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Microbial Degradation of Fuel Oxygenates under Aerobic Conditions

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THESIS

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AFIT/GES/ENV/07-M1

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Abstract

This research determined the rate and extent of aerobic biodegradation of fuel oxygenates ethyl tert butyl ether (ETBE), tert amyl methyl ether (TAME), and ethyl alcohol (ethanol). Biodegradation was measured using gas chromatography (GC), respirometry, and biochemical oxygen demand (BOD) tests. Additionally, the research determined the effects of toluene on degradation rates.

This microcosm study used a microbial consortium obtained from a petroleum refinery wastewater treatment facility. Respirometry data were collected from chambers containing pure oxygenates, or oxygenate/toluene mixtures. Samples were withdrawn periodically for GC analysis. Aerobic conditions were maintained in the chambers at all times. The five-day BOD test was conducted separately using Standard Methods.

Degradation of oxygenates was compared to degradation of toluene, assuming first order decay. Across all experiments TAME degraded at 8.57% the rate of toluene. Similarly, ETBE degraded at 7.86% the rate of toluene. Ethanol was significantly faster, degrading at 158.26% the rate of toluene. GC and respirometry were the most suitable methods for measuring degradation. The BOD_5 test provided acceptable results for toluene and ethanol, but not for the slower degrading oxygenates. Finally, the presence of toluene slowed the degradation of both ETBE and TAME.

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I. Introduction

Background

The use of additives to enhance gasoline performance dates back nearly a century. In the 1920s tetra ethyl lead was added to gasoline to increase the octane rating. The lead effectively prevented premature detonation (knocking) and allowed more efficient engine operation, but evidence of health concerns soon arose. Despite lead poisoning deaths at General Motors, Dupont and Standard Oil tetra ethyl lead manufacturing facilities (Kovarik 1999), leaded gasoline production continued In the 1960s, concern about the health and pollution effects of airborne lead prompted the Environmental Protection Agency (EPA) to mandate lead reduction (EPA 2003). Oxygenates were subsequently introduced as a potential replacement for lead. These oxygen-containing compounds increased the octane rating while burning much cleaner than their predecessor. Methyl tert butyl ether (MTBE) was the first of these oxygenates and was being added to gasoline in small amounts in the late 1970s (Stocking 2000).

The 1990 Clean Air Act (CAA) amendments greatly increased the use of oxygenates. These amendments required the addition of oxygenates to gasoline in certain regions of the country in an attempt to reduce ozone and carbon monoxide emissions (USGS 2002). While the EPA did not specify which of the many possible oxygenates should be used, MTBE quickly became the predominate choice due to its low cost, ease of production, and favorable transfer and blending characteristics (Squillace 1998).

Problem

The increased use of MTBE has led to increased accidental releases from spills and leaks. While MTBE has contributed to a reduction of exhaust emissions (Okeke 2002), the environmental effects of accidental releases are significant. Because of its high solubility in water, MTBE has the ability to migrate through subsurface and groundwater with minimal retardation (Stocking 2000). As a result, releases of fuel with MTBE may spread contamination much faster than traditional,non-oxygenated gasoline (Squillace 1998). Complicating the problem is the fact that MTBE does not appear to degrade easily under natural conditions. Oxygenates typically have one oxygen atom and a chain of carbons with hydrogens. The carbon – oxygen – carbon structure is not easily broken (Dekant 2001). As a result, MTBE resists microbial degradation and has slow natural attenuation, with a half-life of about two years (Fayolle 2001). Long-lasting, fast traveling MTBE plumes have spread from numerous leaks and spill sites. The United States Geological Survey's National Water Quality Assessment (USGS NAWQA) from 1993-1998 documented frequent occurrence of MTBE in shallow urban groundwater (USGS 2006).

Despite the fact that MTBE has become a much-studied chemical, little is definitively known about its health effects. It is widely believed to be a carcinogen, but the link from animal to human toxicity has not been firmly established. The EPA, California Carcinogen Identification Committee, International Agency for Research on Cancer, and National Toxicity Program have all stated that MTBE is clearly an animal carcinogen and potentially a human risk, but there is not enough direct evidence to label it a human carcinogen (Ahmed 2001). In 1998 a Blue Ribbon Panel (created by a charter

from the CAA Advisory Committee) advised the USEPA that MTBE is primarily an odor and taste concern (USGS 2002). While the EPA has only set a drinking water advisory level of 20-40 μg/L based on taste and odor, some states have set much more restrictive health related standards (Fayolle 2001).

Objective

While MTBE is not federally regulated, growing concern is prompting a search for less hazardous oxygenate alternatives. One of the many factors that will contribute to selection of a new oxygenate will be its susceptibility to microbial degradation. This research intends to determine the extent and rate of aerobic biodegradation for potential replacements ethyl tert butyl ether (ETBE), tert amyl methyl ether (TAME), and ethyl alcohol (ethanol). The objective is to establish the rate of degradation for the chemicals alone, as well as chemicals mixed with toluene, a representative of the common gasoline components likely to be present in field conditions.

Biodegradation will primarily be measured by recording the change in oxygenate concentration in aerobic aquatic microcosms over time using the gas chromatograph (GC). Additionally evidence of microbial activity will be measured in the form of O_2 consumption in BOD_5, and CO_2/ O_2 levels measured by respirometry. Finally the BOD/THOD ratio will be determined.

If successful, the information provided could contribute to wise selection of a replacement oxygenate.

Questions

1. Are these oxygenates biodegradable and if so at what rate?
2. Can this biodegradation be measured directly with the GC?

3. Can the microbial activity measured with BOD_5 or respirometry be correlated to reduced concentrations due to degradation?
4. Is the COD/THOD ratio a good indicator of degradation potential?
5. Does the presence of co-contaminant toluene effect biodegradation characteristics?

Limitations

This research is subject to a number of limitations. First, only aerobic degradation is considered. Each release site is different and any site could include both aerobic and anaerobic zones. The microbes, methods, requirements, and degradation rates can vary widely between aerobic and anaerobic systems. As a result the actual rate of contaminant reduction due to microbial activity can vary by site as well.

This is a laboratory study conducted under carefully controlled conditions. While some effort is taken to examine the effects of other chemicals (toluene) on degradation, the research cannot replicate field conditions. Presence of other chemicals, soil characteristics and environmental factors (pH and temperature) can all affect degradation rates in the field.

Finally the measure of degradation used in this study is a reduction in concentration of starting material. The time and steps required for the degradation to go to completion is not considered in this study. There can be numerous steps on a degradation pathway, some of which can contain potentially undesirable substances.

This study is a starting point to determine if, under laboratory settings, microbial degradation of select oxygenates will occur and can be effectively measured.

II. Literature Review

History

The addition of tetra ethyl lead to gasoline as an octane booster was effective, but had significant drawbacks. The acute hazards of airborne lead were readily apparent as manufacturing facilities experienced numerous deaths. As the air pollution effects of lead became more obvious, the decision to continue its use came into question. Finally in 1973, the EPA issued a lead reduction standard requiring a phase down to 0.1gram per gallon of gasoline (Squillace 1998). The next octane booster to emerge was MTBE, added to US gasoline at low levels starting in 1979 (USEPA 2005).

Concerns about air quality continued, leading to the 1990 Clean Air Act Amendments which mandated the use of fuel oxygenates in areas that did not meet ambient air quality standards for CO and ozone (Squillace 1998). In order to meet the mandates of the CAA amendments, oxygenated fuel or OXYfuel, and reformulated gasoline or RFG, were introduced. The 1992 Oxyfuel program required the use of gasoline with 2.7% oxygen (by weight) in 39 different areas with high CO levels, during fall and winter months (USEPA 1998b). The RFG program introduced in 1995 required the year-round use of gasoline with 2.0% oxygen by weight in numerous metropolitan areas (USEPA 1998b).

Current State

The EPA estimates that 34% of all fuel in the US is either OXYfuel or RFG. Oxygenates are also added to many conventional gasoline for octane enhancement.

(USEPA 1998b). As a result, oxygenates are present at some level in about 70% of all US gasoline. The most common oxygenate has historically been MTBE at 80%, followed by ethanol at 15% and all others comprising the last 5% of oxygenates used (USEPA 1998b).

The cleaner-burning oxygenated fuels have been effective in reducing air pollution. The EPA estimates that oxygenate use reduces smog-forming pollutants by approximately 105 thousand tons, and toxics by 24 thousand tons annually (USEPA 2005). Unfortunately, gains made in air pollution seem to have been offset by increased groundwater pollution. Surface runoff and leaks from some of the estimated 3.7million underground storage tanks (USEPA 2004) have allowed large quantities fuel to enter the water table. While leaks and spills are not new occurrences, the physical characteristics of the primary oxygenate, MTBE, have led to a much more widespread contamination.

Of the states that report testing at leaking tank sites, 35 report finding MTBE as well as other oxygenates (USEPA 2004). Additionally MTBE was detected in 5.7% of all NAWQA program well samples (Zogorski 2001). While that is a relatively small percentage, it is significant to note that detection was nearly three times higher in urban wells, serving a much larger population. Drinking water studies in the Northeast and Mid-Atlantic regions of the US found similar results, with 15% of urban sites contaminated (Zogorski 2001). MTBE has also been detected in groundwater of areas not participating in OXYfuel or RFG programs.

While most detection levels are very low, only 1% exceeding 20μg/L, MTBE in groundwater is still a matter of concern. The suspected link between MTBE and adverse health effects has led many to demand its removal. While the federal government has

stopped short of removing MTBE, some states have not. CA has mandated a complete phase out of MTBE. On the federal level, the EPA is reportedly considering changing the requirement for addition of oxygenates to gasoline (Powers 2001).

Oxygenates

The primary purpose of oxygenates is to optimize oxidation during combustion. The more complete burn will result in reduced auto emissions (USEPA 2005). Oxygenates can be divided into two broad categories; alcohols and ethers.

Alcohols are produced from aliphatic hydrocarbons by replacing one or more hydrogen with a hydroxyl group (Cunill 1993). They are produced naturally by fermenting a carbohydrate (typically corn or grain) and distilling. Alcohols can also be produced synthetically using ethylene and an acid catalyst. Alcohols are typically very soluble in water due to the polarity of the oxygen-hydrogen bond. This solubility can make alcohols difficult to blend and transport. Ethanol (ethyl alcohol) is the most commonly produced alcohol. Other alcohols include methanol (methyl alcohol), TBA (tert-butyl alcohol) and TAA (tert-amyl alcohol).

Ethers are organics manufactured from petroleum derivatives that feature an oxygen atom between two carbons (Cunhill 1993). These ethers are generally resistant to biodegradation. The ether linkage, combined with short branching from the base molecule make it hard for microbes to attack and degrade the molecule (Kinner 2001). The most common ether is MTBE (methyl tert-butyl ether). Other ethers include ETBE (ethyl tert-butyl ether), TAME (tert-amyl methyl ether) and DIPE (diisopropyl ether). Chemical properties of the oxygenates are summarized in Table 2.1, below.

MTBE

MTBE, the most commonly used fuel oxygenate, is an aliphatic ether derived from the catalytic reaction of methanol and isobutene (Ahmed 2001). The methanol used in this reaction is generally derived from natural gas. MTBE was developed in the 1940's and had its first commercial use in the 1970's (USEPA 1998a). It was first used in Europe as a gasoline-blending agent, while its initial use in the US was as a replacement for tetra ethyl lead. As previously noted, MTBE is very soluble (43000 mg/L) in water and miscible in gasoline. It has very favorable blending characteristics and can be blended at the refinery and sent through the existing supply systems (Squillace 1998). MTBE is preferred over its rival oxygenate ethanol because of these blending characteristics and the lower volatility of MTBE-blended gasoline. Without considering federal and state subsidies for ethanol, MTBE is also less expensive to produce (USEPA 1998a)

Ethanol

Ethyl alcohol or ethanol, is the second most popular oxygenate in use. It is a volatile, flammable, colorless liquid miscible in water. In the US, it is usually produced by microbial fermentation of corn (CFDC 2006). Ethanol was used in gasoline as early as the 1900's. It was used as a fuel extender in World Wars One and Two, as well as the fuel crisis in 1973 (CFDC 2006). Ethanol has been promoted as a renewable, biomass-based fuel alternative and has considerable political backing. The federal government currently offers a 54-cent per gallon subsidy to promote ethanol markets (Powers 2001).

Health and pollution concerns about MTBE, combined with an aggressive marketing campaign, has led the ethanol output in the US to double since 2000 (Zhang 2006).

For all its benefits, ethanol has some drawbacks as well. It requires separate manufacturing and transport as it will separate from gasoline over time (USEPA 1998b). Its extreme solubility may cause water and impurities to be brought into solution in the gasoline, rendering it unusable (USEPA 1998a). Finally gasoline blended with ethanol has a higher volatility, making it less desirable in warm weather (USEPA 1998b).

ETBE

ETBE is an ethanol-based ether produced by the reaction of ethanol and isobutylene in the presence of heat (CFDC 2006). Isobutylene may be produced from excess butanes already in the refining industry. The main advantage of ETBE is that it allows the use of ethanol, without many of the traditional drawbacks. ETBE blended gasoline is less volatile than MTBE or ethanol blended (CFDC 2006) and it maintains its favorable blending and transporting characteristics. ETBE is actually a better octane enhancer than MTBE (Cunhill 1993) and can be produced with essentially the same refinery equipment.

TAME

TAME is an oxygenate produced by the reaction of methanol and isoamylenes, using a strong acid as a catalyst (Oost 2004). It was first brought into commercial production in the late 1980's (Huttenen 1997). TAME is similar to MTBE except that it

uses existing hydrocarbon feedstocks (converting tertiary olefins to tertiary ethers) and reduces waste in the refinery process (Huttenen 1997).

Table 2.1 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 the name given to a group of volatile organic hydrocarbons found in gasoline. Normally included in the BTEX classification are benzene, toluene, ethylbenzene, and three isomers of xylene (Lovahn 2002). These compounds are generally more volatile and less soluble than gasoline oxygenates; however, they are among the most soluble of the hydrocarbons in gasoline. Since the phase out of lead, the amount of BTEX in gasoline has risen from 26% to 34% by volume (Deeb 2000).

Table 2.2 Chemical properties of select BTEX components

(Deeb 2000)

Component	Pure Phase Solubility (mg/L)	log Kow (log l/kg)	Log Koc (log l/kg)	Vapor Pressure (25C, mmHg)	Henry's Law Constant (Dimensionless)
Benzene	1780	1.6	1.5	76	0.220
Ethylbenzene	161	3.2	2	9.5	0.320
o-xylene	175	2.8	1.7	6.6	0.213
m-xylene	146	3.2	2.2	8.3	0.300
p-xylene	156	3.1	2.1	8.7	0.313
Toluene	534.8	2.73	1.6	28.4	0.242

Health effects of BTEX

Benzene is the most dangerous of the BTEX compounds, having been directly linked to acute myelogenous leukemia or AML (ATSDR 2005a). It is officially listed as a carcinogen by the EPA and the International Agency for Research on Cancer (IARC). Additionally, exposure to high levels of benzene may lead to dizziness, vomiting and rapid heart rate. Toluene exposure at low concentration may cause temporary dizziness, memory loss, and confusion, while higher concentrations may affect the kidneys (ATSDR 2001). Toluene has not been linked to cancer. The health effects of ethyl benzene are similar to toluene, with the added effect of eye irritation in airborne exposure (ATSDR 1997). No studies have shown that ethyl benzene exposure causes cancer. The remaining compounds display the same symptoms after high exposure. Xylenes are more of an inhalation hazard, causing irritation of the eyes, nose and throat. These compounds may also cause changes in the liver and kidneys. There is insufficient evidence to determine whether xylenes are carcinogenic (ATSDR 2005b).

Despite the fact that the health effects of the six BTEX compounds have been studied intensively, no studies specifically address the combination of compounds

(USDHS 2004). It is plausible however to consider the joint action additive, especially in neurotoxic action (USDHS 2004). All six of the BTEX compounds have shown the capability to cause some level of depression of the central nervous system (Lovahn 2002). In addition it should be noted that most health studies of these compounds deal primarily with inhalation, and only secondarily with ingestion.

Health Effect of MTBE

While the detrimental effects of MTBE exposure have been the source of much speculation, there is relatively little data available to support listing MTBE as a toxic substance (Ahmed 2001). Headaches, nausea, and sensory irritation have been reported by personnel exposed to MTBE-gasoline vapors. Controlled studies however, have failed to attribute significant irritation to the MTBE. In fact, no significant difference in irritation was found in exposure to MTBE and non-MTBE gasoline (Ahmed 2001). While there are documented kidney and liver effects on mice, the specific protein affected is not present in the human body, so the linkage is questionable at best (Ahmed 2001).

Formaldehyde and tert-butyl alcohol (TBA), two products formed in the first step of MTBE metabolisms are the main cancer threats (Hong 2001). These compounds have been shown to cause an increase in testicular cancer in male rats and leukemia and lymphoma in female rats. They have also been linked to an increase in mutagenicity in mice (Ahmed 2001). Animal experiments have shown that long term-high level exposure to MTBE via oral or inhalation pathways can lead to cancer (Hong 2001). The difficulty to this point has been translating animal data to reasonable human carcinogenicity

predictions. While it is generally agreed that MTBE is an animal carcinogen, there is insufficient data to label it anything more than a potential risk. As a result, the EPA has only established a drinking water advisory of 20-40 μg/l based on taste and odor. There is no maximum federal contaminant level (USEPA 2004). Despite the lack of hard evidence, 31 states have established their own guidelines, advisory levels or action levels (USEPA 2004).

Health Effects of TAME

There is considerably less data on health effects of TAME than the health effects of MTBE. It is expected however that TAME is very similar to other ether oxygenates. It has primarily been studied as an inhalation and dermal exposure threat from gasoline. Some data suggests that in high concentrations, it can be a skin and eye irritant (NICNAS 2001). Rat inhalation studies have indicated some CNS depression and mortality at extreme doses. Some models have led researchers to believe that TAME can target the liver and kidneys (Davis 2002) but animal studies are inconclusive. One inhalation study using mice and beagles showed some liver effect, but no appreciable CNS depression (NICNAS 2001). Oral studies have shown mortality at extreme doses, but no liver damage or toxic effects at lower doses.

The cancer potential of TAME is largely unstudied and there is no quantitative cancer potency data available (Davis 2002). The metabolism of TAME, however still involves formaldehyde, a possible agent (NICNAS 2001). The cancer threat of TAME may be slightly less than that of MTBE however, because instead of the suspect TBA, the

other TAME metabolite is TAA, a more innocuous compound. TAME also has a lower taste and odor threshold than MTBE (Davis 2002).

Health Effects of ETBE

Similar to the case of TAME, very little is known about the health effects of ETBE. Some studies have demonstrated that inhalation at high levels may cause irritation of the mouth and a 'bad taste', but no significant, dose-dependent nasal or eye effects have been shown (Nihlen 1998). Similarly, a rat inhalation study showed few histopathological effects (Ahmed 2001).

ETBE is metabolized into TBA, so that is a recurring cancer concern. However, a structure-based predictive computer model predicted that ETBE would be neither a genotoxicant nor carcinogen (Ahmed 2001). As with the other ether oxygenates, there is not enough information available to quantify a cancer potency value, or label ETBE a carcinogen (Davis 2002). ETBE is believed to be as bad or worse taste and odor offender than MTBE (Davis 2002).

Health Effects of Ethanol

Of all the oxygenates, ethanol is believed to be the safest from a health perspective. A great deal of data is available on ethanol health effects, most of it based on ingestion in alcoholic beverage consumption (Davis 2002). It is possible that acute inhalation will cause some neurobehavioral effects, but the consensus is that ethanol in groundwater will not create any public health concerns (Davis 2002).

