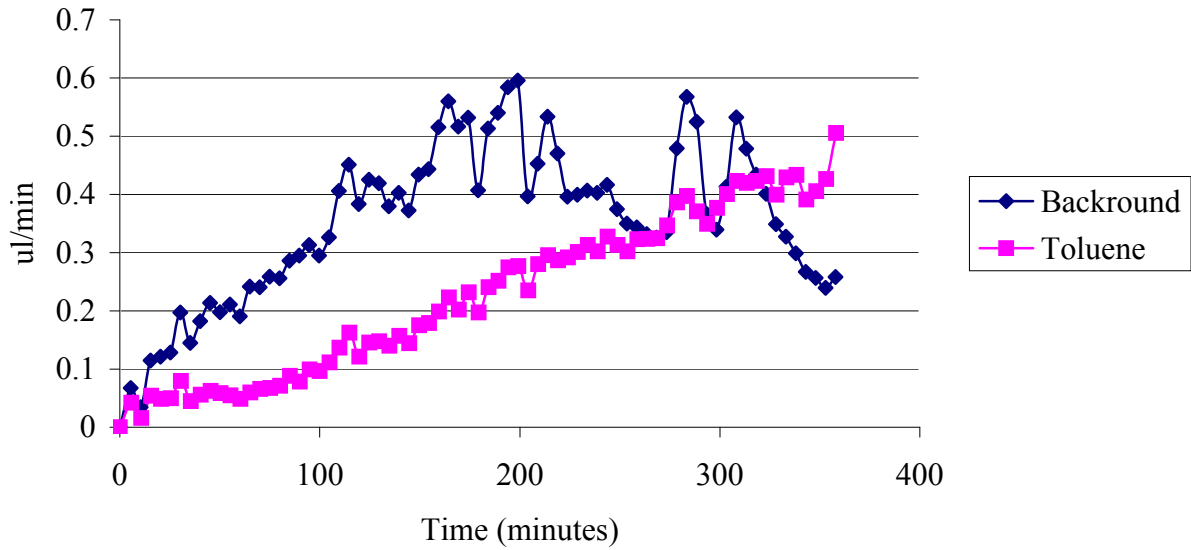


Figure C.11 Respirometry Experiment #1 Toluene Average O<sub>2</sub> Consumption

### Toluene Average CO<sub>2</sub> Production

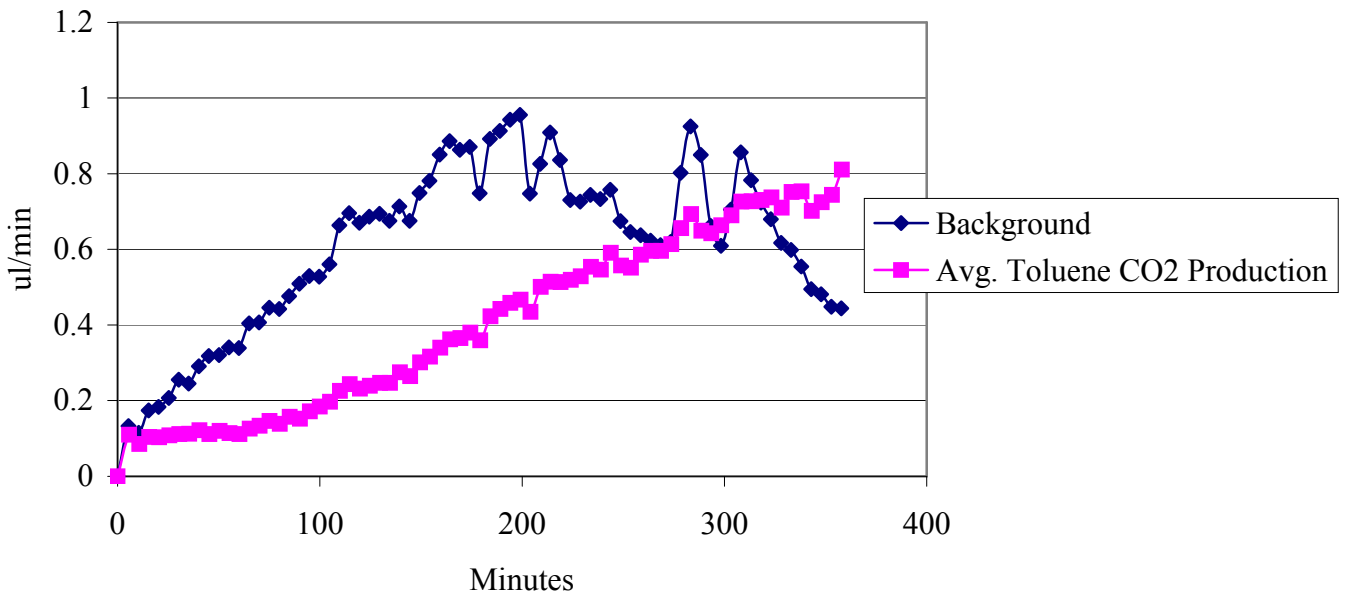


Figure C.12 Respirometry Experiment #1 Toluene Average CO<sub>2</sub> Production

### TBA Average O<sub>2</sub> Consumption

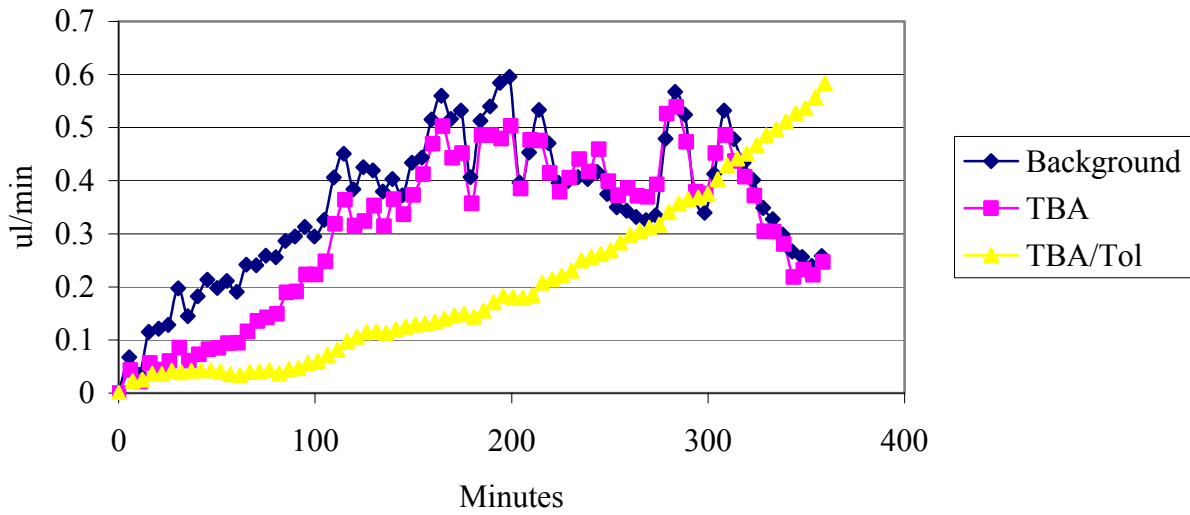


