



The oxygen dependence of the degradation rate of Methyl tert-Butyl Ether by a bacterial isolate
by Elsa Emilie Meiser

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Civil
Engineering

Montana State University

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Abstract:

Methyl tert-Butyl Ether (MTBE) is a fuel oxygenate added to gasoline to boost octane and reduce emissions of volatile organics, nitrogen oxides, and toxics such as benzene. Bacterial degradation represents a viable means of remediating MTBE that is contaminating the environment. The aerobic degradation of MTBE is catalyzed by the enzyme monooxygenase. This enzyme is dependent upon molecular oxygen. The Michaelis-Menton model is a mathematical model that describes the enzyme-mediated dependence of reaction rate on a given substrate. The Hill model further describes enzyme mediated reaction kinetics when cooperativity among enzymes has been introduced.

This study investigated the behavior of MTBE Degradation rate as a function of dissolved oxygen concentration by a bacterial isolate, PMI. Three experiments were run. In each experiment, batch microcosms containing aqueous nutrient media, MTBE, PMI, and oxygen were monitored over a period of 7 days. MTBE and oxygen concentration were determined at each sampling event. These values were used to correlate MTBE degradation rate to dissolved oxygen concentration. This data was fit to the Michaelis-Menton and Hill models using a non-linear regression algorithm.

This study shows that the biodegradation rate of MTBE by PMI is highly dependent on dissolved oxygen concentrations, specifically at concentrations of dissolved oxygen below 2 mg/L. This sensitivity to dissolved oxygen concentration may be an important factor to consider in the design and operation of treatment and remediation systems. Furthermore, PMI was capable of complete mineralization of up to 20 mg/L of MTBE in 7 days with sufficient oxygen present. Both the Hill model and the Michaelis-Menton model represent adequate predictors of the behavior of the data.

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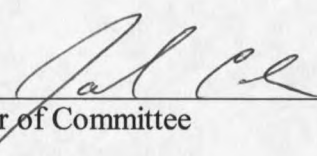
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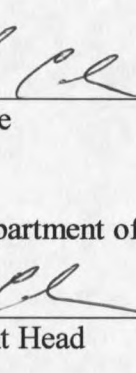
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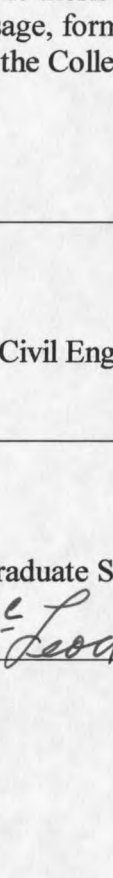
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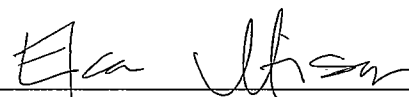
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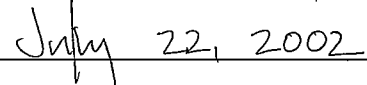


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ABSTRACT

Methyl *tert*-Butyl Ether (MTBE) is a fuel oxygenate added to gasoline to boost octane and reduce emissions of volatile organics, nitrogen oxides, and toxics such as benzene. Bacterial degradation represents a viable means of remediating MTBE that is contaminating the environment. The aerobic degradation of MTBE is catalyzed by the enzyme monooxygenase. This enzyme is dependent upon molecular oxygen. The Michaelis-Menton model is a mathematical model that describes the enzyme-mediated dependence of reaction rate on a given substrate. The Hill model further describes enzyme mediated reaction kinetics when cooperativity among enzymes has been introduced.

This study investigated the behavior of MTBE Degradation rate as a function of dissolved oxygen concentration by a bacterial isolate, PM1. Three experiments were run. In each experiment, batch microcosms containing aqueous nutrient media, MTBE, PM1, and oxygen were monitored over a period of 7 days. MTBE and oxygen concentration were determined at each sampling event. These values were used to correlate MTBE degradation rate to dissolved oxygen concentration. This data was fit to the Michaelis-Menton and Hill models using a non-linear regression algorithm.

This study shows that the biodegradation rate of MTBE by PM1 is highly dependent on dissolved oxygen concentrations, specifically at concentrations of dissolved oxygen below 2 mg/L. This sensitivity to dissolved oxygen concentration may be an important factor to consider in the design and operation of treatment and remediation systems. Furthermore, PM1 was capable of complete mineralization of up to 20 mg/L of MTBE in 7 days with sufficient oxygen present. Both the Hill model and the Michaelis-Menton model represent adequate predictors of the behavior of the data.

CHAPTER 1

INTRODUCTION

Methyl *tert*-Butyl Ether (MTBE) has been used in gasoline since the 1970s to boost octane and reduce emissions of volatile organics, nitrogen oxides, and toxics such as benzene. Over the past decade, MTBE has been detected with increasing frequency in surface and drinking water supplies across the nation, often as a result of gasoline leaks and spills. Traditional techniques that are effective for treatment of other constituents of gasoline are not as effective for remediation of MTBE (EPA 2000). As a result, alternative techniques are currently being explored.