The only potential health effect of ethanol is a result of an ethanol metabolite emitted in vehicle exhaust. Acetyladehyde can undergo a photochemical reaction to make peroxylacetate nitrate (PAN), which is an eye and respiratory system irritant (Ahmed 2001). Despite this fact, ethanol is considered to present no adverse health effects to the general population (Ahmed 2001).

Fate and Transport of Oxygenates

A number of different physical characteristics allow the prediction of a contaminant's behavior in groundwater. The pure phase solubility reflects how much chemical will dissolve into water from a pure product. The organic carbon partition coefficient or K_{oc}, shows how readily a contaminant partitions from the water phase to organic substances. Henry's constant (H) shows readiness to move between dissolved and vapor phases, while the vapor pressure represents readiness to move between pure phase liquid and vapor.

MTBE has a very high pure phase solubility, much higher than the highest BTEX component. Its solubility means that it is much more likely to enter dissolved phase than other ingredients in the gasoline mixture (Squillace 1998). MTBE has a relatively low K_{oc}, making it unlikely to sorb onto organic solids. Finally, while MTBE is actually more volatile, with a higher vapor pressure than BTEX components from the pure phase, it has a low Henry's number and doesn't rapidly volatilize from the dissolved state. These unique physical characteristics allow MTBE to dissolve into and move along with groundwater with little or no retardation (Stocking 2000).

The other ether oxygenates display chemical properties similar to MTBE and can be expected to behave similarly. Once released, TAME is expected to penetrate the soil and reach the groundwater quickly without much sorption to organic matter (NICNAS 2001). ETBE can be expected to react similarly and should dissolve into groundwater nearly as fast as MTBE. In a groundwater model, (Huttenen 1997) showed MTBE with a relative mobility (compared to groundwater) of 1.0, while TAME had a mobility of 0.69 and BTEX components ranged from 0.11 to 0.33 (Huttenen 1997).

Ethanol is miscible, or infinitely soluble in water. It also displays little propensity to sorb into organics or to volatilize out of groundwater. Ethanol blended gasoline has a relatively high volatility that limits its use in some areas (GEC 1999) but when dissolved in groundwater ethanol tends to stay dissolved.

Fate and Transport of BTEX Compounds

BTEX compounds are generally not easily dissolved into groundwater (Jean 2002). They are also much more likely to sorb onto organic surfaces and volatilize into interstitial pores (Jean 2002).

Biodegradation of Oxygenates

Physical characteristics allow prediction of contaminant travel, but don't offer much insight into the contamination level. Biodegradation is significant because it offers the actual reduction of contaminant levels through destruction of material. This reason, along with the fact that it is generally less expensive, makes bio-remediation an attractive choice in many situations (Goudar 1998). Additionally, while many of the traditional

physical and chemical remediation techniques used for BTEX can work for oxygenates, it is usually at much higher cost and much longer duration (Zein 2006).

Early studies on ether oxygenates have shown them to be resistant to degradation under field conditions. MTBE's natural attenuation is very slow, with a reported half-life of two years (Squillace 1998, Kinner 2001) in groundwater. TAME is also reported to be officially non degradable (less than 60 percent degradation in ten days) (Huttenen 1997). Despite early assertions that MTBE was recalcitrant, laboratory work has demonstrated that it and other oxygenates can be degraded under certain conditions (Zein 2006). Zein's membrane- based bio reactor showed 99% removal of MTBE, TAME, DIPE, ETBE, and BTEX components. Similarly Kharoune degraded ETBE, MTBE and TAME using specialized innoculum and an upflow fixed bed reactor (Kharoune 2000). Numerous studies have demonstrated that specific strains or isolates can degrade many different oxygenates under various aerobic and anaerobic conditions. Admittedly, the challenge is to replicate laboratory conditions in the subsurface environment (Zein 2006).

While MTBE has been a carefully examined chemical, little is known about the other oxygenates' relative degradability. Kharoune showed that, in his reactor, MTBE and TAME had a reaction rate of 29 mg/L -d while ETBE was faster at 73 mg/L - d. (Kharoune 2000).

Ethanol is readily degraded both aerobically and anaerobically. Even in field conditions, it can exhibit 97% biodegradation in soil within one month (Zhang 2006). Ethanol can actually degrade too readily, causing excessive microbial growth that clogs aquifers (Powers 2001). Ethanol has been determined to have a half-life in groundwater

of 2-3 days under aerobic conditions and 1-7 days under anaerobic conditions (Powers 2001).

Biodegradation of BTEX Compounds

BTEX components are all fairly susceptible to biodegradation. There are numerous organisms that can degrade toluene, and most of those are thought to be effective with other monoaromatic hydrocarbons (Chakraboty 2005). Toluene has been shown to have a half-life of 2-3 months in ground water (Kinner 2001). Microbial cultures have shown the need to adjust to toluene, creating a lag time of up to 186 hrs before the onset of biodegradation, in addition there is no degradation above the threshold level of 200 μg/g (Davis 1996). Other BTEX components can degrade as quickly as toluene. Laboratory experiments showed toluene with a 0.8 day -1 decay rate, benzene with a 1.3 day-1, and the xylenes with 1.8 day -1 (Jean 2002). All other BTEX components displayed lag times under certain conditions, ranging from 3 to 74 days (Goudar 1998).

Biodegradation of Mixtures

Despite the fact that these components are very often found in mixed releases, little research has been done on the degradation of the mixtures. Early research seems to show that biodegradation of BTEX as a mixture, is slower than biodegradation of its components separately (Goudar 1998). Also the degradation of BTEX and oxygenate mixtures have not been widely studied. Initial results indicate that the outcome differs by BTEX component. When mixed, toluene and MTBE both degrade, but at a much lower

rate while ethyl benzene and xylene completely inhibited MTBE degradation (Deeb 2000). The same study indicated that when mixed with MTBE, benzene would be preferentially consumed, after which time MTBE would return to normal degradation rate (Deeb 2000).

The combination that has been examined extensively is the combination of ethanol and BTEX components. The presence of ethanol seems to be detrimental to degradation of other materials. One reason is that ethanol degrades so rapidly that it exerts a large biochemical oxygen demand. The ethanol consumes electron acceptors and nutrients making the degradation of remaining products very slow (Powers 2001). While microbes can degrade ethanol simultaneously with BTEX , they normally consume ethanol preferentially over BTEX compounds, leaving the BTEX until all the ethanol is consumed (Lovahn 2002). In some cases, especially along plume borders, ethanol may cause an increase in overall microbial population that temporarily boosts BTEX degradation, but generally ethanol hinders natural attenuation of BTEX (Lovahn 2002).

In general the fate and transport of gasoline contamination is dependent on the particular oxygenate in the fuel. MTBE and the other ether oxygenates are more likely to travel farther and faster than BTEX components of gasoline (USEPA 2004). The physical characteristics of ethers that allow minimal retardation, combined with the resistance to degradation, make oxygenate plumes a serious concern. Ethanol mixed gasoline has the opposite effect. While ethanol is also able to dissolve into groundwater, it is degraded so rapidly that it rarely lasts long in the environment. The problem with the ethanol-blended gasoline is that BTEX plumes seem to migrate farther than they would in non-blended gasoline. During the initial travel time, ethanol is being exclusively

degraded while the BTEX is largely untouched, causing its plume to migrate and spread (USEPA 2004)

Specific Loss

Specific loss of a chemical can be accurately measured with an analytical method such as gas chromatography or high-pressure liquid chromatography (Pagga 1997). The official method required by the EPA for measurement of MTBE and BTEX compounds is method 8015 GC/FID (USEPA 2004), a gas chromatography with flame ionization detector method. While it specifically indicates that a chemical is no longer present in its initial form, it does not indicate complete degradation. If the degradation by-products are known, they can be detected separately. This can give valuable information about how long a contaminant stays in each step of its degradation pathway.

CO_2 production

CO_2 production can be accurately measured by respirometry. Measurement of CO_2 production is a reliable technique to determine how much of a chemical substance has progressed completely to mineralization (Miles 2001). This technique may, however, underestimate the rate and extent of biodegradation (Davis 1996). CO_2 measurement does not take into account that substrate that is incorporated into the microbial biomass. Additionally, there are numerous sinks and sources of CO_2 in many environments that can lead to inaccuracy (Miles 2001). In a comparison of CO_2 production to actual substrate disappearance, Davis found that 100 percent substrate removal translated into between 29 and 56 percent mineralization (Davis 1996).

O_2 consumption

O_2 consumption, as an indicator of microbial activity, can also be used to measure biodegradation. O_2 consumption measured by respirometry was found to be an effective indicator of substrate removal (Miles 2001). Miles showed in a soil microcosm test of 14 hydrocarbons, that O_2 consumption represented as percent Theoretical Oxygen Demand, correlated to analytically measured reduction in chemical levels (Miles 2001).

Measurement of O_2 consumption by a standard Biochemical Oxygen Demand test has been done since the late 19th century (Min 2004). It is widely regarded as a good way to measure the amount of biodegradable organic matter in water. Typically oxygen demand is exerted faster in respirometry than in BOD tests, mostly due to higher concentrations of both substrates and microbes (Min 2004).

Rapid screening models have also been used to predict O_2 consumption and potential for biodegradation based on physiochemical properties. Babeu used three different equations to predict biodegradability and compare the results to actual BOD_5 measurements. His model showed the ability to predict BOD_5 results under certain starting concentrations (Babeu 1987). It should be noted that all O_2 measurement techniques are not necessarily indicators of complete mineralization (Miles 2001).

III. Research Methodology

General experimental design

The experiments used three main techniques. Direct measurement of oxygenate level by gas chromatograph (GC) was done in conjunction with respirometric monitoring. Biochemical oxygen demand (BOD_5) measurements were conducted separately.

Aqueous samples containing deionized water, BOD buffer, microbial seed and one or more oxygenates were prepared and connected to the respirometer. Periodic measurements were taken of each sample to determine rate of oxygen consumption and rate of carbon dioxide production. Samples from these same mixtures were also analyzed periodically by GC to determine actual oxygenate concentration. Similar aqueous samples were prepared separately for the BOD_5 experiments.

All chemicals used were manufactured by Sigma Aldrich of Milwaukee, WI. Table 3.1 summarizes the chemical information.

Table 3.1 Summary of Chemical Information

Name	CAS no	Grade	Lot no
Toluene	108-88-3	HPLC grade 99.8%	01546EC
Ethanol	64-17-5	HPLC/Spectrophotometric grade (200 proof)	03348PC
ETBE	637-92-3	Reagent grade 99%	06820HA
TAME	994-05-8	Reagent grade 97%	46697-1

The selection of microbial seed differed over the course of the experiments. Initially, the supernate from activated sludge samples obtained from a local municipal wastewater treatment plant was used. After the first five BOD_5 experimental runs, the decision was made to obtain a microbial seed that was more able to consume the samples.

The next two BOD_5 experiments, as well as the first respirometer and GC experiment, utilized supernate from activated sludge samples obtained from a petroleum refinery's industrial wastewater treatment facility. The remaining experiments utilized microbial seed obtained from the refinery but acclimated and maintained in the lab.

The microbial seed was stored in two 1000mL bottles. The seed was fed 0.01mL toluene and 0.003mL of an ETBE, TAME, and ethanol mixture every four days. Toluene was the predominate feed to reflect the fact that in most gasoline releases, BTEX compounds would be present in much higher amounts than oxygenates. The seed was also fed one gram of yeast or beef extract every eight days.

Every two weeks 300mL of the seed was poured off and the bottles were refilled to 1000mL with double strength BOD buffer and deionized water. This replaced water lost to evaporation and reduced toxic waste product accumulation in the bottles. Table 3.2 summarizes the seed sources for each of the experiments.

Table 3.2 Seed sources for experiments

Seed Source	Experiment
Municipal Activated Sludge	**BOD_5 numbers 1 - 5**
Industrial Activated Sludge	**BOD_5 numbers 6-7** **GC experiment 1** **Respirometer experiment 1**
Acclimated Seed	**BOD_5 number 8** **all remaining GC and respirometer experiments**

Biochemical Oxygen Demand (BOD_5)

The BOD_5 experiment was conducted in accordance with the commonly accepted procedures established in the Standard Methods (Greenberg 2005). Deionized water was

incubated at 20 degrees C and aerated for a period of 24 hours prior to each BOD_5 test. BOD buffer was added to the water and it was thoroughly mixed to make the dilution water. A glucose/glutamic acid standard solution was prepared by adding 75 mg of each and diluting to 500 mL.

The bottles used for the experiment were 300mL glass BOD bottles with ground glass stoppers and a flared mouth. Three bottles were filled with dilution water to serve as controls. Five seed control bottles were filled with dilution water and seed at various dilutions. These seed control bottles establish the dissolved oxygen uptake of the seed itself. Three glucose/glutamic acid check bottles were filled with 6mL of the glucose/glutamic acid solution, 3mL seed and dilution water. Finally groups of five bottles were filled with 3mL seed, dilution water and oxygenates at various dilutions.

For dilutions greater than 1:100, a primary dilution was made first. Each bottle was initially filled approximately two thirds full with dilution water. The oxygenate was added next, followed by the seed. The 3mL seed addition consisted of 1.5mL from each of the two seed storage bottles. The bottle was then filled with dilution water until the insertion of the stopper displaced all air in the bottle.

The initial dissolved oxygen was measured using a YSI model 5100 Dissolved Oxygen Meter and YSI 5010 BOD Probe with automatic stirrer from Yellow Springs, Instrument Co, Yellow Springs, OH. Each test day, the DO meter was allowed to equalize for a period of one to two hours. It was then calibrated using the auto calibration feature. After calibration, a dissolved oxygen reading was taken from deionized water blank. The blank was sampled again in the middle of the initial batch readings and upon completion of the initial readings to ensure that the probe was consistent. After the initial

dissolved oxygen concentrations were recorded, stoppers were inserted, a water seal was created with deionized water, and plastic caps were put over the stoppers to prevent introduction of ambient oxygen and loss of liquid due to evaporation. The bottles were then placed in a dark incubator at 20 degrees C and left for five days.

After the five-day incubation period, the final dissolved oxygen concentrations were determined using the same technique as the initial values. BOD_5 values were calculated as per the Standard Methods (Greenberg 2005).

Respirometry

The Micro-Oxymax respirometer from Columbus Instruments was used for this portion of the experiment. It was used as a closed circuit system to measure the oxygen and carbon dioxide levels of individual chambers. The oxygen sensor had a range of 10-21 percent, while to carbon dioxide sensor had a range of 0-1 percent. With the installed expansion interface, it could monitor up to 20 chambers. The experiment sampled 19 chambers during each cycle. 18 chambers consisted of 250mL sample jars. Two of the jars were seed controls, filled with 130ml of deionized water and BOD buffer solution, and 20mL of microbial seed. The other jars were filled with 130mL buffer solution, 20mL microbial seed and oxygenate. The remaining chamber was a length of silicone tubing exposed to laboratory air. To minimize cross contamination in the sampling process, each inlet line to the respirometer had a Millipore, Millex-FG 0.20 µm hydrophobic PTFE 50 mm filter. In addition, the air was circulated through one of two drying columns to prevent moisture from reaching the sensors.

Before data collection, calibration of oxygen and carbon dioxide sensors was conducted using calibration gas from Weiler Welding Company. This calibration gas contained 20.4% oxygen, 0.704% carbon dioxide and the remainder nitrogen. Chamber volume was measured, and chambers were tested for excessive leakage or restriction prior to data collection. Typical values are listed in Appendix A.

Instrument settings were a mix of default and user-defined settings. A complete cycle consists of samplings each of the 19 chambers. A more frequent sampling cycle allows for more data points, while a less frequent cycle allows for better sensor response and higher quality data. Initially, the sample frequency, or interval was set at ten hours. After the first trial, it was reduced to five hours for the remainder of the experiments. A complete list of respirometer settings is found in Appendix A.

Gas Chromatography

An HP 5890 Series II Gas Chromatograph with Flame Ionization Detector (GC-FID) was used to analyze the aqueous samples. The capillary column used was a J&W Scientific DB-624 (#123-1334, length: 30m, ID: 0.32mm, Film: 1.8um) with a DuraGuard deactivated fused silica column guard (#160-2325-5, length: 5m, ID:0.32mm). The MSD Chemstation Build 75 (August 26, 2003) was used to control the GC-FID. Chemstation software translated the GC-FID response into an area that was converted to concentration using calibration curves.

GC analytical operating parameters were based on methods developed by Mares (2004) and Torres (2005). Based on trial and error, methods were adjusted to achieve better separation and to overcome equipment difficulties. Due to auto sampler and inlet

inconsistencies, the first methods developed were manual injection methods for each separate chemical. Detailed methods are listed in Appendix A. After equipment maintenance and repair, an auto sampler method suitable for all the chemicals was developed.

After each method was developed, a calibration curve was established for it. Standard solutions were prepared using serial dilutions. Each standard was prepared using deionized water, glass pipettes, and volumetric flasks. Samples were transferred using a Finnpipette with disposable tips into two ml amber vials sealed with PTFE/rubber lined crimped caps. Samples were analyzed using the GC-FID and the response plotted against the known concentration of the standard. Using Microsoft Excel, a best fit line and equation was developed. Calibration curves and equations can be found in Appendix B.

The method detection limit (MDL) was determined as per the Code of Federal Regulations (CFR) (40 CFR 136, 1993). This identifies the lowest quantifiable analytical results for the specific GC-FID method and was determined using the equation below.

$MDL = SD \times t_{0.99}$

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

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

MDL calculations for each method can be found in Appendix B.

At predetermined time intervals, samples from the respirometer chambers were measured using the GC-FID. After the respirometer test cycle was complete, each 250 mL sample jar was opened and 1mL samples were removed using a Finnpipette and disposable tip. Each of the two samples was placed into a separate two ml amber vial sealed with a PTFE/rubber lined crimped cap. Samples were also obtained from the eight 40mL control bottles that contained chemical, BOD buffer, deionozed water, and killed seed. The amber vials were then loaded into the auto sampler and analyzed using the GC-FID.

IV. Data Analysis

Introduction

In this Chapter the results of the BOD_5, respirometer and GC experiments will be covered. The BOD_5 experiments will be discussed separately as they were done first and independent of the other techniques.

The four remaining combination experiments consist of both respirometer and GC data collected concurrently. The first two experiments tested pure oxygenates while experiments three and four tested mixtures of toluene and oxygenates. GC and respirometer data is discussed for each of the four experiments. Finally the results of the abiotic control experiments are covered.

BOD_5 Results

The first four BOD_5 experimental runs produced no valid BOD_5 values for the experimental materials. The experiments started with ethanol dilutions as high as 40 ml per 300 ml bottle and worked down as low as 0.01 ml per bottle. In each case, less than 1 mg/L of oxygen (the minimum residual required for a valid test) remained in the bottle. In contrast, the same range of dilutions for toluene resulted in very little oxygen uptake (2.0 mg/l is the minimum uptake required for a valid test).

The fifth BOD_5 experiment used further reduced dilutions for ethanol and produced valid BOD_5 values. These values were consistent with published ethanol oxygen demand values (Vaishnav 1987) and were expressed in terms of percent of Theoretical Oxygen Demand (THOD). Detailed BOD_5 data are presented in Appendix C, while a summary of percent THOD is listed in Table 4.1.

The sixth and seventh BOD_5 experiments, using the refinery industrial water treatment activated sludge, produced similar ethanol values over a range of small dilutions. Using identical dilutions, the toluene samples produced BOD_5 values consistent with published literature (Ford 1971 , Lund 1971). ETBE and TAME were tested at similar dilutions and produced no appreciable oxygen uptake and no valid results in either experiment.