Figure C.13 Respirometry Experiment #1 TBA and TBA/Toluene Average O<sub>2</sub> Consumption

### TBA Average CO<sub>2</sub> Production

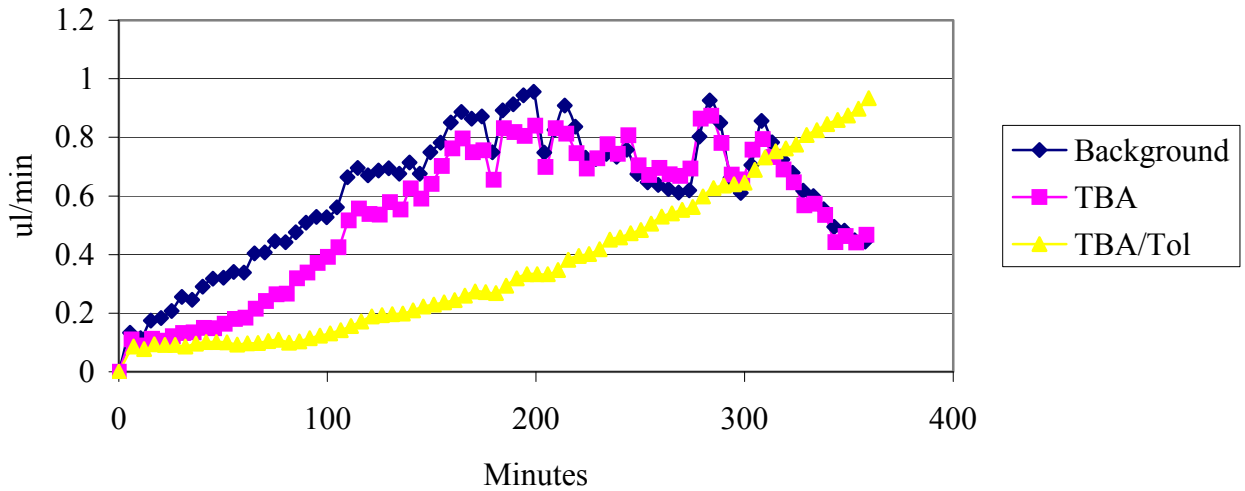


Figure C.14 Respirometry Experiment #1 TBA and TBA/Toluene Average CO<sub>2</sub> Production

### MTBE/Tol & MTBE Average O<sub>2</sub> Consumption

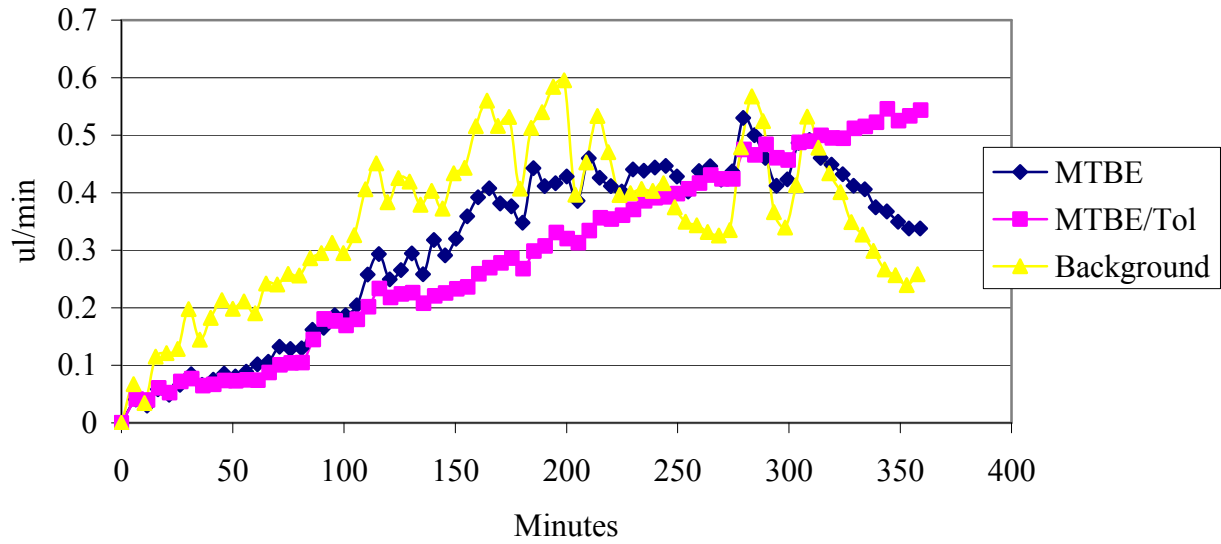


Figure C.15 Respirometry Experiment #1 MTBE and MTBE/Toluene Average O<sub>2</sub> Consumption