The final BOD_5 experiment produced ethanol values that were slightly lower than those previously recorded while toluene levels were slightly higher. ETBE and TAME still produced no valid results.

Table 4.1 Average BOD_5 data by chemical and experiment number

Chemical	BOD_5 Experiment Number	Percent THOD
Ethanol	5	66.39
	6	73.81
	7	77.06
	8	39.39
Toluene	6	12.68
	7	18.47
	8	22.58

Seven of the eight BOD_5 experiments experienced excessive dilution water dissolved oxygen (DO) uptake (greater than 0.2 mg/L). The first experiment demonstrated a dilution water DO uptake of 1.04 mg/L. For remaining tests, the dilution water was aerated and incubated for a period of 24 hours prior to the start of the experiment. DO uptake was reduced significantly to a range of 0.3 to 0.7 mg/L. Only

experiment seven produced a dilution water blank DO uptake of an acceptable level by Standard Methods criteria (Greenberg 2005).

Due to improper preparation of the glucose/glutamic acid check solution, BOD_5 experiments one through four did not have valid glucose/glutamic acid check BOD_5 values. Three of the remaining tests (numbers four, six and eight) had valid glucose/glutamic acid checks, while two (numbers five and seven) had BOD_5 values that were slightly below the minimum acceptable. Data is presented in Appendix C.

Respirometry and Gas Chromatography Results

The first respirometry and gas chromatography experiment used no chemical mixtures, only pure oxygenates. Once the sample chambers were filled and connected to the respirometer, the data collection began. Test compound concentration was measured directly with the gas chromatograph at the start, 12 hours, and one day for ethanol and toluene samples. Anticipating slower degradation, the concentration was measured at start, 2, 4, 10 and finally 15 days for ETBE and TAME. The GC integration function was used to find the area under the curve. Sample chromatographs are located in Appendix C. This area was converted to a concentration using the calibration curves established for each analyte. Detailed results of the GC analysis can be found in Appendix C. Assuming first order decay, the decay constant λ was calculated using the following equation:

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

Where C= concentration at time t
C_0= concentration at time zero
t = elapsed time

Respirometry data, specifically the rates of O_2 consumption and CO_2 production, were collected until the oxygenate concentration in the test chamber dropped below the MDL. Rates of consumption and production, listed in μL per min were then converted to total O_2 and CO_2 produced in μls. The totals were then expressed as a percentage of the theoretical CO_2 production and O_2 consumption of the given amount of oxygenate. Detailed respirometry data are included in Appendix C.

The second respirometry and gas chromatography experiment, similar to the first, also used only pure oxygenates. A sumary of the first and second experiments is shown in Table 4.2.

Table 4.2 Summary of First and Second Respirometry and GC Experiment Data

Experiment	Chemical	Duration (days)	Average λ (day-1)	CO_2 production (% theoretical)	O_2 consumption (% theoretical)
1	ethanol	1	3.445	28.5	51.3
1	toluene	1	2.18	10.2	22.9
1	ETBE	15	0.1312	12.1	19.2
1	TAME	15	0.1065	14.7	11.9
2	ethanol	1.5	4.24	46.3	60.1
2	toluene	1.5	2.73	18.6	24.8
2	ETBE	15	0.208	8.6	9.7
2	TAME	15	0.337	7.1	3.2

Additionally, the first and second experiments collected respirometry data for three days beyond the point where the GC indicated oxygenate concentrations below MDL. Results, listed as percentage of theoretical CO_2 production and O_2 consumption, are summarized in Table 4.3.

Table 4.3 Respirometry Data for Extended Experiment

Experiment	Chemical	O_2 consumption (% of theoretical) at conc < MDL	O_2 consumption (% of theoretical) at 88hrs	CO_2 production (% of theoretical) at conc < MDL	CO_2 production (% of theoretical) at 88hrs
1	Ethanol	51.325	92.88	28.5	70.75
1	Toluene	22.9	64.4	10.25	53.5
2	Ethanol	60.1	73.5	46.3	49.4
2	Toluene	24.8	45.4	18.6	27

Experiments three and four tested degradation rates of both pure oxygenates and oxygenate/toluene combinations. A summary of the results is listed in Table 4.4.

Table 4.4 Summary of Third and Fourth Experiment Data

Experiment	Chemical	Duration (days)	Average λ (day-1)	CO_2 production (% theoretical)	O_2 consumption (% theoretical)
3	ETBE	18	0.165	16	22.275
3	ETBE/toluene	18	0.155	65.45	78.72
3	TAME	18	0.231	23.13	25.23
3	TAME/toluene	18	0.141	53	15.73
4	ETBE	18	0.246	10.775	17.37
4	ETBE/toluene	18	0.225	54.625	68
4	TAME	18	0.247	17	55.17
4	TAME/toluene	18	0.212	45.125	48.92

Experimental controls, consisting of oxygenate and mixed samples treated with microbiocide were also maintained for the duration of the experiments. Concentration was measured at the start and finish of each experiment to determine if any of the

oxygenate was being degraded by non-microbial means. Most of the abiotic controls retained 90% or more of the original concentration, the lone exception being toluene which displayed only a 76.7% retention rate. A complete summary of the experimental controls is listed in Appendix C.

Respirometry data were also analyzed to ensure that aerobic conditions were maintained. Sample chambers showed an O_2 percent ranging from approximately 20.5 to 21.5 during the experiment. Sample O2 percentages over the experiments are listed in Appendix C.

V. Conclusions and Recommendations

Summary

The focus of this research was to determine if the oxygenates tert amyl methyl ether (TAME) and ethyl tert butyl ether (ETBE) would degrade under aerobic conditions. In addition to determining the degradation rate of isolated oxygenates, experiments were conducted to determine the degradation rate of oxygenates in the presence of a co-contaminant, toluene. Additionally, the research compared gas chromatography with respirometry and BOD_5 tests to determine applicability of the latter techniques in measurement of degradation.

Answers to Specific Questions

1. Are the oxygenates biodegradable and if so at what rate?

Gas Chromatography data from each of the four experiments seem to indicate that the oxygenates are degradable under the experimental conditions and within the given concentration range. Each experiment resulted in the reduction of initial concentrations 96 to 20 ppm), to levels below the MDL established for the GC methods. The reduction in oxygenate concentration in the experiments was significantly greater than the reduction in the abiotic controls.

Some portion of the concentration reduction could potentially be attributed to volatilization in the respirometer chambers. Assuming that the oxygenate and water solution had time to reach equilibrium with the air in the headspace of the respirometer

bottle, toluene could have lost up to 5 ppm to volatilization. Due to smaller Henry's constants, ETBE and TAME would only have lost 1-2 ppm and ethanol much less than 1 ppm. This loss could have been repeated each time the respirometer conducted a refresh, or replacement of sample chamber air with outside air. Respirometer data indicates, however, that the respirometer did not reach the required oxygen draw down to conduct the refresh function. Even with the probable loss of some chemical to volatilization, the amount of reduction still greatly exceeded that of the abiotic controls.

The rate of biodegradation varied greatly by experiment. It appears that as the microbial consortium became acclimated to the established feeding routine, it became more efficient at degrading the oxygenates. While a particular rate could not be established, a consistent relationship between the degradation rate of toluene and that of the other oxygenates was found across all experiments. In all four experiments, ETBE was found to have a first order degradation rate constant 7.86 % of that of toluene (95% C.I. +/- 3.91). TAME was found to have a first order degradation rate constant of 8.57% of that of toluene (95% C.I. +/- 4.97). While both ETBE and TAME appeared to degrade more than an order of magnitude slower than toluene, ethanol was significantly faster with a rate constant of 158.26 % of toluene (95% C.I. +/- 18.74).

2. Can biodegradation be measured directly with the GC?

It appears that biodegradation, in the form of reduced oxygenate concentration, can be measured directly using gas chromatography. The GC methods developed produced consistent results for all chemicals involved. ETBE and TAME produced the most statistically precise results in all experiments, with relatively low standard

deviations. The method was least precise for the detection of ethanol. Statistical data for the GC experiments can be found in Appendix D.

It should be noted that the GC only measures the reduction in concentration of the original chemical. Oxygenate degradation can be a multi step process and a reduction in the original chemical does not address the time required for complete mineralization. Additionally, the GC analysis does not separate degradation loss from loss due to volatilization or other factors.

3. Can microbial activity measured with BOD_5 or respirometry be correlated to reduced concentrations due to degradation?

The BOD_5 test appears to be a good indicator of the biodegradability of rapidly-degrading substances such as ethanol. In BOD_5 tests 1 through 4, ethanol consumed all available dissolved oxygen before the end of the five day incubation period. After settling on a range of appropriate dilutions, the ethanol samples in BOD_5 test 5 through 8 produced fairly consistent results of 59.88 percent of THOD (95% C.I. +/- 11.8). These are consistent with published values of 60% THOD (Vaishav 1987).

The toluene samples provided useful data as well. The BOD_5 tests 1 through 4 used high toluene dilutions that proved to be inhibitory to the microbes. This is consistent with studies that have shown inhibitory effects at concentrations above 29 mg/L (Bringmann 1980). In BOD_5 tests 5 through 8, the use of acclimatized seed combined with appropriate dilution levels led to toluene BOD_5 levels of 17.42 percent of THOD (95% C.I. +/- 3.7). These results fall within the range of other published values of 5% THOD (Ford 1971) and 27% THOD (Lund 1971).

The BOD_5 data was consistent with GC results indicating that ethanol degraded much faster than toluene. To provide a good correlation, the BOD_5 data should have resulted in ETBE and TAME BOD_5 values approximately one tenth the BOD_5 value of toluene. In fact, neither ETBE nor TAME samples provided good BOD_5 values. It appears that the BOD_5 test is not suitable for slower degrading chemicals. A possible explanation is that to meet the Standard Method requirement of 2 mg/L DO uptake in five days would require a starting dilution too high for the microbes.

In all cases, respirometry data did not appear to correlate well to GC data. As previously discussed, a GC concentration measurement of nearly zero did not correspond to complete theoretical O_2 consumption or complete theoretical CO_2 production. At the ending point for the GC data collection, ethanol had the highest O_2 consumption at 51-60 percent of theoretical. ETBE, TAME and toluene were significantly less. CO_2 production was highest for ethanol at 45 percent of theoretical. Again ETBE, TAME and toluene were significantly less. Even at three days after measuring GC concentrations below MDL for ethanol and toluene, O_2 and CO_2 percentages were still below theoretical levels. This indicates that there may be significant microbial activity involved in breaking the chemicals down beyond the first step in the degradation pathway. Also the fact that CO_2 production was consistently below O_2 consumption may indicate that some amount of CO_2 was incorporated into the microbial biomass. While this data may be a more accurate representation of the degradation process, it does not correlate well to the concentration reductions measured by the GC.

The degradation rate ratios found in the GC data do not appear to hold true with respirometry data. While ethanol predictably consumes O_2 and produces CO_2 faster than

toluene, the rates for ETBE, TAME and toluene are all roughly equal. Additionally, overall respirometry data was much less precise than GC data, as indicated in Appendix D.

4. Is BOD/THOD ratio a good indicator of degradation potential?

Because the BOD_5 test used in this thesis did not produce acceptable data for ETBE or TAME, it does not appear to be useful in determining BOD/THOD ratios for those and other similar oxygenates. It is possible that 10 or 30 day BOD tests may be more appropriate.

5. Does the presence of the co contaminant toluene effect biodegradation characteristics?

It appears that the presence of toluene as a co contaminant has an effect on the degradation rate of both ETBE and TAME. In each experiment with the combined chemicals, despite the fact that toluene was added at roughly one third the concentration of the primary oxygenate, the rate constant for the combination was lower than the oxygenate alone. ETBE appears to have been least affected, with the combination degrading at 92.75 % of the pure oxygenate rate. The TAME combination degraded at 73.28 % of the pure oxygenate rate.

While the data suggests that there is an effect, it should be noted that only one of the four combination versus pure chemical comparisons were statistically different with a 90% confidence interval, and none with a 95 % C.I. Statistical data can be found in Appendix D.

Conclusion

This thesis showed that useful data about the degradation of ETBE and TAME can be obtained using the GC. It also demonstrated that under controlled aerobic conditions, the oxygenates will degrade consistently. Finally it suggested that the presence of other chemicals can have a negative effect on the degradation of the oxygenates.

Limitations

The selection of the BOD_5 test proved to be problematic. A longer duration measure of BOD would have been more appropriate. Respirometry data should have been collected for a longer period of time. Rather than stopping when GC concentrations dropped, respirometer data should have been collected until O_2 and CO_2 levels neared theoretical maximums.

Opportunities for further research

1. Repeat the same study with different oxygenates. Using the same procedures with MTBE and additional potential replacements would create a broader basis for comparison.
2. Expand the study of co-contaminants. Experiment with different starting concentrations of toluene in the combinations. Also experiment with different co-contaminants.
3. Investigate the degradation pathway. Broaden GC methods to include detection of

daughter chemicals and breakdown products. Compare respirometer data to more complete GC analysis.

4. Start with the same microbial seed and experiment with different acclimation routines to determine effects on degradation.

Appendix A

Table A.1 Typical Micro-Oxymax Respirometer Values

Channel Label	Channel	Volume (ml)	Restriction (mmHg)	Leakage (ml/min)
seed control	1	175	34.05	0.05
silicon tube	2	15	28.56	-0.04
seed control	3	171	32.42	-0.16
oxygenate 1	4	169	26.3	-0.43
oxygenate 1	5	169	16.49	-0.02
oxygenate 1	6	170	31.14	-0.02
oxygenate 1	7	184	28.79	-0.06
oxygenate 2	8	169	34.19	-0.08
oxygenate 2	9	169	24.66	-0.08
oxygenate 2	10	172	20.01	-0.06
oxygenate 2	11	168	35.05	-0.1
oxygenate 3	12	168	34.69	-0.18
oxygenate 3	13	165	34.66	-0.11
oxygenate 3	14	176	36.76	-0.05
oxygenate 3	15	164	20.06	-0.04
oxygenate 4	16	178	30.02	-0.33
oxygenate 4	17	167	25.38	-0.07
oxygenate 4	18	166	32.76	-0.12
oxygenate 4	19	175	34.8	-0.09
unused	20	NA	NA	NA

Table A.2 Micro-Oxymax Respirometer Equipment Settings

Parameter	Value
Start Channel	1
Stop Channel	19
Sample Interval	10
Sample Duration	0
Refresh Interval	0
Refresh Threshold	0.5
Refresh Window	23
Auto Volume Measurement	N
Purge Sensor Enabled	Y
Switch Drier Enabled	Y
Gas Data Units	µL
Time Units	MIN
Normalization Units	N.A.
Aux Temp start at Ch	0
Enable Open Flow	N

Table A.3 GC-FID Manual injection method for ethanol

6890 Gas Chromatograph

Serial Number	US 10339021

Oven

Initial temperature	35 °C
Maximum temperature	260°C
Initial time	3.30 min
Equilibration time	1.00 min
Post temperature	40 °C
Post time	1.00 min
Run time	3.30 min

Ramp	Rate (°C/min)	Final Temperature	Final Time
none			

Rear Inlet (Split/Splitless)

Mode	split
Initial temperature	175 °C
Pressure	17.00 psi
Split ratio	41.2:1
Split flow	232.3 ml/min
Total flow	240.2 ml/min
Gas saver	off
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	5m

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

Table A.3 GC-FID Manual injection method for ethanol (Continued)

Nominal film thickness	1.80 µm
Mode	constant pressure
Pressure	17.0 psi
Nominal initial flow	5.6 ml/min
Average velocity	86 cm/sec
Inlet	back
Outlet	front detector
Outlet pressure	vacuum
Flame Ionization Detector	
Temperature	250 °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
Injection Parameters	
Injector location	back
Sample washes	2
Sample pumps	2
Injection volume	2 µl
Post injection solvent washes	2

Table A.4 GC-FID Manual injection method for ETBE

6890 Gas Chromatograph

Serial Number	US 10339021

Oven

Initial temperature	45 °C
Maximum temperature	260 °C
Initial time	2.30 min
Equilibration time	1.00 min
Post temperature	50 °C
Post time	0.30 min
Run time	2.30 min

Ramp	Rate (°C/min)	Final Temperature	Final Time
none			

Rear Inlet (Split/Splitless)

Mode	split
Initial temperature	175 °C
Pressure	40.66 psi
Split ratio	25:1
Split flow	407.2 ml/min
Total flow	425.8 ml/min
Gas saver	off
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 A.4 GC-FID Manual injection method for ETBE (Continued)

Nominal film thickness	1.80 µm
Mode	constant pressure
Pressure	40.66 psi
Nominal initial flow	16.3 ml/min
Average velocity	147 cm/sec
Inlet	back
Outlet	front detector
Outlet pressure	vacuum
Flame Ionization Detector	
Temperature	250 °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
Injection Parameters	
Injector location	back
Sample washes	2
Sample pumps	2
Injection volume	3 µl
Post injection solvent washes	2

Table A.5 GC-FID Manual injection method for toluene and TAME

6890 Gas Chromatograph

Serial Number	US 10339021

Oven

Initial temperature	130 °C
Maximum temperature	260 °C
Initial time	3.0 min
Equilibration time	1.00 min
Post temperature	130 °C
Post time	0.50 min
Run time	3.0 min

Ramp Rate (°C/min)	Final Temperature	Final Time
none		

Rear Inlet (Split/Splitless)

Mode	splitless
Initial temperature	175 °C
Pressure	13.23 psi
Purge flow	52.5 ml/min
Purge time	0.00 min
Total flow	57.1 ml/min
Gas saver	off
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 A.5 GC-FID Manual injection method for toluene and TAME (Continued)

Nominal film thickness	1.80 µm
Mode	constant pressure
Pressure	13.23 psi
Nominal initial flow	2.0 ml/min
Average velocity	39 cm/sec
Inlet	back
Outlet	front detector
Outlet pressure	ambient
Flame Ionization Detector	
Temperature	250 °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
Injection Parameters	
Injector location	back
Sample washes	2
Sample pumps	2
Injection volume	3 µl
Post injection solvent washes	2

Table A.6 GC-FID Auto sampler 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 A.6 GC-FID Auto sampler method (Continued)

Nominal film thickness	1.80 µm
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
Flame Ionization Detector	
Temperature	250 °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
Injection Parameters	
Injector location	back
Sample washes	2
Sample pumps	2
Injection volume	1 µl
Syringe size	10 µl
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

Appendix B

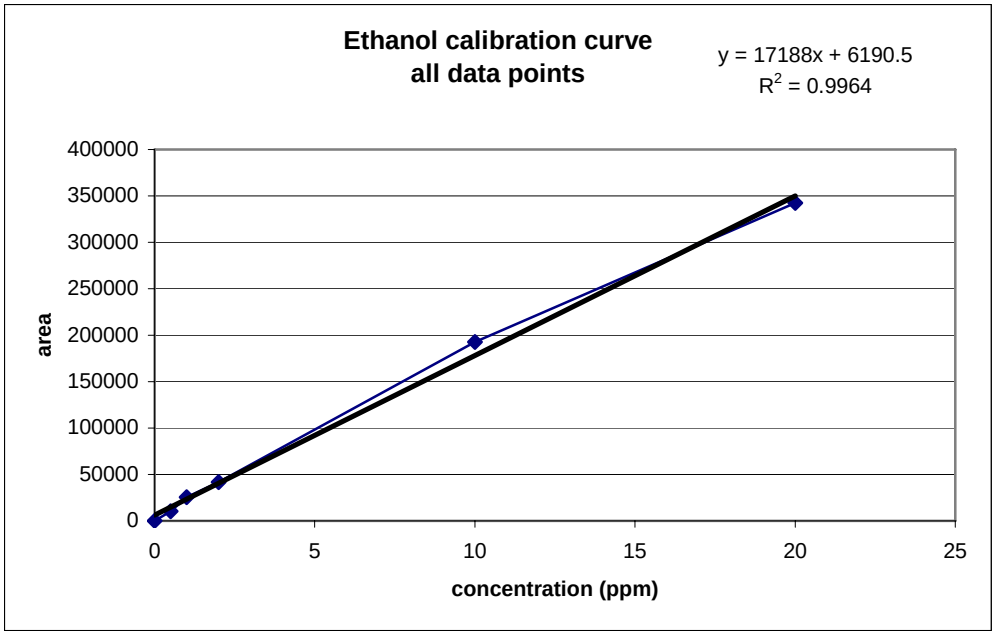


Figure B.1 Manual Injection Ethanol Calibration Curve

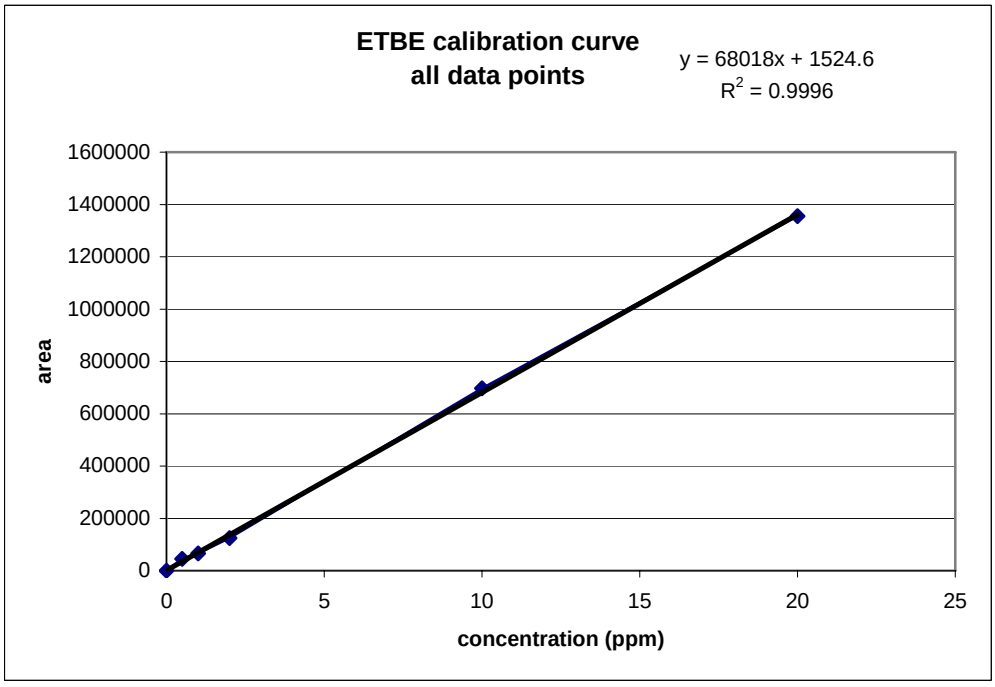


Figure B.2 Manual Injection ETBE Calibration Curve

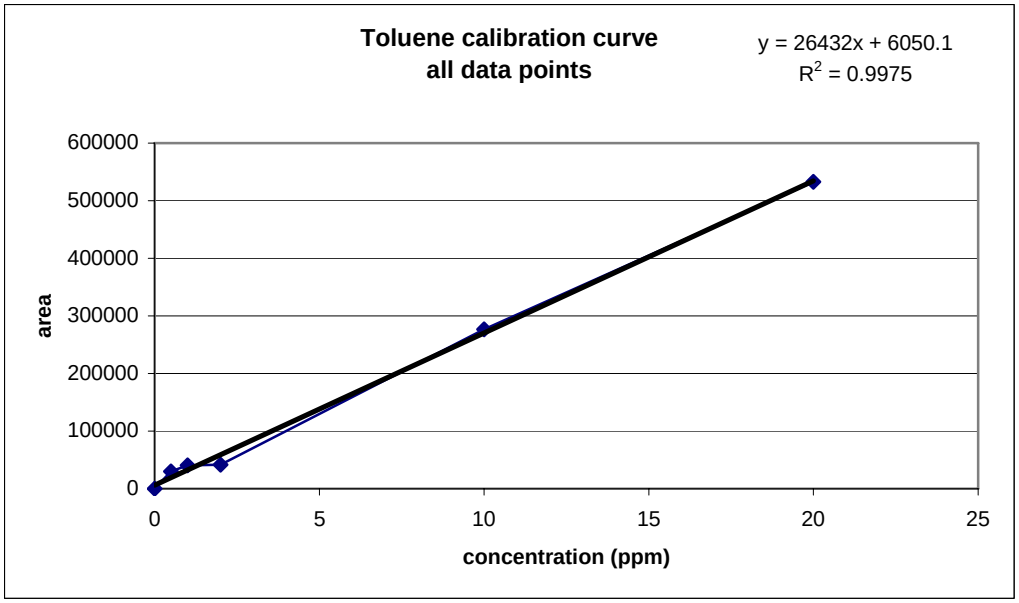


Figure B.3 Manual Injection Toluene Calibration Curve

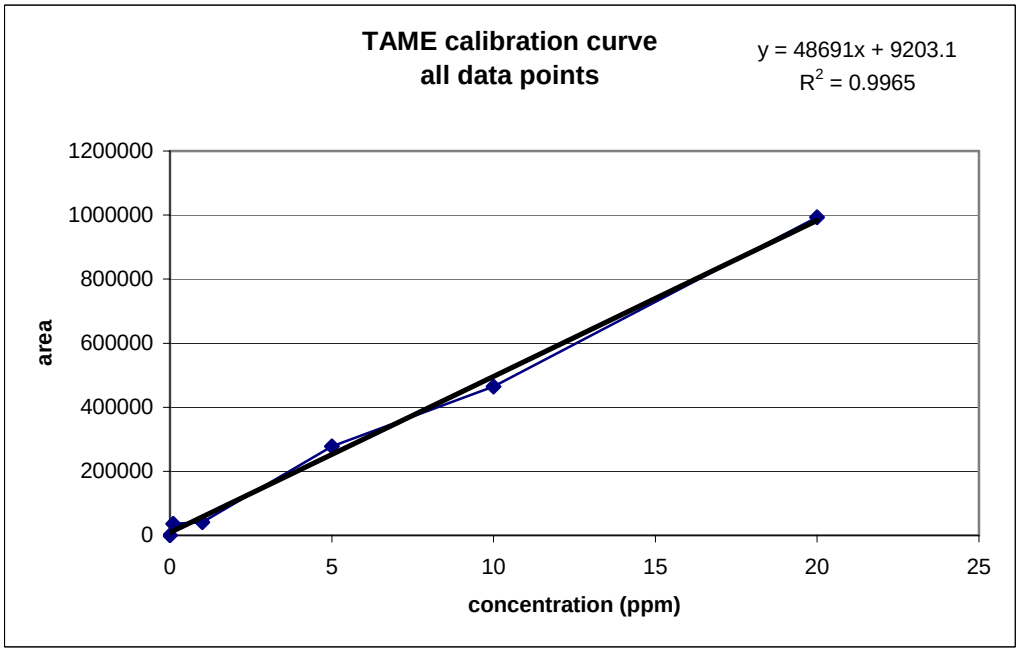


Figure B.4 Manual Injection TAME Calibration Curve

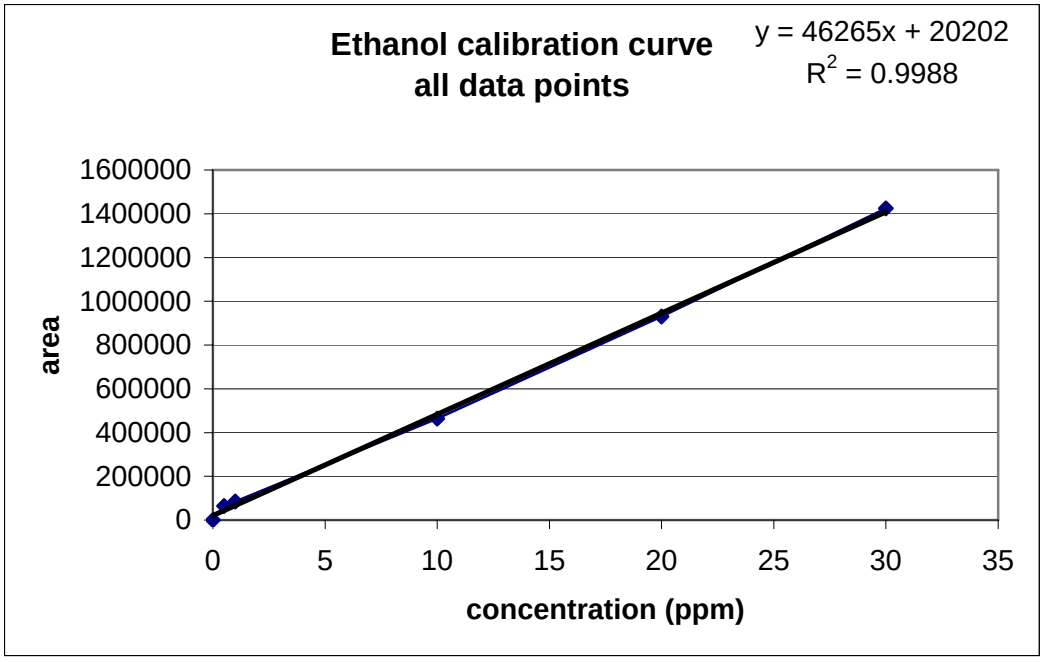


Figure B.5 Auto injector Method Ethanol Calibration Curve

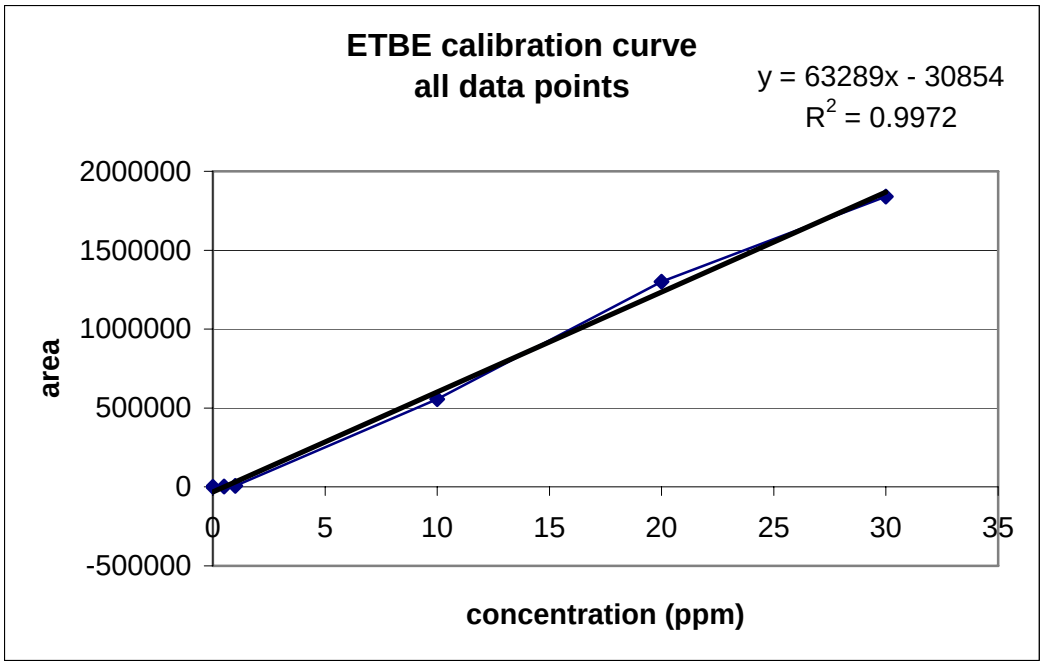


Figure B.6 Auto injector Method ETBE Calibration Curve

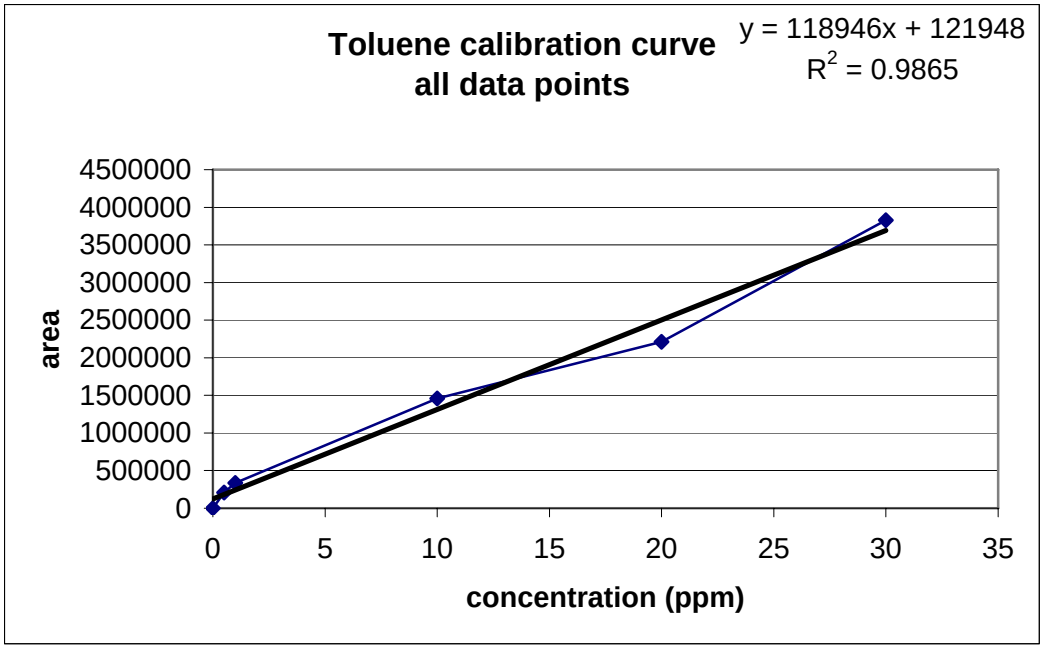


Figure B.7 Auto injector Method Toluene Calibration Curve

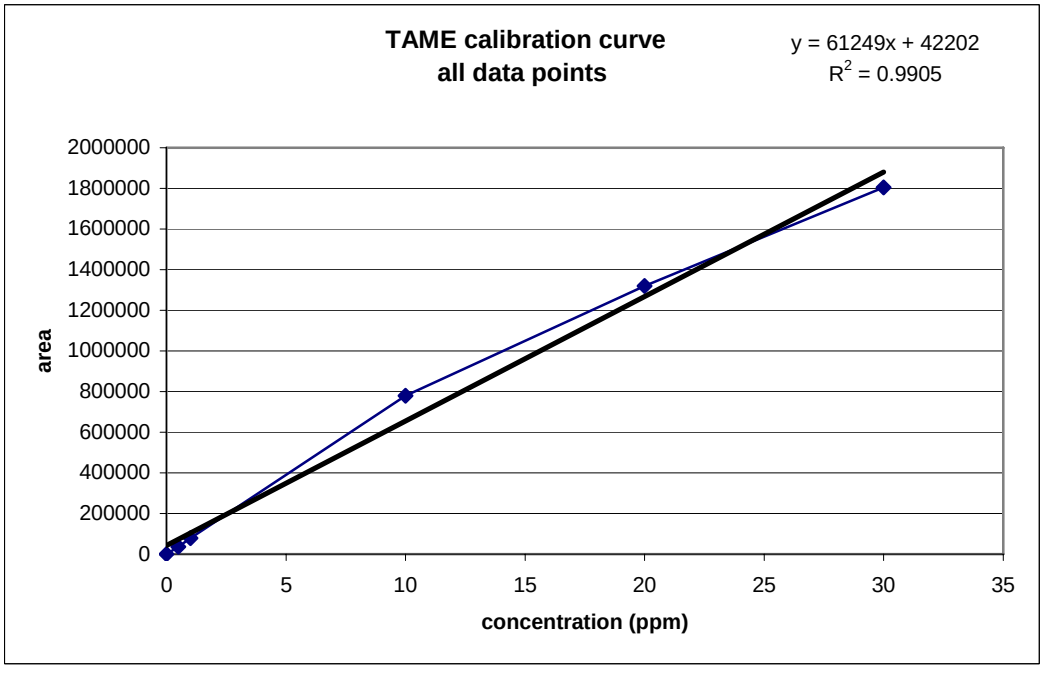


Figure B.8 Auto injector Method TAME Calibration Curve

Table B.1 Manual Injection Method MDL Calculations

Toluene

Sample	Concentration(ppm)	$(xi-X)^2$
1	1.11	0.038
2	1.03	0.012
3	0.75	0.028
4	0.57	0.12
5	0.73	0.036
6	1.32	0.16
SD	0.28	
t_{99}	3.36	
MDL	0.95	

TAME

Sample	Concentration(ppm)	$(xi-X)^2$
1	0.76	0.055
2	0.51	0.0002
3	0.47	0.003
4	0.37	0.022
5	0.36	0.025
6	0.66	0.019
SD	0.12	
t_{99}	3.36	
MDL	0.39	

ETBE

Sample	Concentration(ppm)	$(xi-X)^2$
1	0.45	5.29exp-4
2	0.47	9exp-6
3	0.54	0.0041
4	0.41	0.0038
5	0.34	0.019
6	0.63	0.026
SD	0.10	
t_{99}	3.36	
MDL	0.35	

Table B.1 Manual Injection Method MDL Calculations (Continued)

Ethanol

Sample	Concentration(ppm)	$(xi-X)^2$
1	1.13	0.088
2	2.005	0.33
3	1.31	0.013
4	1.57	0.02
5	1.12	0.094
SD	0.33	
$\mathbf{t_{99}}$	3.07	
MDL	1.24	

Table B.2 Auto injector Method MDL Calculations

Toluene

Sample	Concentration(ppm)	$(xi-X)^2$
1	1.56	0.0015
2	1.59	9.06E-06
3	1.62	0.00037
4	1.59	4.93E-05
5	1.41	0.035
6	1.82	0.047
SD	0.13	
t_{99}	3.36	
MDL	0.44	

TAME

Sample	Concentration(ppm)	$(xi-X)^2$
1	0.74	0.54
2	0.92	0.84
3	0.95	0.89
4	0.81	0.66
5	0.77	0.59
6	0.69	0.48
SD	0.10	
t_{99}	3.36	
MDL	0.34	

ETBE

Sample	Concentration(ppm)	$(xi-X)^2$
1	1.70	0.0022
2	1.68	0.00065
3	1.61	0.0018
4	1.61	0.0022
5	1.61	0.0017
6	1.71	0.0033
SD	0.049	
t_{99}	3.37	
MDL	0.16	

Table B.2 Auto injector Method MDL Calculations (Continued)

Ethanol

Sample	**Concentration(ppm)**	**$(xi-X)^2$**
1	1.84	0.093
2	1.51	0.00066
3	1.57	0.0018
4	1.16	0.14
5	1.71	0.033
6	1.40	0.017
SD	0.24	
t_{99}	3.36	
MDL	0.80	