### MTBE & MTBE/Toluene Average CO<sub>2</sub> Production

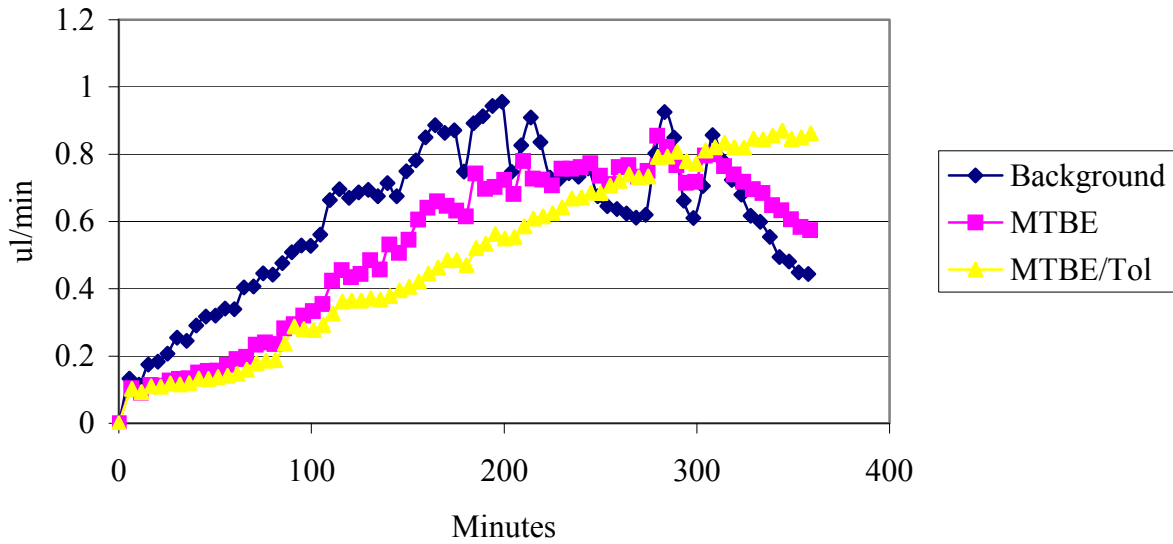


Figure C.15 Respirometry Experiment #1 MTBE and MTBE/Toluene Average CO<sub>2</sub> Production