Appendix C

Table C.1 BOD_5 Data by experiment

Sample	Test Number	Dilution (mL per 300 mL)	Initial DO (mg/L)	Final DO (mg/L)	BOD_5	g O_2 per g chemical	Percent of THOD
Ethanol	5	0.001	9.13	5.58	1032960	1.31	65.47
	5	0.002	9.13	1.94	1062480	1.35	67.32
	6	0.0005	9.09	6.47	1532760	1.94	97.13
	6	0.001	9.1	5.39	1093380	1.39	69.29
	6	0.0015	9.1	3.65	1076920	1.36	68.25
	6	0.002	9.09	1.66	1104690	1.40	70.01
	6	0.0025	9.09	0.56	1015752	1.29	64.37
	7	0.0015	9.62	3.46	1216100	1.54	77.07
	8	0.001	9.8	7.12	788700	0.99	49.98
	8	0.0015	9.77	6.95	553800	0.70	35.09
	8	0.0015	9.8	7.58	433800	0.55	27.49
	8	0.001	9.79	7.03	812700	1.03	51.50
	8	0.002	9.73	6.22	518850	0.66	32.88
Toluene	6	0.0005	9.08	8.33	410760	0.47	15.14
	6	0.001	9.1	8.15	265380	0.31	9.78
	6	0.0015	9.1	6.31	544920	0.63	20.08
	6	0.002	9.09	7.31	257190	0.30	9.48
	6	0.0025	9.1	7.02	241752	0.28	8.91
	7	0.0015	9.55	6.81	532100	0.61	19.61
	7	0.003	9.55	4.81	466050	0.54	17.18
	7	0.0045	9.55	1.88	506033.3	0.58	18.65
	8	0.001	9.8	7.52	668700	0.77	24.64
	8	0.0015	9.79	7.02	543800	0.63	20.04
	8	0.002	9.8	5.94	571350	0.66	21.06
	8	0.003	9.79	3.07	666900	0.77	24.58

Notes:
BODs 1-4 resulted in no valid data
BOD 1-5 used Fairborn city sludge
BOD 6 and 7 used fresh Lima refinery sludge
BOD 8 used acclimated Lima refinery sludge (fed toluene and small amounts of ETBE and TAME)
No valid data was obtained for ETBE or TAME

Table C.2. Gas Chromatography data by experiment

First Experiment

	day0 ppm	**day.5 ppm**	**day1 ppm**			**λ (day-1)**	
ethanol	21.61	16.98	1.03			3.04	ethanol
ethanol	18.52	13.75	0.39			3.85	ave 3.45
toluene	1.77	1.73	0.19			2.24	
toluene	2.64	2.32	0.19			2.64	toluene
toluene	4.02	1.31	0.75			1.68	ave 2.18

	day0 ppm	**day2 ppm**	**day4 ppm**	**day10 ppm**	**day15 ppm**	**λ (day-1)**	
ETBE	6.68	4.16	2.25	0.97	0.81	0.13	
ETBE	5.69	3.945	2.23	0.96	0.72	0.14	
ETBE	4.68	3.58	2.28	1.03	0.73	0.14	ETBE
ETBE	5.63			0.78	0.91	0.12	ave .13
TAME	4.61	4.22	2.10	1.18	0.97	0.10	
TAME	5.63	3.73	2.25	1.28	1.03	0.11	
TAME	4.00	3.75	2.29	1.48	0.96	0.095	TAME
TAME	5.87			0.99	1.06	0.11	ave .11

Second Experiment

	day0 ppm	**day.5 ppm**	**day1 ppm**	**day1.5 ppm**		**λ (day-1)**	
ethanol	23.05	15.35	0.42			4.37	
ethanol	23.04	18.29	4.89	0.17		3.30	ethanol
ethanol	23.54	16.49	0.15			5.06	ave 4.24
toluene	30.84	19.59	2.56	0.41		2.88	
toluene	44.47	21.88	15.02	0.61		2.85	toluene
toluene	4.92	2.77	0.52			2.45	ave 2.73

	day0 ppm	**day6 ppm**	**day10 ppm**	**day15 ppm**		**λ (day-1)**	
ETBE	19.01	3.50	1.05	0.76		0.22	
ETBE	18.23	3.49	1.15	0.59		0.23	
ETBE	15.56	3.98	1.53	1.00		0.18	ETBE
ETBE	18.73	3.52	1.32	1.23		0.18	ave .20
TAME	17.66	3.82	0.45 4	0.13		0.33	
TAME	18.46	4.34	1.10	0.09		0.36	
TAME	19.79	4.63	1.32	0.18		0.32	TAME
TAME	18.92	3.71	1.19	0.10		0.35	ave .34

Table C.2. Gas Chromatography data by experiment (cont.)

Third Experiment

	day0 ppm	(toluene)	day1 ppm	(toluene)	day3 ppm	day6 ppm	day11 ppm	day18 ppm	λ (day-1)	
ETBE	19.44		13.74		9.31	4.64	2.00	0.87	0.17	
ETBE	19.67		13.53		8.74	4.36	1.8	0.97	0.17	ETBE only
ETBE	18.85		13.7		8.98	4.51	2.18	1.13	0.16	ave .17
TAME	20.87		14.92		9.86	4.65	1.58	0.15	0.27	
TAME	17.73		14.11		9.85	5.44	2.12	0.33	0.22	TAME only
TAME	18.05		13.94		9.57	5.42	1.88	0.49	0.20	ave .23
ETBE/tol	17.9	9.73	12.81	3.25	8.46	4.51	2.04	1.16	0.15	
ETBE/tol	19.07	4.08	13.31	0.04	8.75	4.69	1.97	0.85	0.17	ETBE/toluene
ETBE/tol	16.76	4.28	12.85	0.06	9.03	5.17	1.84	1.30	0.14	ave .16
TAME/tol	18.6	5.00	14.34	1.23	9.14	5.94	3.09	0.91	0.17	
TAME/tol	18.64	3.36	14.12	0.13	10.41	5.86	2.79	0.47	0.20	TAME/toluene
TAME/tol	16.8	2.98	14.07	1.04	10.26	6.04	2.83	1.33	0.14	ave .14

Fourth Experiment

	day0 ppm	(toluene)	day1.5 ppm	(toluene)	day3.5 ppm	day6 ppm	day11 ppm	day18 ppm	λ (day-1)	
ETBE	17.66		9.695		5.79	2.92	1.12		0.25	
ETBE	18.04		10.09		5.36	2.52	1.11		0.25	ETBE only
ETBE	18.06		10.43		6.05	3.06	1.39		0.23	0.25
TAME	19.4		11.03		6.86	2.75	0.55	0.30	0.23	
TAME	18.38		11.43		5.12	3.42	1.103	0.22	0.25	TAME only
TAME	18.87		10.77		6.82	3.19	1.04	0.16	0.27	0.25
ETBE/tol	15.66	6.3	9.48	0.52	6.10	2.87	1.32		0.23	
ETBE/tol	16.38	6.4	9.85	0.14	5.32	3.15	1.49		0.22	ETBE/toluene
ETBE/tol	16.36	6.8	9.72	0.18	5.94	3.18	1.28		0.23	0.23
TAME/tol	17.87	6.23	11.89	0.40	7.68	4.66	2.01	0.67	0.18	
TAME/tol	17.21	6.45	10.4	0.141	6.14	3.40	1.08	0.11	0.28	TAME/toluene
TAME/tol	20.97	6.28	13.24	0.22	9.07	4.62	1.83	0.94	0.17	0.212

Table C.3 Respirometry data by experiment

First Experiment

Sample	Total O_2 consumed (µL)	starting concentration (ppm)	Percent of Theoretical
ethanol	3630.72	21.6	88.42
ethanol	3743.04	18.5	106.43
ethanol	3746.16	22	89.57
ethanol	3360.96	20.3	87.09
toluene	886.56	2.6	108.82
toluene	500.16	1.8	88.68
toluene	297.12	2.6	36.47
toluene	296.4	4	23.65
ETBE	428.5	6.7	24.83
ETBE	216.82	5.7	14.77
ETBE	222.34	4.8	17.99
TAME	425.14	4.6	37.11
TAME	70.42	5.6	5.05
TAME	33.7	4	3.17
TAME	36.82	5.9	2.61

Sample	Total CO_2 produced (µL)	starting concentration (ppm)	Percent of Theoretical
ethanol	1874.04	21.6	68.46
ethanol	1931.88	18.5	82.40
ethanol	1853.64	22	66.48
ethanol	1688.76	20.3	65.64
toluene	558.6	2.6	88.15
toluene	331.8	1.8	75.63
toluene	204.36	2.6	32.25
toluene	175.8	4	18.03
ETBE	207.36	6.7	18.03
ETBE	67.2	5.7	6.87
ETBE	94.08	4.8	11.42
TAME	215.52	4.6	28.22
TAME	11.04	5.6	1.19

Table C.3 Respirometry data by experiment (cont.)

Second Experiment

Sample	Total O_2 consumed (µL)	starting concentration (ppm)	Percent of Theoretical
ethanol	3123.52	23	71.44
ethanol	3184	23	72.82
ethanol	3016.96	23.5	67.53
ethanol	3641.2	23.3	82.20
toluene	5724.16	30.8	59.31
toluene	5117.68	44.4	36.78
toluene	816.16	4.9	53.16
toluene	3251.44	32.1	32.33
ETBE	513.56	19	10.50
ETBE	298.52	18.2	6.37
ETBE	486.44	15.6	12.11
TAME	144.44	17.7	3.28
TAME	365.72	18.5	7.94
TAME	69.08	19.8	1.40

Sample	Total CO_2 produced (µL)	starting concentration (ppm)	Percent of Theoretical
ethanol	1536.6	23	52.71
ethanol	1497	23	51.36
ethanol	1352.28	23.5	45.40
ethanol	1421.4	23.3	48.13
toluene	2773.56	30.8	36.95
toluene	2266.44	44.4	20.94
toluene	408.84	4.9	34.24
toluene	1264.68	32.1	16.17
ETBE	294.38	19	9.02
ETBE	137.18	18.2	4.39
ETBE	328.22	15.6	12.25
TAME	126.62	17.7	4.31
TAME	296.06	18.5	9.64
TAME	240.38	19.8	7.31

Table C.3 Respirometry data by experiment (cont.)

Third Experiment

Sample	Total O_2 produced (µL)	starting concentration oxygenate(ppm)	starting concentration toluene (ppm)	Percent of total Theoretical
ETBE	1331.55	19.5		26.52
ETBE	978.15	19.6		19.38
ETBE	1142.55	18.5		23.98
ETBE	958.05	19.2		19.38
TAME	1336.95	20.8		25.81
TAME	1497.15	17.7		33.96
TAME	717.15	18		15.99
ETBE/toluene	4355.25	17.9	9.7	56.94
ETBE/toluene	5309.55	19.1	4.7	83.07
ETBE/toluene	5806.05	16.7	4.3	102.80
ETBE/toluene	4260.45	17.2	4.7	72.18
TAME/toluene	4912.35	19	5	77.99
TAME/toluene	2556.45	18.6	5	41.24
TAME/toluene	2135.55	18.6	3.4	37.48
TAME/toluene	1908.15	16.8	3	37.24
TAME/toluene	6468.45	20.1	3.7	104.92

Sample	Total CO_2 produced (µL)	starting concentration oxygenate(ppm)	starting concentration toluene (ppm)	Percent of total Theoretical
ETBE	797.4	19.5		23.82
ETBE	561.3	19.6		16.68
ETBE	558.6	18.5		17.59
ETBE	374.7	19.2		11.37
TAME	648.6	20.8		18.78
TAME	780.9	17.7		26.57
TAME	233.4	18		7.81
TAME	2728.8	19	5	62.40
ETBE/toluene	2665.5	17.9	9.7	49.02
ETBE/toluene	3284.7	19.1	4.7	74.24
ETBE/toluene	3478.8	16.7	4.3	88.86
ETBE/toluene	2453.7	17.2	4.7	59.87
TAME/toluene	1626	18.6	5	37.75
TAME/toluene	1368.3	18.6	3.4	34.93
TAME/toluene	1258.5	16.8	3	35.75
TAME/toluene	3699.3	20.1	3.7	87.27

Table C.3 Respirometry data by experiment (cont.)

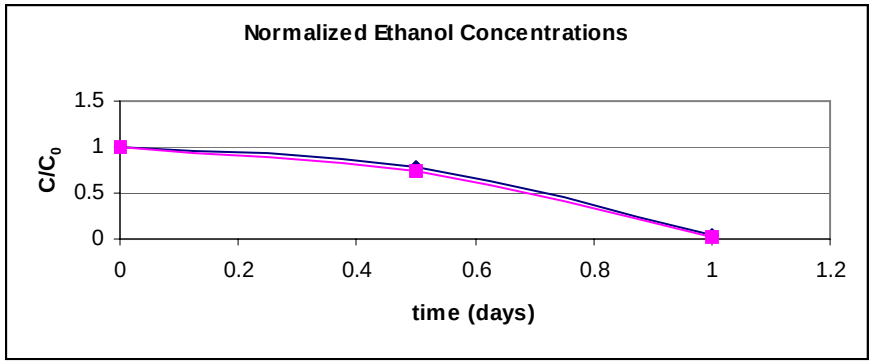
Fourth Experiment

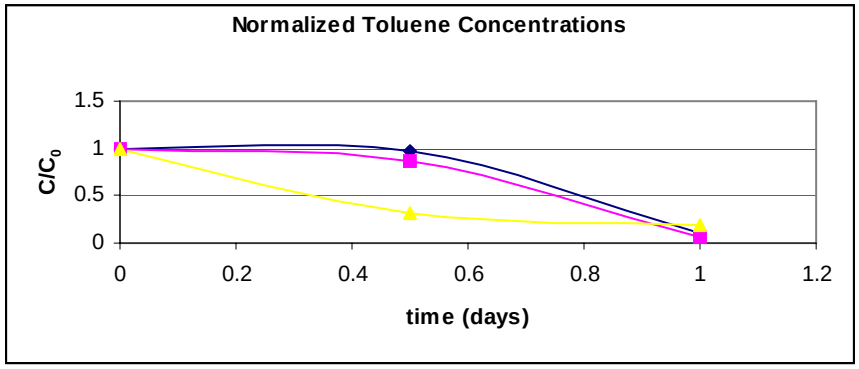
Sample	Total O_2 produced (µL)	starting concentration oxygenate(ppm)	starting concentration toluene (ppm)	Percent of total Theoretical
ETBE	921.7	17.7		20.22
ETBE	568.3	18		12.26
ETBE	873.4	18.1		18.74
ETBE	514.9	15.6		12.82
TAME	1168.3	19.4		24.18
TAME	1337.5	18.4		29.19
TAME	753.4	18.9		16.01
TAME	1450	13		44.79
ETBE/toluene	4758.1	15.7	6.3	79.07
ETBE/toluene	4705	16.4	6.4	75.53
ETBE/toluene	3666.4	16.3	6.8	57.93
ETBE/toluene	3263.2	18.4	6.3	48.61
TAME/toluene	3084.7	17.9	6.2	48.19
TAME/toluene	3423.7	17.2	6.4	54.44
TAME/toluene	2469.1	20.9	6.3	34.39
TAME/toluene	4983.1	19	6.1	75.01

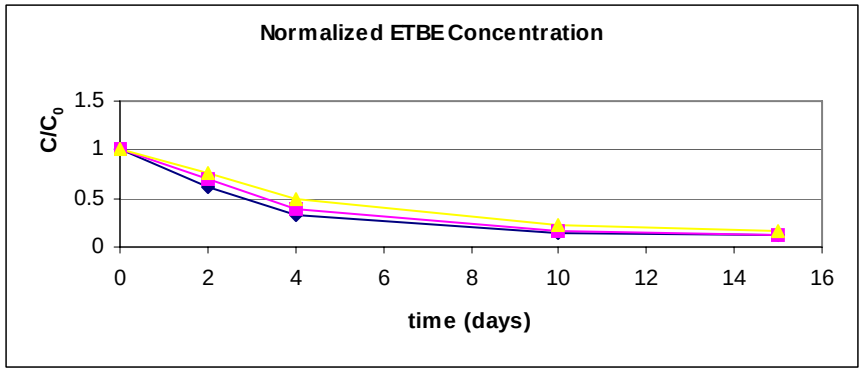
Sample	Total CO_2 produced (µL)	starting concentration oxygenate(ppm)	starting concentration toluene (ppm)	Percent of total Theoretical
ETBE	474.15	17.7		15.60
ETBE	295.65	18		9.57
ETBE	394.35	18.1		12.69
ETBE	135.75	15.6		5.07
TAME	544.65	19.4		16.91
TAME	750.15	18.4		24.55
TAME	304.05	18.9		9.69
TAME	793.65	13		36.77
ETBE/toluene	2775.75	15.7	6.3	65.61
ETBE/toluene	2843.55	16.4	6.4	64.99
ETBE/toluene	2117.85	16.3	6.8	47.53
ETBE/toluene	1900.35	18.4	6.3	40.48
TAME/toluene	1881.15	17.9	6.2	41.96
TAME/toluene	2026.05	17.2	6.4	45.88
TAME/toluene	1591.35	20.9	6.3	31.79
TAME/toluene	2825.85	19	6.1	60.88

Figure C.1 Oxygenate Concentrations over time

First Experiment







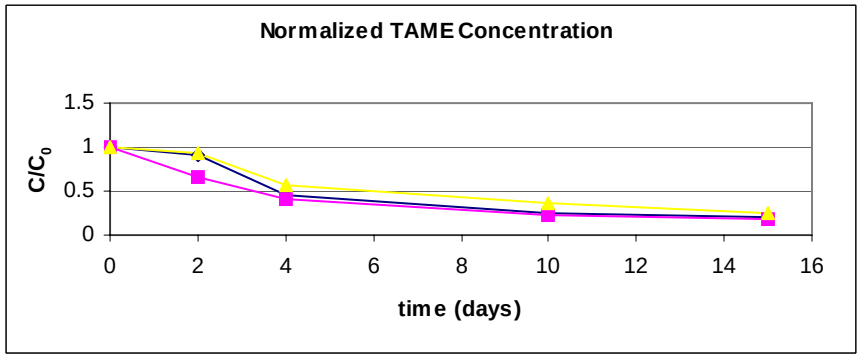
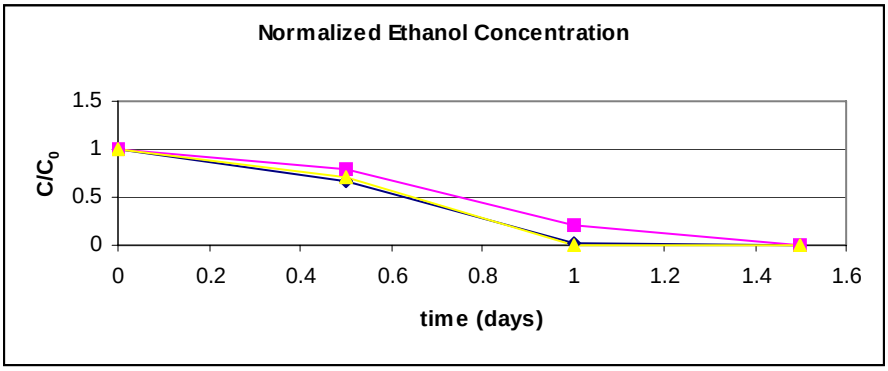
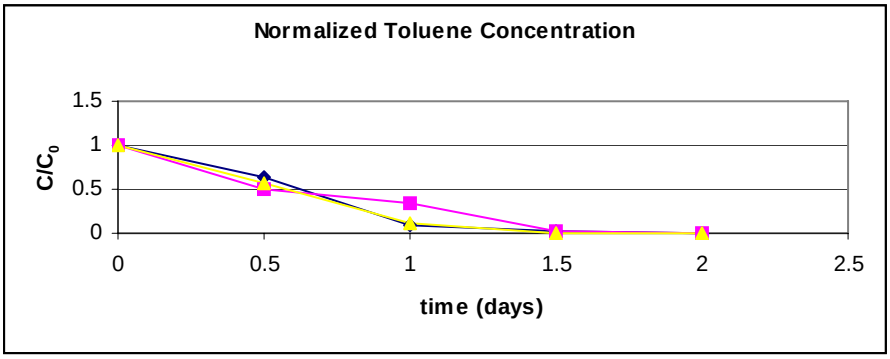
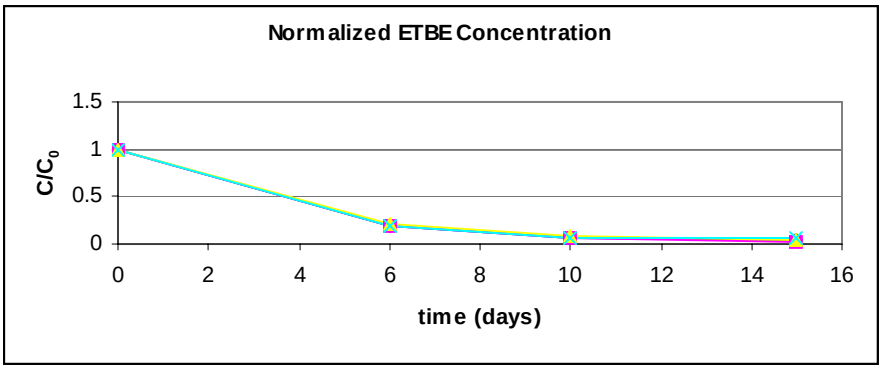


Figure C.1 Oxygenate Concentrations over time (cont.)

Second Experiment







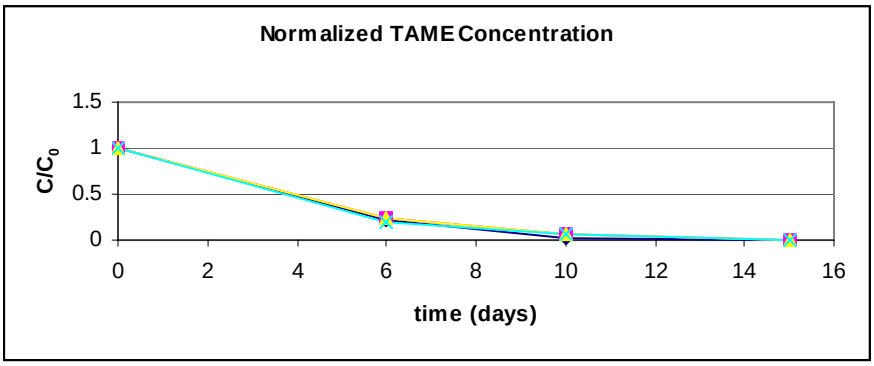
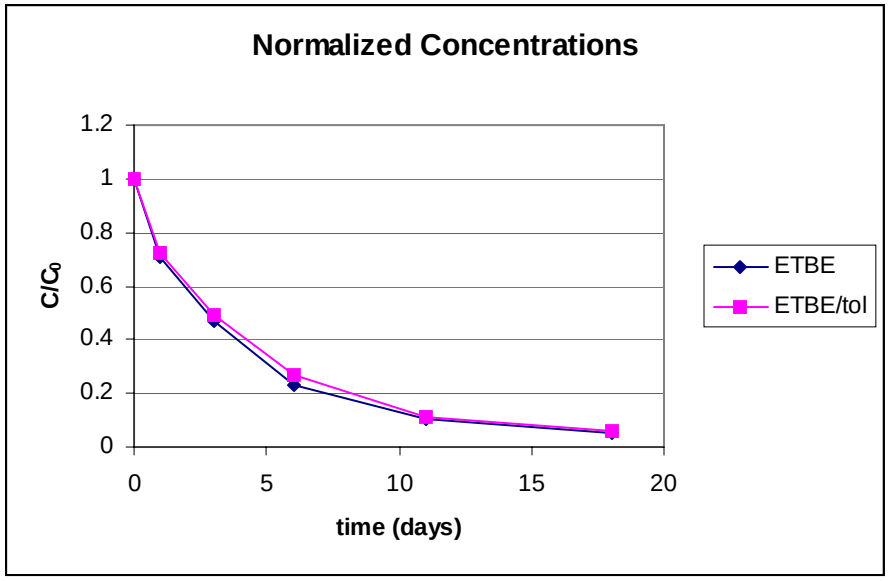


Figure C.1 Oxygenate Concentrations over time (cont.)

Third Experiment



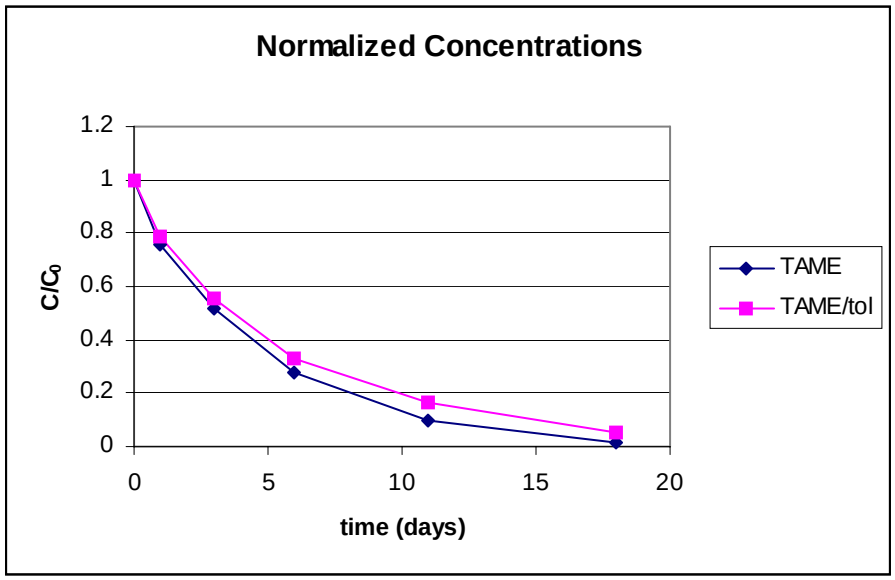
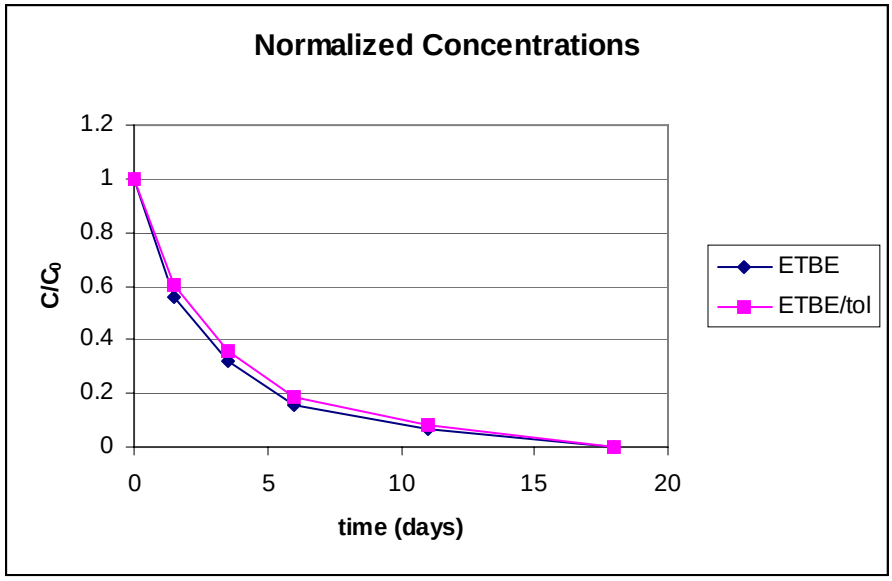


Figure C.1 Oxygenate Concentrations over time (cont.)

Fourth Experiment



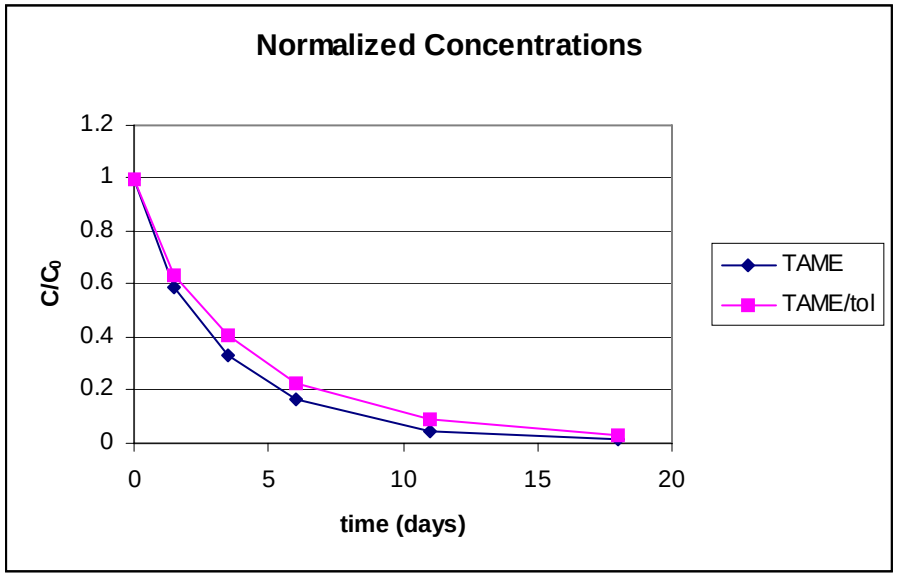
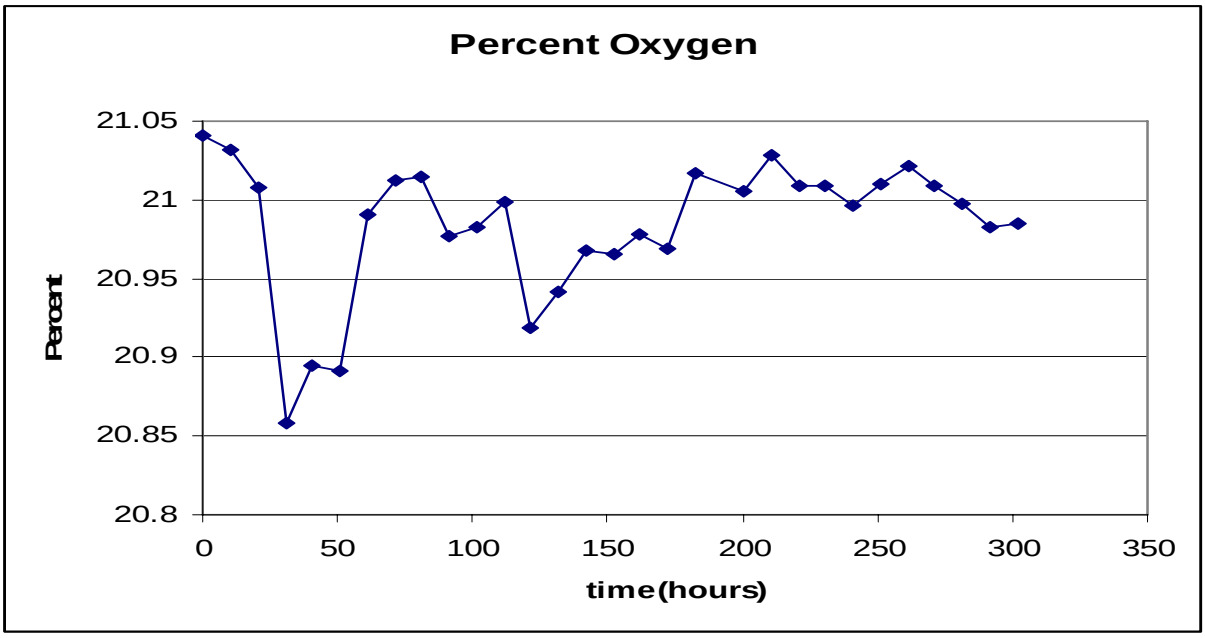


Figure C.2 Sample Chamber Oxygen Concentration over time

First Experiment



Third Experiment

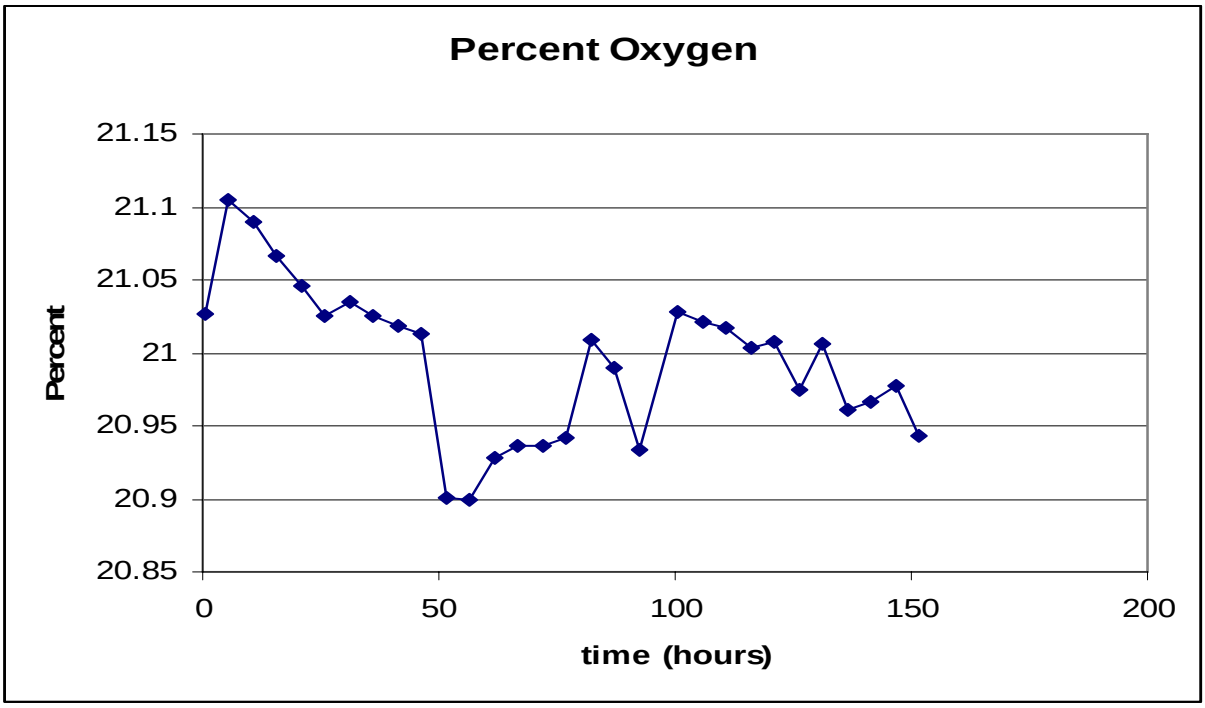


Figure C.3 Sample Ethanol Chromatograph

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Acquired	: AcqMethod DIETZ4.M
Instrument	: HPG1530A

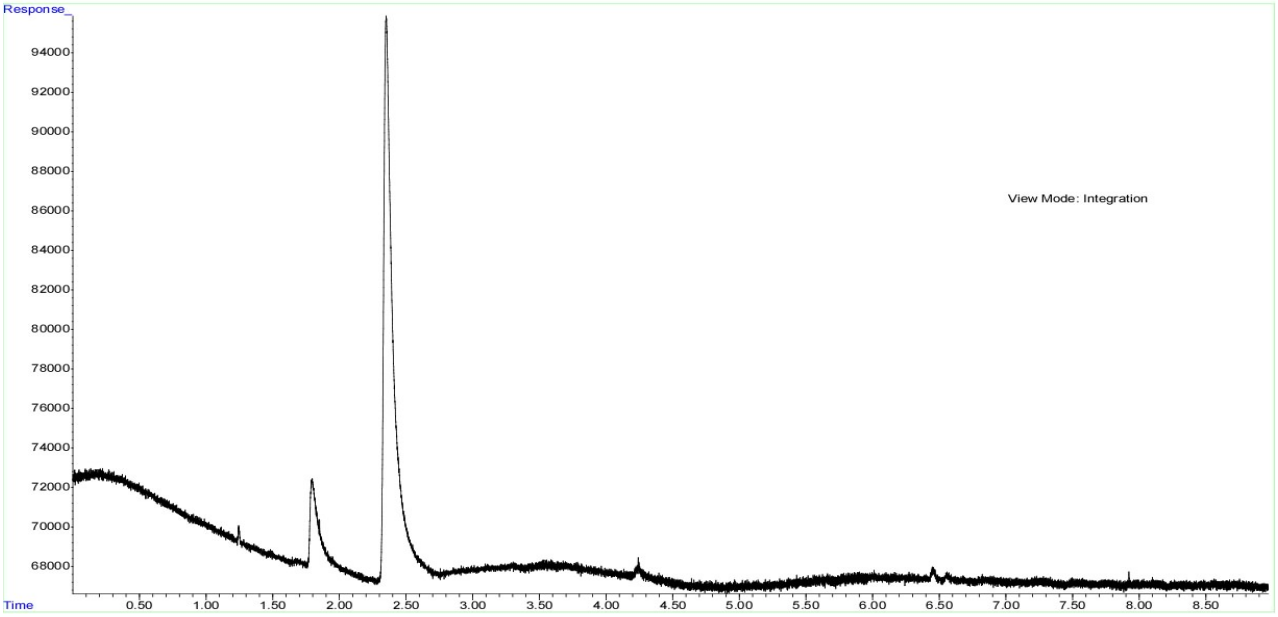

Figure C.4 Sample Toluene Chromatograph

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Acquired	: AcqMethod DIETZ4.M
Instrument	: HPG1530A