### Toluene Average O<sub>2</sub> Consumption

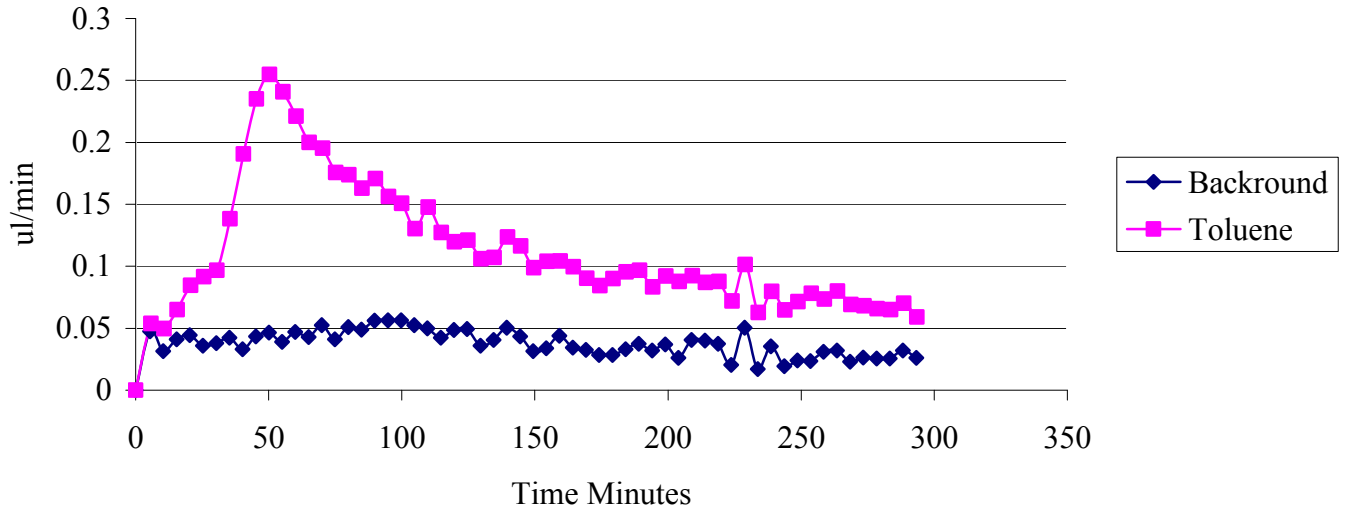


Figure C.17 Respirometry Experiment #2 Toluene Average O<sub>2</sub> Consumption

### Toluene Average CO<sub>2</sub> Production

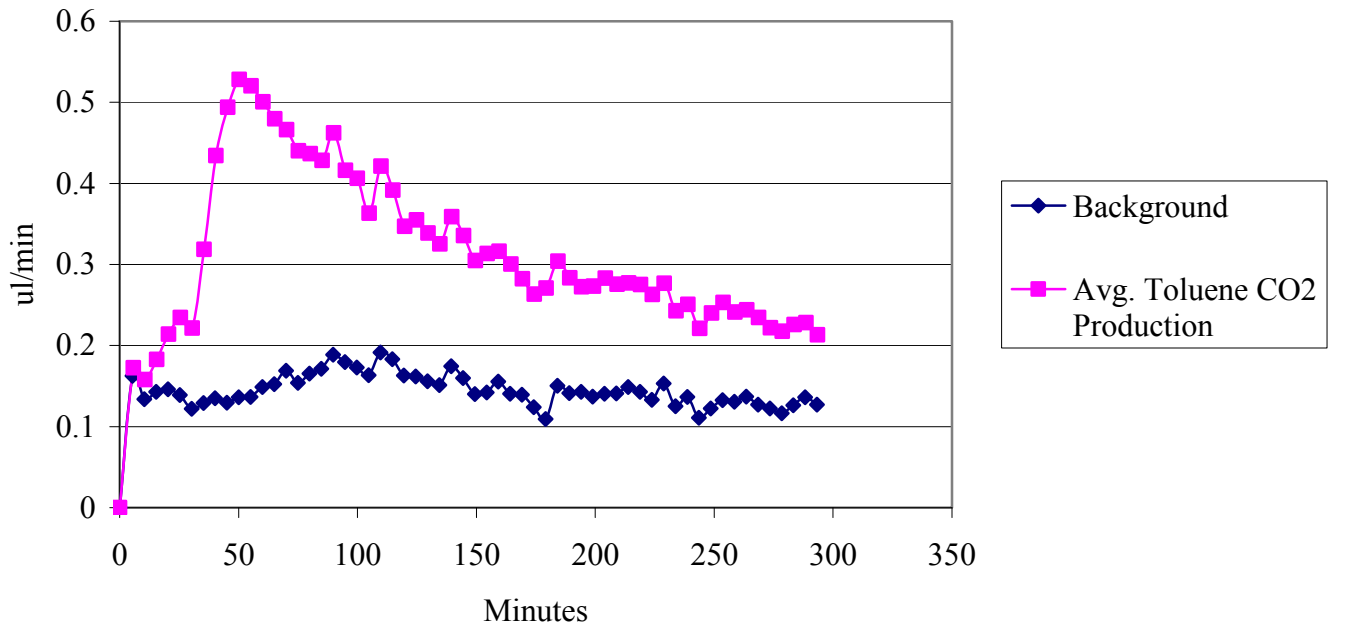


Figure C.18 Respirometry Experiment #2 Toluene Average CO<sub>2</sub> Production

### TBA/Toluene & TBA Average O<sub>2</sub> Consumption

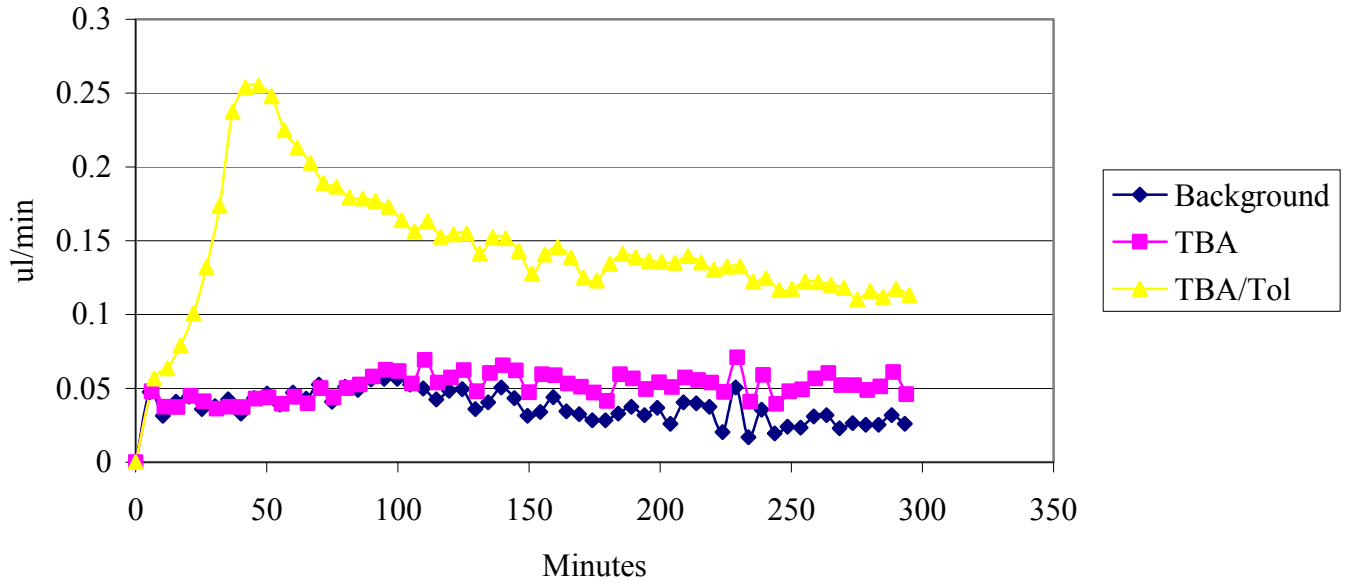


Figure C.19 Respirometry Experiment #2 TBA and TBA/Toluene Average O<sub>2</sub> Consumption