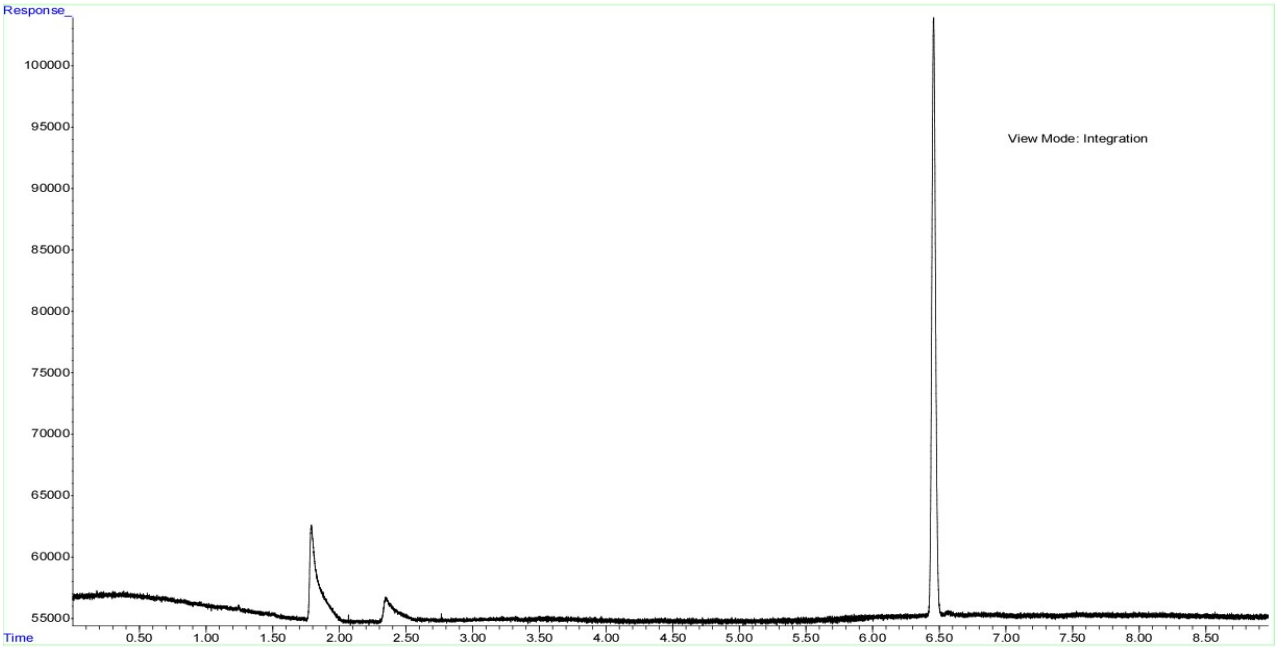


Figure C.5 Sample ETBE Chromatograph

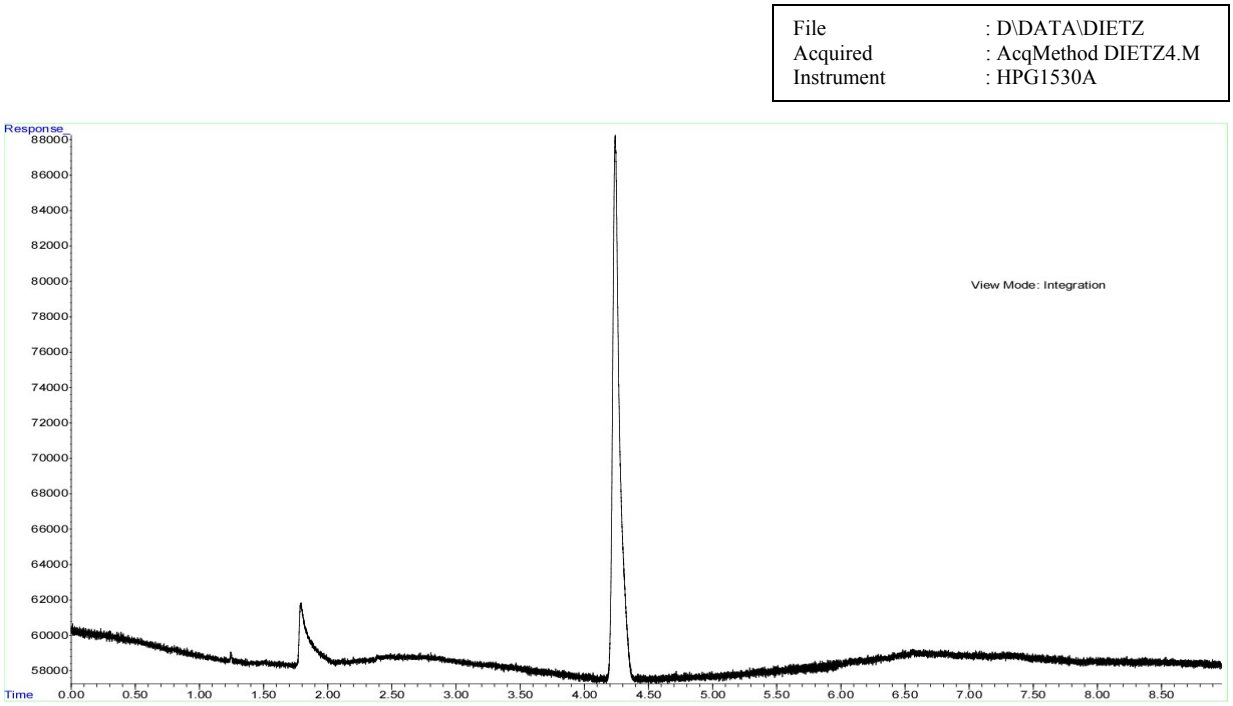


Figure C.6 Sample TAME Chromatograph

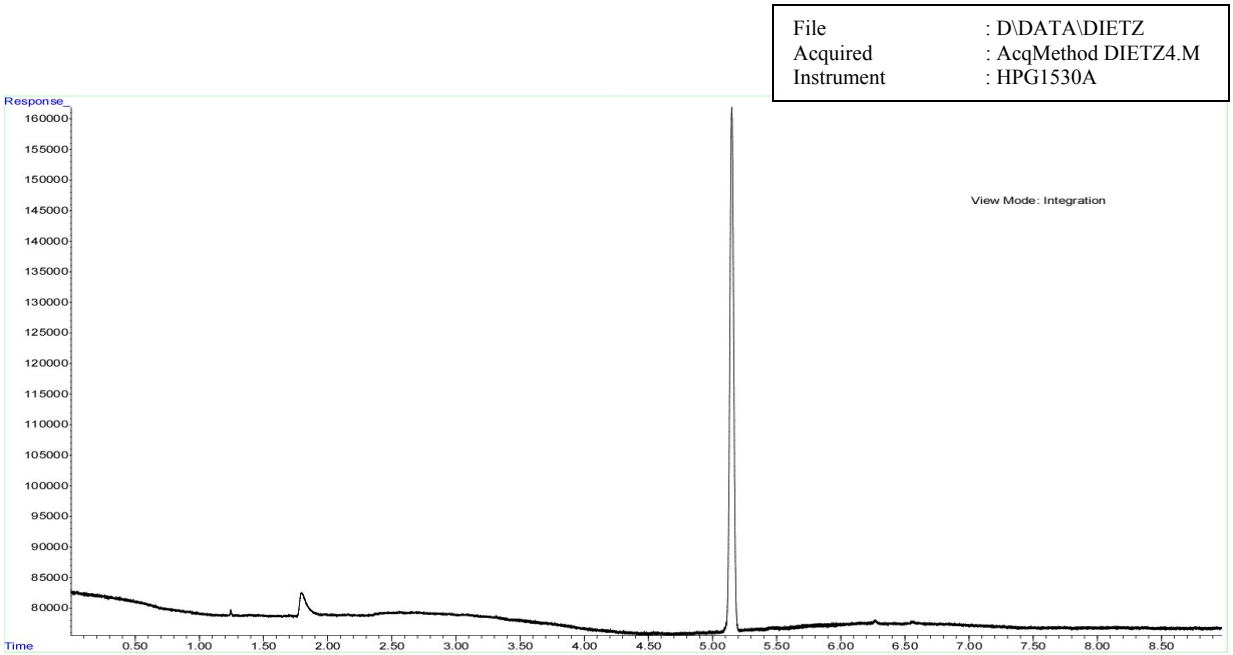


Figure C.7 Sample ETBE/toluene chromatograph

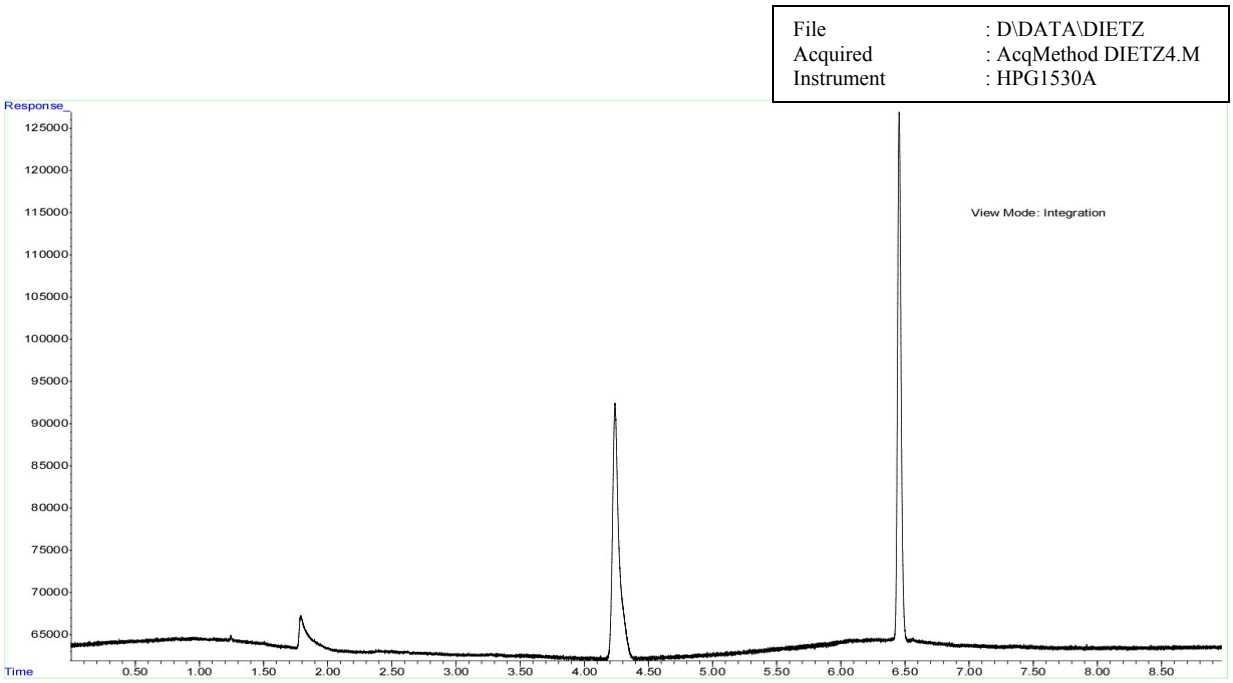


Figure C.8 Sample TAME/toluene Chromatograph

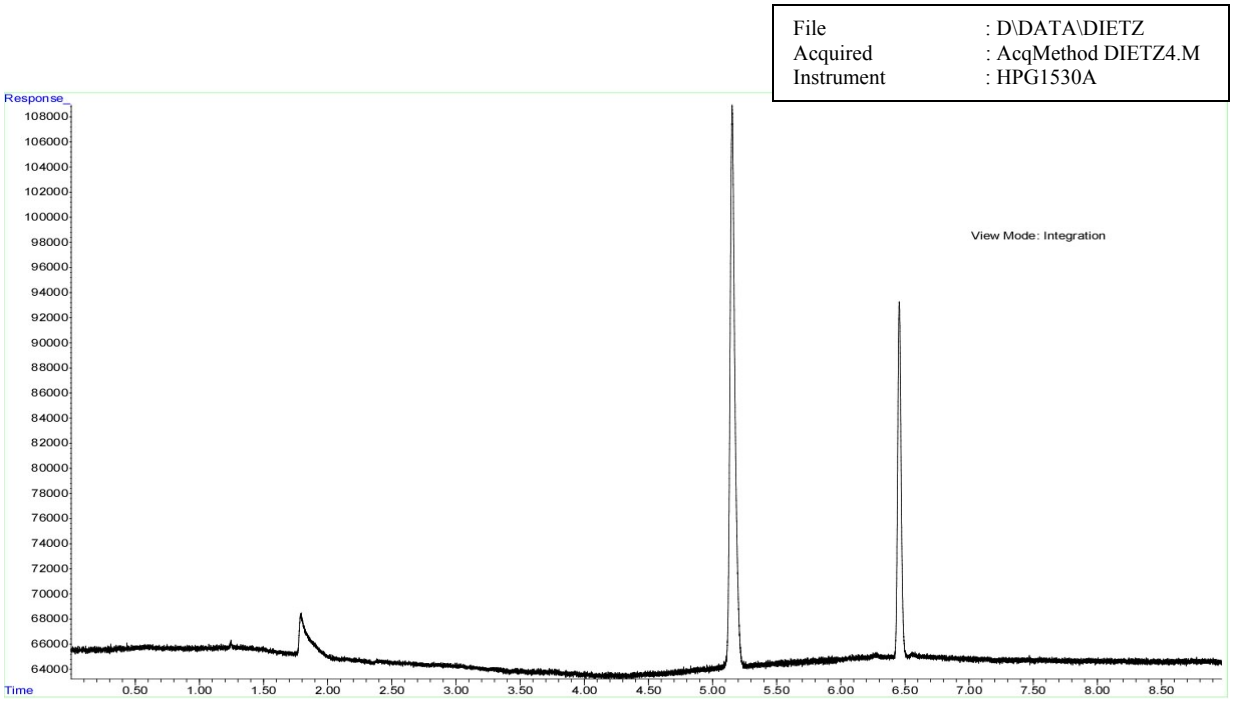


Table C.4 Experimental Control Gas Chromatography Data

First Experiment (15 day duration)

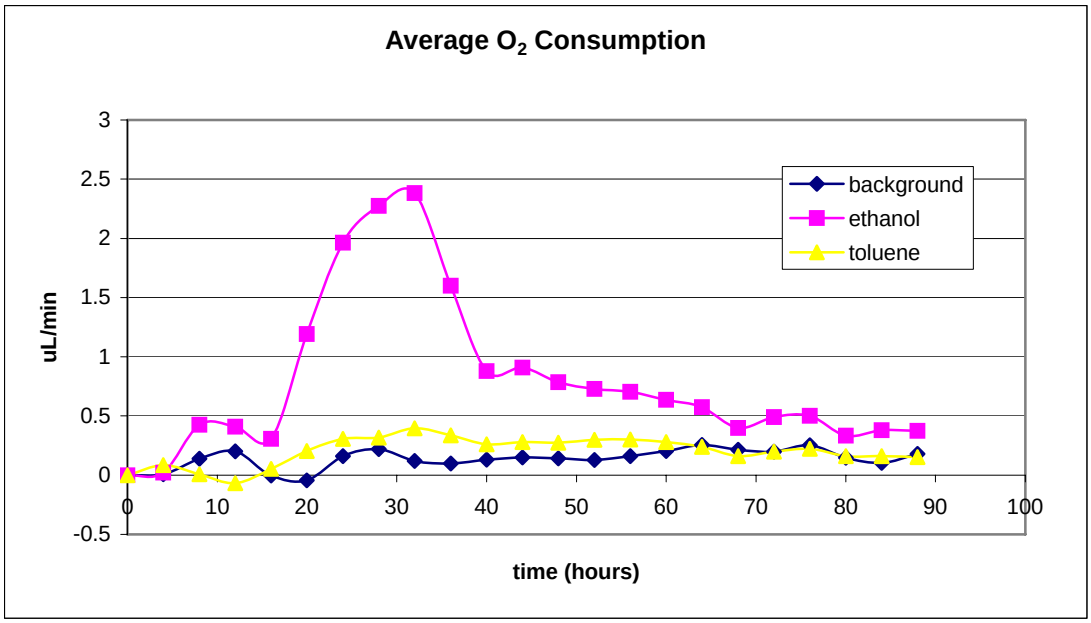
Sample	Starting Concentration (ppm)	Final Concentration (ppm)	Percent Remaining
ETBE	16.68	16.76	100.48
ETBE	15.19	14.21	93.55
TAME	16.9	16.98	100.47
TAME	16.96	17.3	102.00
toluene	18.57	11.9	64.08
toluene	15.51	13.25	85.43
toluene	9.65	7.77	80.52
toluene	9.69	7.15	73.79
ethanol	28.51	29.27	102.67
ethanol	29.19	29.72	101.82
ethanol	28.67	28.41	99.09
ethanol	28.92	28.63	98.99

Second Experiment (18 day duration)

Sample	Starting Concentration (ppm)	Final Concentration (ppm)	Percent Remaining
ETBE	12.65	10.801	85.38
ETBE	10.44	9.53	91.28
ETBE/toluene	13.45	13.29	98.81
ETBE/toluene	12.24	11.93	97.47
TAME	16.74	16.37	97.79
TAME	16.28	16.07	98.71
TAME/toluene	16.3	16.61	101.90
TAME/toluene	13.49	13.79	102.22

Figure C.9 O_2 Consumption and CO_2 Production

First Experiment



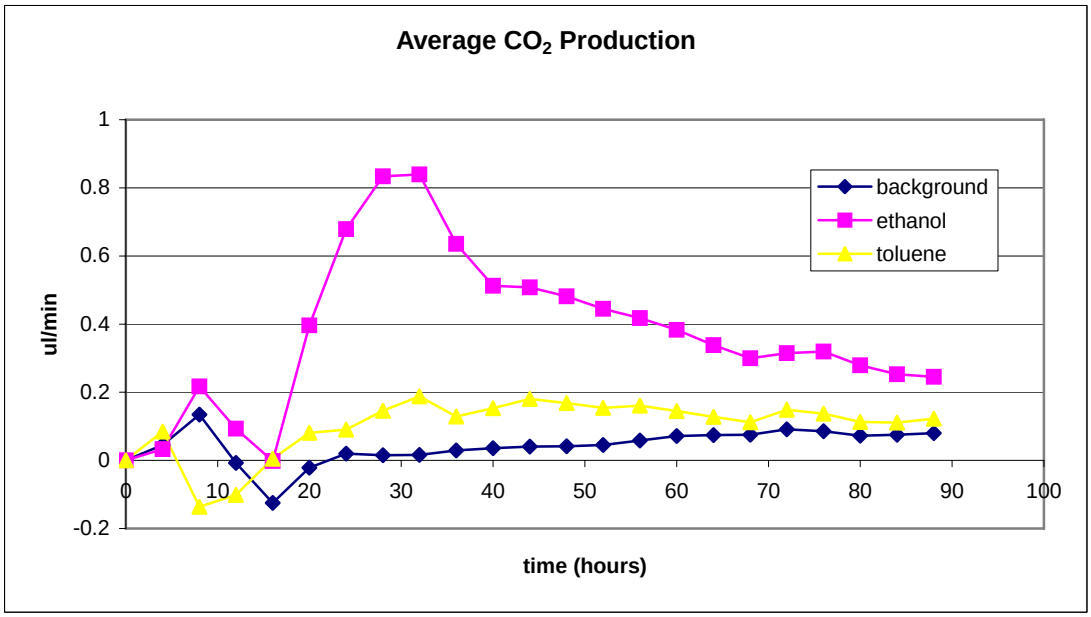
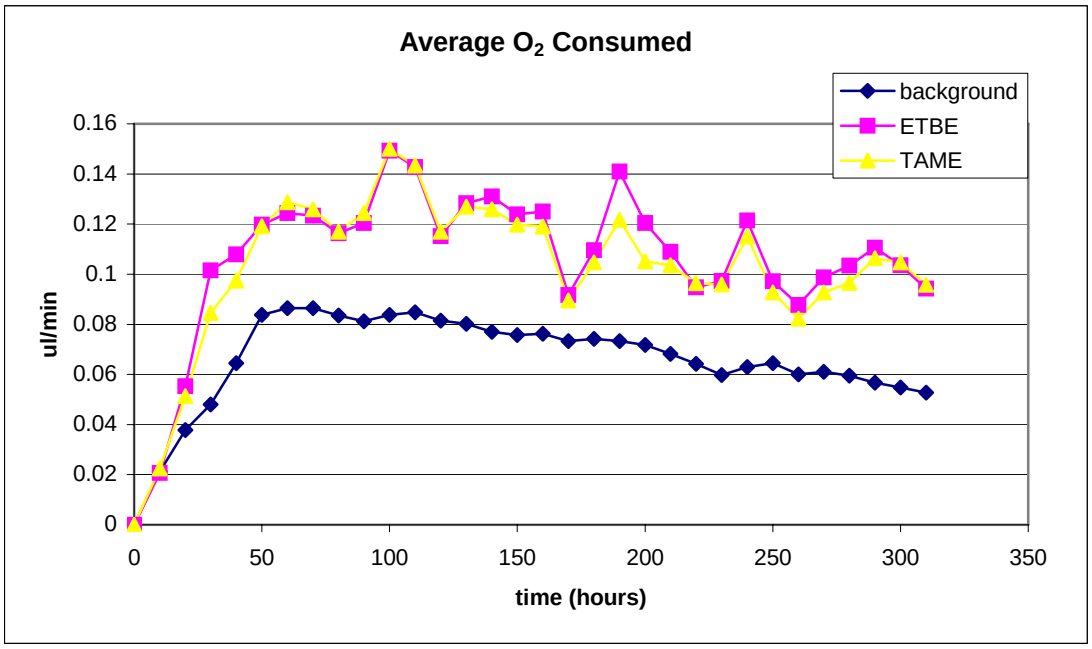


Figure C.9 O_2 Consumption and CO_2 Production (cont.)