### TBA/Toluene & TBA Average CO<sub>2</sub> Production

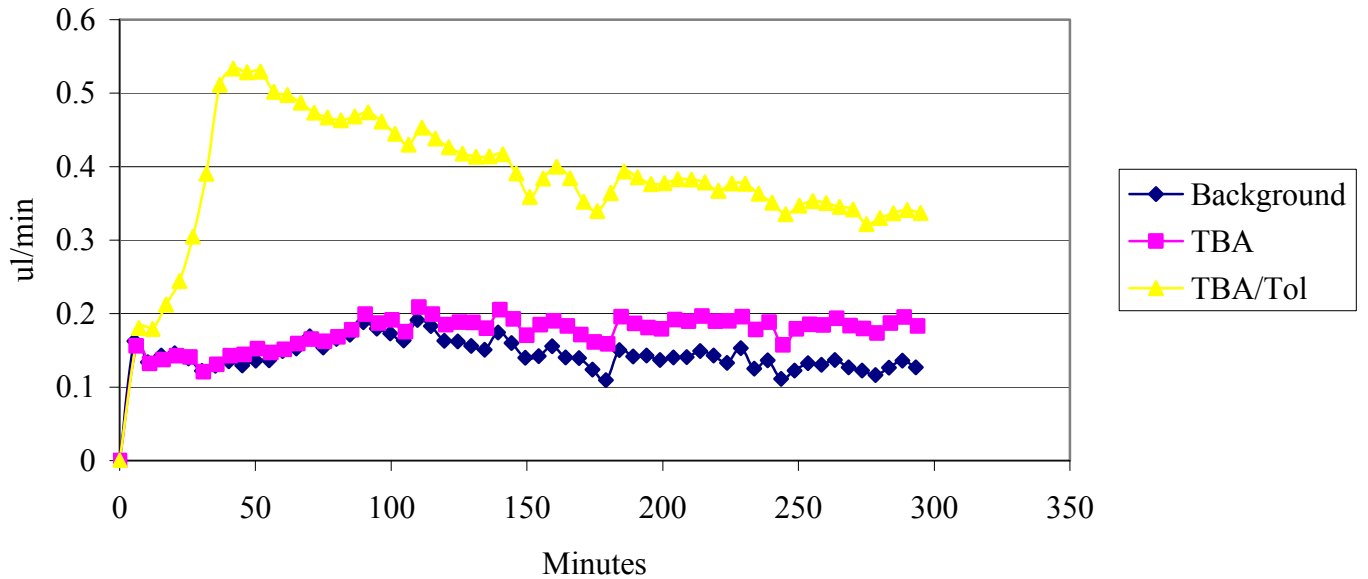


Figure C.20 Respirometry Experiment #2 TBA and TBA/Toluene Average CO<sub>2</sub> Production



### MTBE/Tol & MTBE Average O<sub>2</sub> Consumption

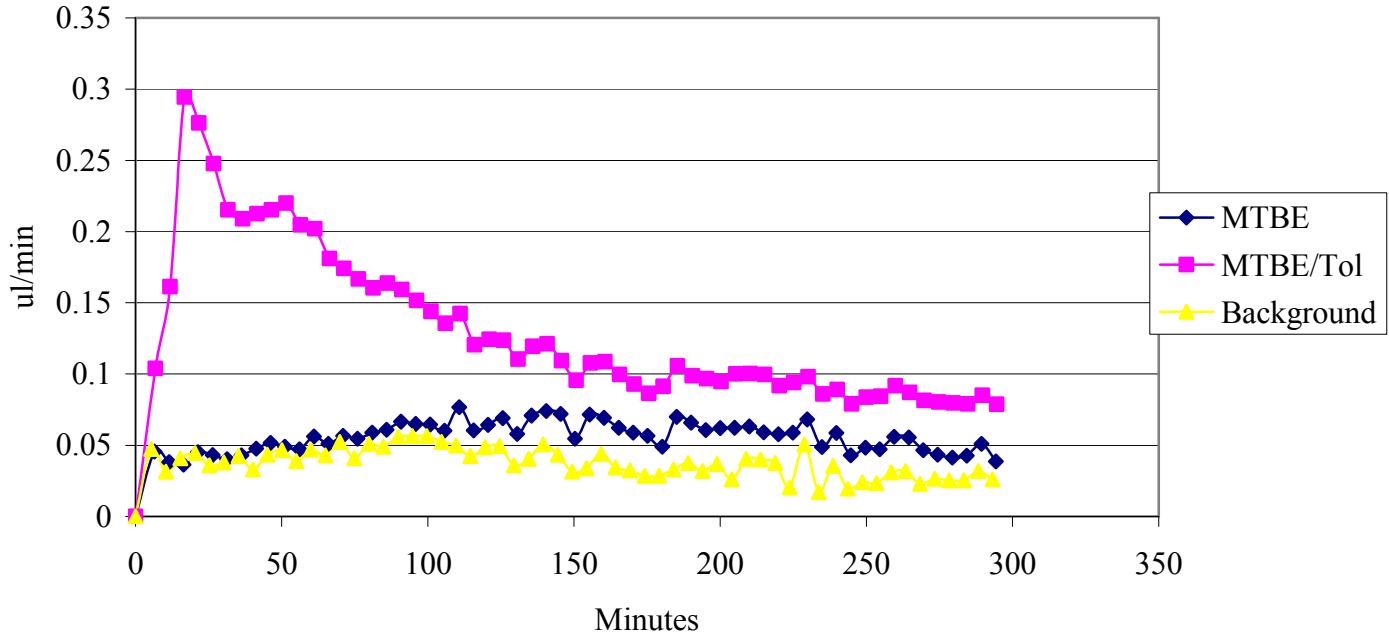


Figure C.21 Respirometry Experiment #2 MTBE and MTBE/Toluene Average O<sub>2</sub> Consumption

### MTBE & MTBE/Toluene Average CO<sub>2</sub> Production

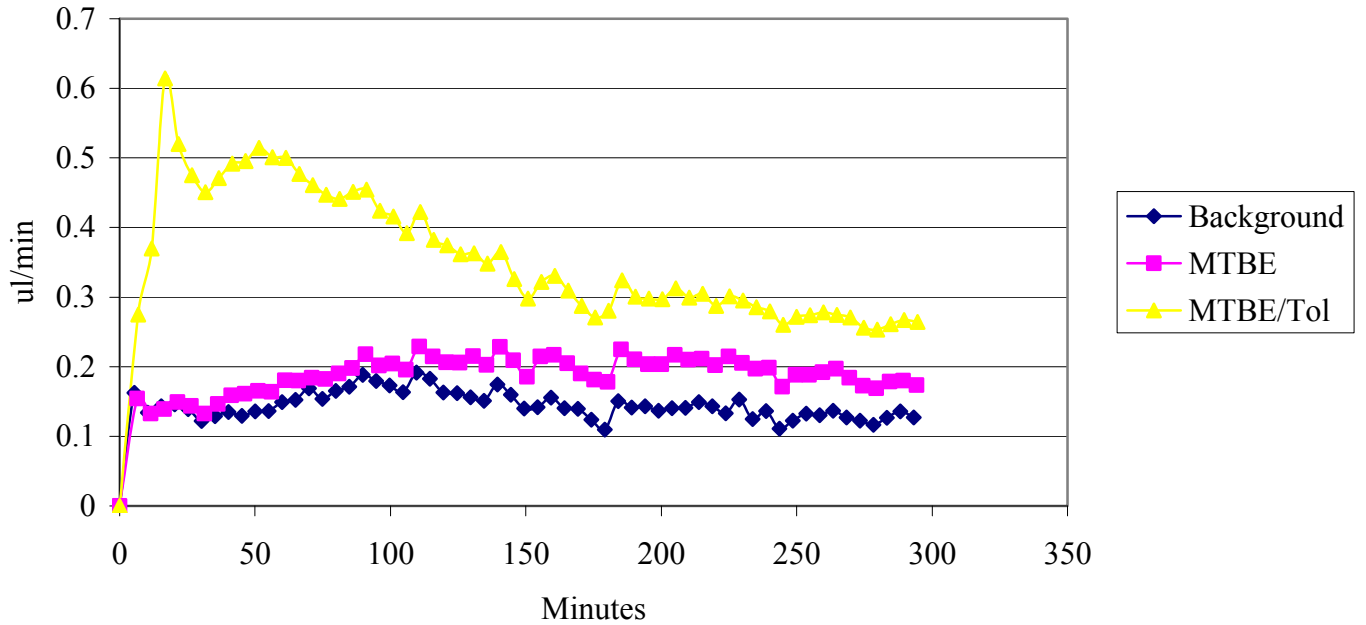


Figure C.22 Respirometry Experiment #2 MTBE and MTBE/Toluene Average CO<sub>2</sub> Production

## References

- Adam, G., Gamoh, K., Moris, D., & Duncan, H. (2002). Effect of alcohol addition on the movement of petroleum hydrocarbon fuels in soil. *The Science of the Total Environment*, 286, 15-25.
- Ahmed, F. E. (2001). Toxicology and human health effects following exposure to oxygenated or reformulated gasoline. *Toxicology Letters*, (123), 89-113.
- ATSDR: ToxFAQs for methyl *tert*-butyl ether (MTBE). (1997). *Agency for Toxic Substances and Disease Registry*,
- ATSDR ToxFAQs for ethylbenzene.(1999). *Agency for Toxic Substances and Disease Registry*,
- ATSDR: Interaction profile for benzene, toluene, ethylbenzene, and xylenes (BTEX).(2004). *Agency for Toxic Substances and Disease Registry*
- ATSDR: ToxFAQs for toluene.(2001). *Agency for Toxic Substances and Disease Registry*
- ATSDR: ToxFAQs for benzene.(2005a). *Agency for Toxic Substances and Disease Registry*
- ATSDR: ToxFAQs for xylene.(2005b). *Agency for Toxic Substances and Disease Registry*,
- Bradley, P. M., Landmeyer, J. E., & Chapelle, F. H. (1999). Aerobic mineralization of MTBE and *tert*-butyl alcohol by stream-bed sediment microorganisms. *Environmental Science and Technology*, 33, 1877-1879.
- Chiang, C. F., Wu, Y. S., & Young, J. C. (2006). Critical analysis of computing equations for determination of BOD by dilution methods. *Journal of Environmental Engineering*, 132(5), 470-478.
- Cirvello, J. D., Radovsky, A., Heath, J. E., Fornell, D. R., & Lindamood, C. L. I. (1995). Toxicity and carcinogenicity of *t*-butyl alcohol in rats and mice following chronic exposure in drinking water. *Toxicology and Industrial Health*, (11), 151-165.
- Clark, J. J. (2002). *Tert*-butyl alcohol: Chemical properties, production and use, fate and transport, toxicology, and detection in groundwater and regulatory standards. In A. F. Diaz, & D. L. Drogos (Eds.), *Oxygenates in gasoline, environmental aspects* (1st ed., pp. 92-106). Washington D.C.: American Chemical Society.
- Davis, J. W., & Madsen, S. (1996). Factors affecting the biodegradation of toluene in soil. *Chemosphere*, 33(1), 107-130.
- Davis, M. J. (2002). Fuel oxygenates: How do their health effects compare? *ASTSWMO State Symposium on Fuel Oxygenates: Analysis, Assessment, and Remediation*. Arlington, VA.

- Day, M. J., & Gulliver, T. (2003). Natural attenuation of tert-butyl alcohol at a Texas chemical plant. In E. E. Moyer, & P. T. Kostecki (Eds.), *MTBE remediation handbook* (pp. 541-560). Amherst, MA: Amherst Scientific Publishers.
- Deeb, R. A., Scow, K. M., & Alvarez-Cohen, L. (2000). Aerobic MTBE biodegradation: An examination of past studies, current challenges and future research directions. *Biodegradation*, *11*, 171-186.
- Dietz, J., (2007). *Microbial degradation of fuel oxygenates under aerobic conditions*. MS Thesis AFIT/GES/ENV/07-M1. School of Engineering, Air Force Institute of Technology (AU), Wright Patterson AFB.
- Drogos, D. L. (2000). MTBE v. the other oxygenates. *Proceedings of Mealy's MTBE Conference*, Marina Del Rey, CA.
- Ellis, P. (2001). A Circle vicious what do we know about the other oxygenates? *LUSTLine, Bulletin 37*, 17-21.
- Fayolle, F., & Monot, F. (2005). Biodegradation of fuel ethers. In B. Olliver, & M. Magot (Eds.), *Petroleum microbiology* (1st ed., pp. 301-316). Herndon, VA: American Society for Microbiology.
- Fayolle, F., Vandecasteele, J. P., & Monot, F. (2001). Microbial degradation and fate in the environment of methyl tert-butyl ether and related fuel oxygenates. *Applied Microbiology and Biotechnology*, (56), 339-349.
- Goudar, C. T., & Strevett, K. A. (1998). Comparison of relative rates of BTEX biodegradation using respirometry. *Journal of Industrial Microbiology and Biotechnology*, (21), 11-18.
- Greenberg A., (2005). *Standard methods for the examination of water and wastewater* (21<sup>st</sup> ed.) American Public Health Association, American Water Works Association, Water Federation.
- HEI. (2001). *Metabolism of ether oxygenates added to gasoline* (Research Report No. 102). Cambridge, MA: Health Effects Institute.
- Jean, J. S., Tsai, C. L., Ju, S. H., Tsao, C. W., & Wang, S. M. (2002). Biodegradation and transport of benzene, toluene, and xylenes in a simulated aquifer: Comparison of modelled and experimental results. *Hydrological Processes*, (16), 3151-3168.
- Johnson, P., Bruce, C., & Miller, K. (2003). *In situ bioremediation of MTBE in groundwater (ESTCP project no. CU-0013)* (Final No. TR-2222-ENV). Port Hueneme, CA: Naval Facilities Engineering Service Center.