First Experiment



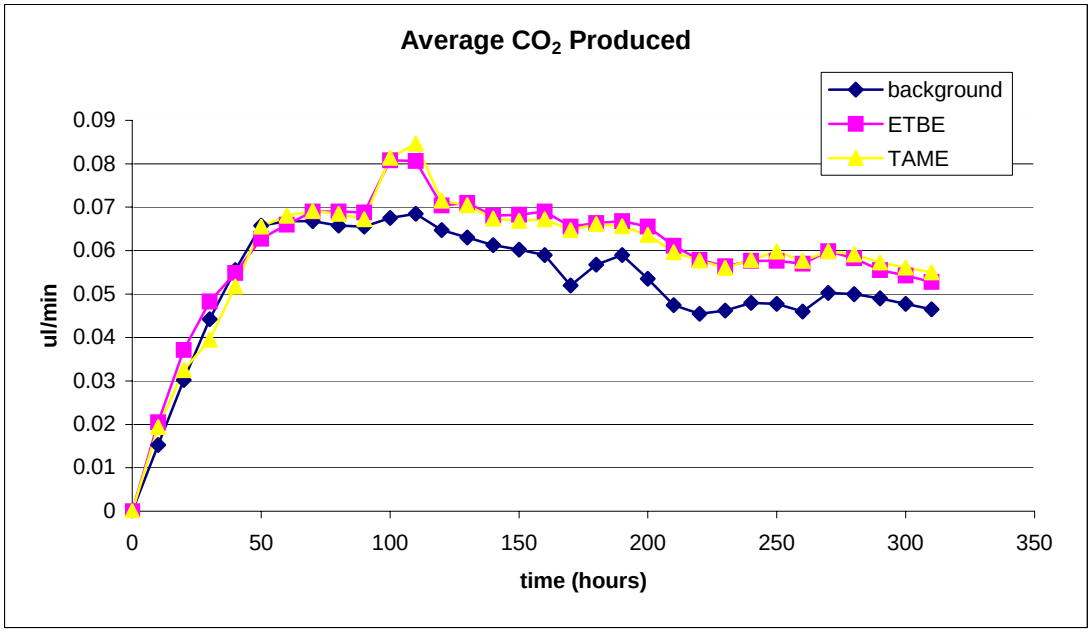
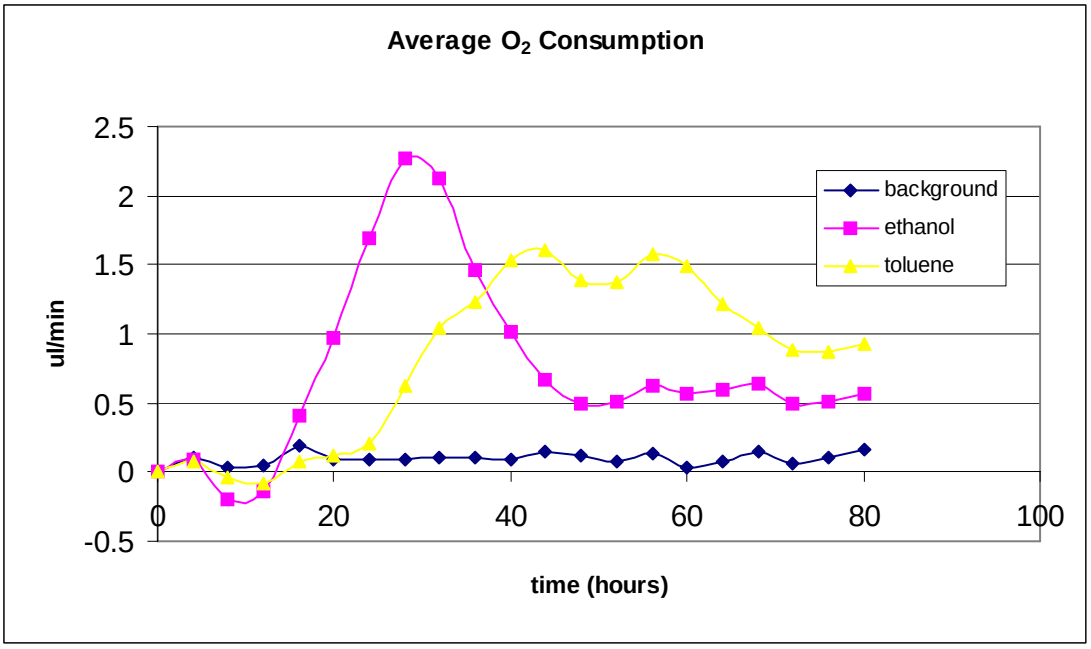


Figure C.9 O_2 Consumption and CO_2 Production (cont.)

Second Experiment



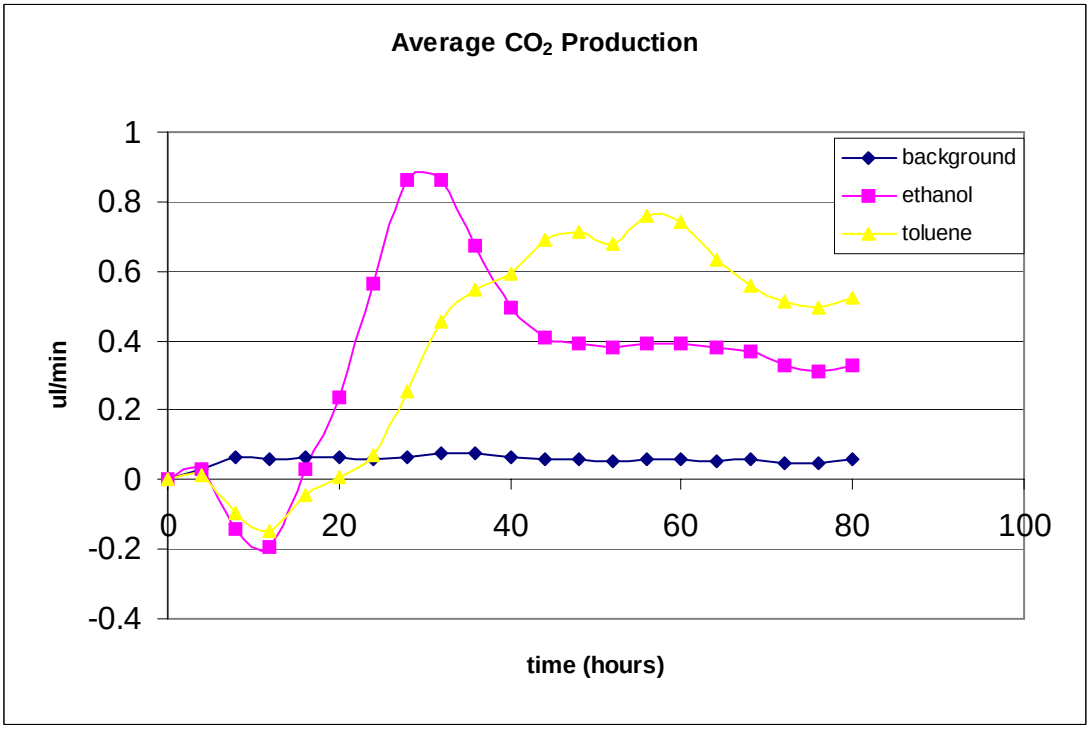
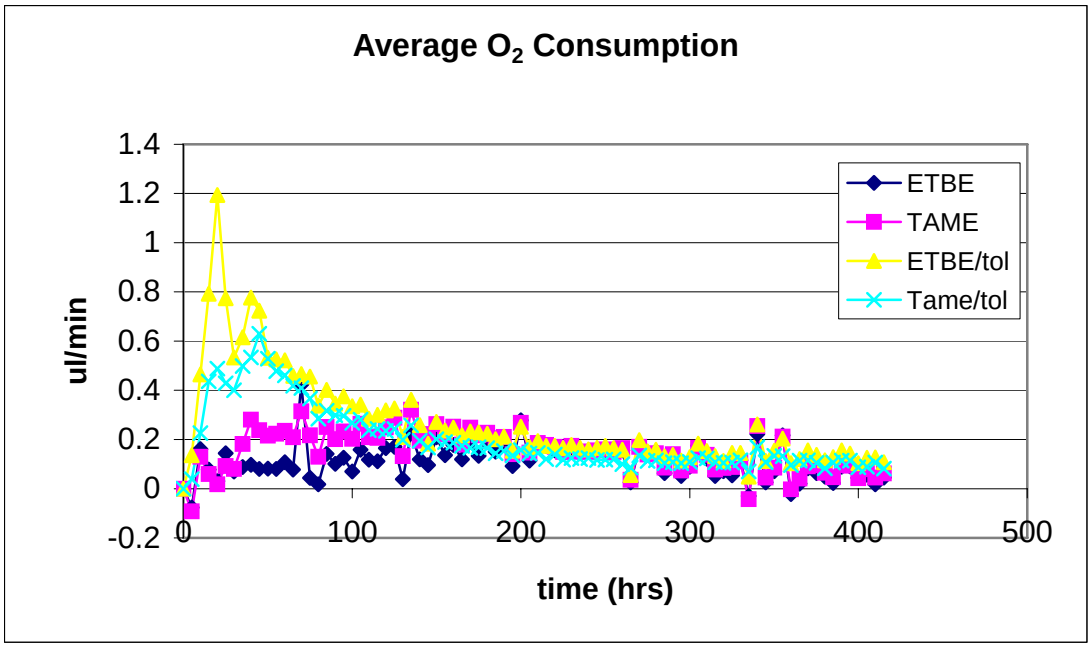


Figure C.9 O_2 Consumption and CO_2 Production (cont.)

Third Experiment



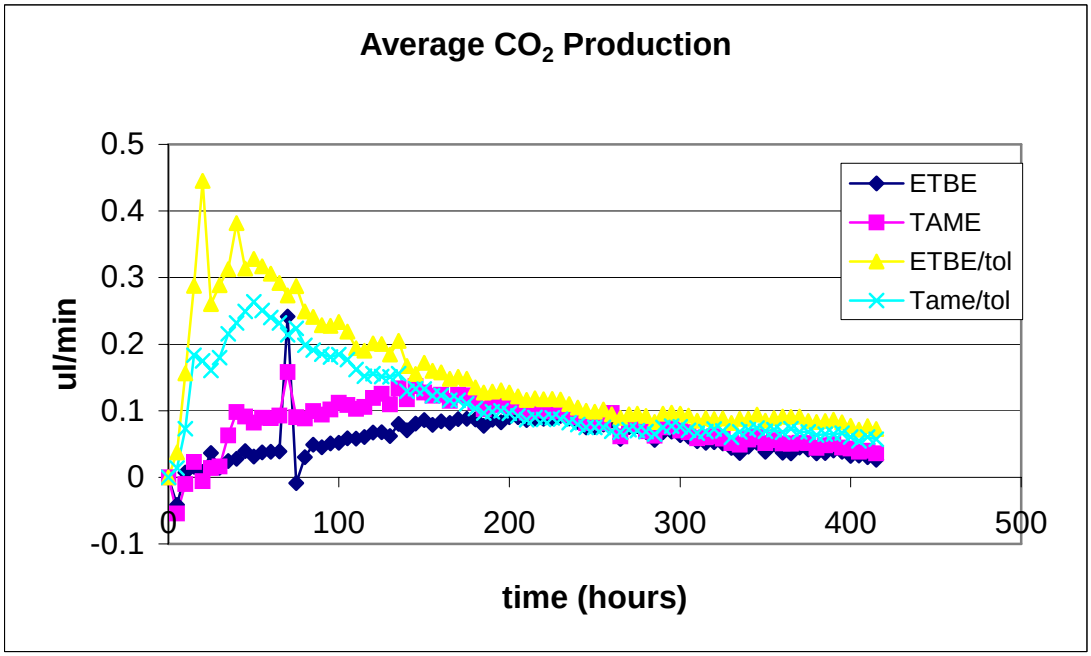
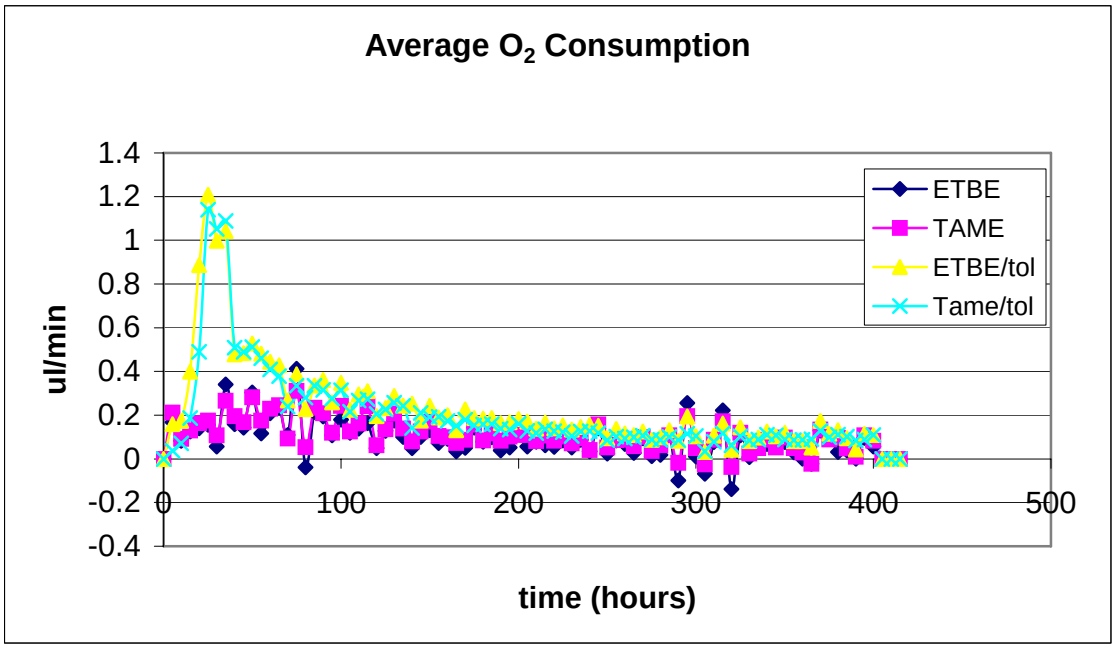
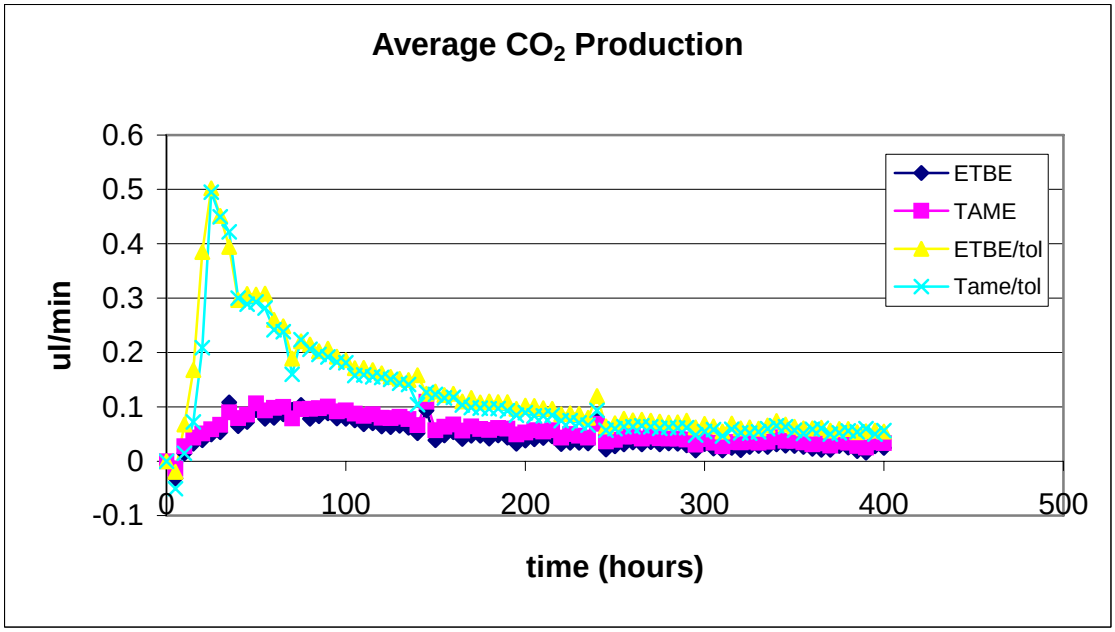


Figure C.9 O_2 Consumption and CO_2 Production (cont.)

Fourth Experiment





Appendix D

Table D.1 Consolidated GC experiment statistical data

	Mean λ	**Standard Deviation**	**95% C.I. +/-**
ETBE	0.13	0.009	0.015
	0.2	0.02	0.037
	0.17	0.008	0.021
	0.25	0.011	0.027
TAME	0.11	0.009	0.014
	0.34	0.018	0.029
	0.23	0.037	0.092
	0.25	0.017	0.042
Toluene	2.19	0.48	1.19
	2.73	0.24	0.59
Ethanol	3.44	0.57	1.24
	4.24	0.88	2.19
ETBE/Toluene	0.15	0.016	0.039
	0.26	0.007	0.018
TAME/Toluene	0.17	0.032	0.079
	0.21	0.06	0.15

Table D.2 Consolidated Respirometry experiment statistical data

	Mean % Theoretical	**Standard Deviation**	**95% C.I. +/-**
ETBE O_2	19.19	5.14	12.78
	9.65	2.95	7.35
	22.28	3.54	5.63
	17.34	5.08	8.09
ETBE CO_2	12.1	5.61	13.93
	8.55	3.935	9.82
	16	4.03	6.42
	10.78	4.48	7.13
TAME O_2	11.98	16.78	26.7
	4.24	3.37	8.36
	25.23	8.96	22.26
	15.73	6.29	17.23
TAME CO_2	14.7	19.11	17.72
	7.09	2.67	6.63
	23.13	6.66	16.56
	17	7.45	18.5

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14. ABSTRACT

This research determined the rate and extent of aerobic degradation of fuel oxygenates ethyl tert butyl ether (ETBE), tert amyl methyl ether (TAME) and ethyl alcohol (ethanol). Biodegradation was measured using gas chromatography (GC), respirometry, and biochemical oxygen demand (BOD) tests. Additionally, the research determined the effects of toluene on degradation rates.

This microcosm study used a microbial consortium obtained from a petroleum refinery wastewater treatment facility. Respirometry data were collected from chambers containing pure oxygenates, or oxygenate/toluene mixtures. Samples were withdrawn periodically for GC analysis. Aerobic conditions were maintained in the chambers at all times.

Degradation of oxygenates was compared to degradation of toluene, assuming first order decay. Across all experiments TAME degraded at 8.65 % the rate of toluene, ETBE at 8.17% the rate of toluene and ethanol at 156.79 % the rate of toluene. GC and respirometry data were the most suitable methods for measuring degradation. BOD provided acceptable results for toluene and ethanol but not for the slower degrading oxygenates. Finally, the presence of toluene slowed the degradation of both ETBE and TAME.

15. SUBJECT TERMS
Fuel Oxygenates, Ethanol, TAME, ETBE, Toluene

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