- Kane, S. R., Beller, H. R., Legler, T. C., Koester, C. J., Pinkart, H. C., Halden, R. U., (2001). Aerobic biodegradation of methyl tert-butyl ether by aquifer bacteria from leaking underground storage tank sites. *Applied and Environmental Microbiology*, 67(12), 5824-5829.
- Kinner, N. (2001). *Fate, transport and remediation of MTBE: Testimony Before the United States Senate Committee on Environment and Public Works*.
- Kisiel, B. D. (2000). Gasoline oxygenates attract attention of NHDES. *GeoInsighter Fall / Winter 2000 Newsletter*, 5(3), 1-2.
- Kovarik, B. (1994). Charles F. Kettering and the 1921 discovery of tetraethyl lead in the context of technological alternatives. *Society of Automotive Engineers Fuels & Lubricants Conference*, Baltimore, MD. 1-47.
- Lindamood, C. L. I., Farrell, D. R., Giles, H. D., Prejean, J. D., Collins, J. J., Takahashi, K., (1992). Subchronic toxicity studies of t-butyl alcohol in rats and mice. *Fundamentals of Applied Toxicology*, (19), 91-100.
- Lovanh, N., Hunt, C. S., & Alvarez, P. J. J. (2002). Effect of ethanol on BTEX biodegradation kinetics: Aerobic continuous culture experiments. *Water Research*, 36(15), 3739-3746.
- Mackay, D., Einarson, M., Wilson, R., Fowler, B., Scow, K., & Wood, I. (2001). *In situ* remediation of MTBE at vanderberg air force base, california. *Contaminated Soil Sediment and Water*, (Spring), 43-46.
- McCarthy, J., & Tiemann, M. (2003). *MTBE in gasoline: Clean air and drinking water issues* (Congressional Research Service Report for Congress No. 98-290 ENR). Washington D.C.: U.S. Library of Congress.
- Miles, R. A., & Doucette, W. J. (2001). Assessing the aerobic biodegradability of 14 hydrocarbons in two soils using a simple Microcosm/Respiration method. *Chemosphere*, (45), 1085-1090.
- Min, B., Kohler, D., & Logan, B. (2004). A simplified headspace biochemical oxygen demand test protocol based on oxygen measurements using a fiber optic probe. *Water Environmental Research*, (76), 29-36.
- Moyer, E. E. (2003). In Moyer E. E., Kostecki P. T. (Eds.), *MTBE remediation handbook, introduction & chemical and physical properties*. Amherst, MA: Amherst Scientific Publishers.
- OSTP (Office of Science and Technology Policy). (1997). *Interagency assessment of oxygenated fuels* (Final. Washington D.C.: Executive Office of the President, National Science and Technology Council.

- Pagga, U. (1997). Testing biodegradability with standard methods. *Chemosphere*, 35(12), 2953-2972.
- Powers, S. E., Hunt, C. S., Heermann, S. E., Corseuil, H. X., Rice, D., & Alvarez, P. J. J. (2001). The transport and fate of ethanol and BTEX in groundwater contaminated by gasohol. *Critical Reviews in Environmental Science and Technology*, 31(1), 79-123.
- Reisch, M. S. (1994). Top 50 chemicals production rose modestly last year. *Chemical and Engineering News*, (72), 12-16.
- Reuschenbach, P., Pagga, U., & Strotmann, U. (2003). A critical comparison of respirometric biodegradation tests based on OECD 301 and related test methods. *Water Research*, (37), 1571-1582.
- Salanitro, J. P., Johnson, P. C., Spinnler, G. E., Maner, P. M., Wisneski, H. L., & Bruce, C. (2000). Field-scale demonstration of enhanced MTBE bioremediation through aquifer bioaugmentation and oxygenation. *Environmental Science and Technology*, 34(19), 4152-4162.
- Sedran, M. A., Pruden, A., Wilson, G. J., Suidan, M. T., & Venosa, A. D. (2002). Effect of BTEX on degradation of MTBE and TBA by mixed bacterial consortium. *Journal of Environmental Engineering*, 128(9), 830-835.
- Squillace, P. J., Pankow, J. F., Korte, N. E., & Zogorski, J. S. (1998). Environmental behavior and fate of methyl *tert*-Butyl ether (MTBE). *U.S. Geological Survey, National Water Quality Assessment Program (NAWQA)*,
- Steffan, R. J., McClay, K., Vainberg, S., Condee, C., & Zhang, D. (1997). Biodegradation of the gasoline oxygenates methyl *tert*-butyl ether, ethyl *tert*-butyl ether, and *tert*-amyl methyl ether by propane-oxidizing bacteria. *Applied and Environmental Microbiology*, 63(11), 4216-4222.
- Stephenson, J. (2002). *Environmental protection, MTBE contamination from underground storage tanks*: U.S. General Accounting Office Testimony Before the Subcommittee on Environment and Hazardous Materials Committee on Energy and Commerce, U.S. House of Representatives.
- Stocking, A. J., Deeb, R. A., Flores, A. E., Stringfellow, W., Talley, J., Brownell, R., et al. (2000). Bioremediation of MTBE: A review from a practical perspective. *Biodegradation*, 11, 187-201.
- U.S. Department of Labor, Occupational Safety and Health Administration. (1996). *Occupational safety and health guideline for tert-butyl alcohol*. Retrieved 3/21, 2007, from <http://www.osha.gov/sltc/healthguidelines/tertbutylalcohol/recognition.html>

- U.S. EPA. (1997). *Drinking water advisory: Consumer acceptability advice and health effects analysis on methyl tertiary-butyl ether (MTBE)* No. EPA-822-F-97-009). Washington D.C.: U.S. EPA Office of Water.
- U.S. EPA. (1998). *MTBE fact sheet #3, use and distribution of MTBE and ethanol*. Retrieved 3/29, 2007, from <http://www.epa.gov/swrust1/mtbe/mtbefs3.pdf>
- U.S. EPA. (1999). *Achieving clean air and clean water: The report of the blue ribbon panel on oxygenates in gasoline* No. EPA420-R-99-021). Washington D.C.: USEPA.
- U.S. EPA. (2000). Methyl tertiary butyl ether (MTBE); advance notice of intent to initiate rulemaking under the toxic substance control act to eliminate or limit the use of MTBE as a fuel additive in gasoline. *U.S. Federal Register*, 65(58), 16093-16109.
- U.S. EPA. (2003). *Toxic release inventory (2001)* No. EPA 260-R-03-001). Washington D.C.: U.S. EPA Office of Environmental Information (2810A).
- U.S. EPA. (2004). *Technologies for treating MTBE and other fuel oxygenates*. Washington DC: U.S.EPA: Office of Solid Waste and Emergency Response, Office of Superfund Remediation and Technology Innovation.
- U.S. EPA. (2005). *Contaminant focus: Methyl tertiary butyl ether (MTBE)*. Retrieved 3/21, 2007, from [http://www.cluin.org/contaminantfocus/default.focus/sec/methyl\\_tertiary\\_butyl\\_ether\\_\(mtbe\)/cat/overview](http://www.cluin.org/contaminantfocus/default.focus/sec/methyl_tertiary_butyl_ether_(mtbe)/cat/overview)
- U.S. EPA. (2006a). *Methyl tertiary butyl ether (MTBE): Overview*. Retrieved 03/21, 2007, from <http://www.epa.gov/mtbe/faq.htm>
- U.S. EPA. (2006b). *Methyl tertiary butyl ether (MTBE) gasoline*. Retrieved 9/21, 2006, from <http://www.epa.gov/mtbe/gas.htm>
- U.S. EPA. (2007). *EPA takes final step in phase out of leaded gasoline (EPA press release-january 29, 1996)*.<http://www.epa.gov/history/topics/lead/02.htm>
- U.S. EPA. (2007). *UST program facts*. Retrieved 3/21, 2007, from <http://www.epa.gov/OUST/pubs/ustfacts.pdf>
- Wilson, J. T. (2003). Fate and transport of MTBE and other gasoline components. In E. E. Moyer, & P. T. Kosteki (Eds.), *MTBE remediation handbook* (pp. 19-61). Amherst, MA: Amherst Scientific Publishers.
- Wilson, G. J. (2002). Biodegradation kinetics of MTBE in laboratory batch and continuous flow reactors. *Journal of environmental engineering (New York, N.Y.)*, 128(9), 824-829.

- Young, J. C., Clesceri, L. S., & Kamhawy, S. M. (2005). Changes in the biochemical oxygen demand procedure in the 21st edition of standard methods for the examination of water and wastewater. *Water Environmental Research*, 77(4), 404-410.
- Zanardini, E., Pisoni, C., Ranalli, G., Zucchi, M., & Sorlini, C. (2002). Methyl *tert*-Butyl ether bioremediation studies. *Annals of Microbiology*, (52), 207-221.
- Zhuang, W., Tay, J., Yi, S., & Tay, S. (2005). Microbial adaptation to biodegradation of *tert*-butyl alcohol in a sequencing batch reactor. *Journal of Biotechnology*, (118), 45-53.
- Zogorski, J. S., Moran, M. J., & Hamilton, P. A. (2001). MTBE and other volatile organic compounds-new findings and implications on the quality of source waters used for drinking-water supplies. *U.S. Geological Survey*

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<b>14. ABSTRACT</b> Methyl <i>tert</i> -Butyl Ether (MTBE) is the most commonly used fuel oxygenate in the world. Its recalcitrant nature as well as its chemical properties have led to widespread groundwater contamination. Questions regarding its toxicity have spurred a search for viable oxygenate alternatives. Since biodegradability is a key indicator of a chemical's environmental impact, this research used three different well-known methods, BOD <sub>5</sub> , respirometry, and GC analysis, to examine the extent and rates of aerobic biodegradation of MTBE along with <i>tert</i> -butyl alcohol (TBA). The common fuel component toluene was added to some of the samples to determine if the presence of a co-contaminant would effect aerobic microbial degradation of TBA or MTBE. This group of experiments used an acclimatized microbial consortium to enhance degradation of the oxygenates. BOD <sub>5</sub> experiments were performed separately from the GC and respirometric analyses. The respirometry used 250ml microcosms containing a mix of microbial seed, BOD buffer, and varying concentrations of the oxygenates or oxygenate/toluene mixtures. The respirometer also maintained the microcosms in aerobic conditions for the duration of each experiment. For GC analysis, samples were drawn from the respirometer microcosms at predetermined intervals and first order degradation rate constants were calculated from established calibration curves. The oxygenates degraded much slower than toluene in all experiments. This degradation characteristic made BOD <sub>5</sub> analysis impractical for MTBE or TBA. BOD <sub>5</sub> did provide valid results for toluene. The respirometer data was not as good as gas chromatography to provide specific measurements of degradation. To facilitate comparison of degradation across experiments with differing seed, oxygenate degradation was compared to toluene. MTBE was effectively degraded under these experimental conditions and degraded at 13.94% the rate of toluene. TBA was more recalcitrant and only degraded at 1.37% of toluene.					
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