Bioaugmentation and natural attenuation represent two remediation techniques in which bacteria are used to degrade MTBE. Often, low levels of dissolved oxygen characterize aquifer environments contaminated by MTBE. In these cases the addition of oxygen would stimulate aerobic degradation activity, and thus enhance the remediation effort.

MTBE can be difficult to degrade due to its chemical characteristics. While other gasoline constituents such as the BTEX compounds (benzene, toluene, ethylbenzene, and the xylenes) will readily sorb to soils and are quite amenable to microbial degradation, MTBE resists retardation and decay in natural systems. MTBE is also more soluble than the BTEX compounds. As a result, MTBE plumes at contaminated sites extend farther down gradient than BTEX plumes. MTBE travels at virtually the speed of groundwater due to its solubility and low sorption coefficient, thus increasing its potential to impact domestic water supplies (US EPA Office of Pollution Prevention and Toxics 1994).

MTBE is used almost exclusively as a fuel additive for motor gasoline (US EPA 1997). MTBE is classified as an "oxygenate" because it raises the oxygen content of gasoline, helping it to burn more completely and thus reducing harmful tailpipe emissions. Oxygenates are helpful in reducing emissions in two ways. Firstly, the oxygen that they contain dilutes or displaces gasoline components such as aromatics and sulfur. Secondly, additional oxygen increases oxidation during the combustion of gasoline, thus making the gasoline burn more cleanly.

Under the Clean Air Act Amendments of 1990, Congress mandated the use of reformulated gasoline (RFG) in areas of the country with the worst ozone or smog problems. The Act required that RFG must contain a minimum of 2 percent oxygen by weight for the Year-round Reformulated Gasoline Program and 2.7 percent oxygen by weight for the Winter Oxyfuel Program (US EPA 2000).

Reformulated gasoline has been effective in the United States since 1995. The use of RFG as compared to conventional gasoline has resulted in annual reductions of smog forming pollutants (volatile organic compounds and nitrogen oxides) and toxics (such as benzene). With the second phase of the RFG program, which began in January 2000, the EPA estimates that smog-forming pollutants are being reduced annually by at least 105 thousand tons, and toxics by at least 24 thousand tons. RFG accounts for approximately 30% of gasoline nationwide (US EPA 1999).

The Clean Air Act Amendments of 1990 do not specifically require the use of MTBE. Refiners may choose to use other oxygenates such as ethanol or *tert*-amyl ether (TAME). However, MTBE is popular because it can be produced within existing

refineries, it blends well with gasoline without phase separation, and can be transferred through existing pipelines (Sufliya and Mormile 1993). MTBE is produced in very large quantities in the United States. In 1999, over 200,000 barrels per day of MTBE were produced. This is not surprising, since as of 1997 MTBE was used in over 84% of reformulated gasoline supplies (US EPA 1998).

MTBE has been found in urban water supplies, in surface waters such as recreational lakes, and in drinking water wells (Squillace 1998; Delzer 1996). However, the question of whether or not MTBE is harmful when ingested by humans remains unanswered. The EPA has classified MTBE as a possible human carcinogen based on laboratory experiments in which MTBE was found to cause cancer in lab rats (US EPA 1997). MTBE is also generally unpleasant in taste and odor. Studies conducted to determine the concentrations at which individuals can detect the taste and odor of the chemical led to the establishment of an EPA Advisory recommendation of acceptable MTBE levels at or below 20 to 40 $\mu\text{g/L}$ (US EPA 1997). However, this level is not an enforceable standard.

Some states have chosen to mandate their own enforceable MTBE standards. The most notable of these is California. Currently, California has a secondary maximum contaminant level of 13 $\mu\text{g/L}$ and a primary MCL of 5 $\mu\text{g/L}$. Montana has adopted a Human Health Standard of 30 $\mu\text{g/L}$ (MT DEQ 2002).

While it has been instrumental in reducing smog-forming and toxic air pollutants, MTBE is undesirable in water supplies. In fact, MTBE was included in The Clean Air Act's lists of hazardous air pollutants. Leaking Underground Storage Tanks (LUSTs) are

a major source of environmental contamination that directly contribute to the introduction of MTBE into water supplies. As of September 30, 2001 state and local UST programs had submitted information to the EPA confirming 418,918 UST releases, prompting 379,243 cleanup initiatives. Nearly all USTs contain petroleum (US EPA 2002).

The Federal Government is currently considering legislation to limit or even eliminate MTBE from reformulated gasoline supplies. California, where high population density has exacerbated widespread MTBE contamination, has already mandated the complete phase-out of MTBE by the year 2010.

Bioaugmentation and natural attenuation are attractive remediation alternatives because of their use of microorganisms to completely degrade MTBE to inert compounds. It is now widely accepted that aerobic degradation of MTBE involves oxidation by the monooxygenase (MO) enzyme. The MO enzyme is dependent on molecular oxygen.

The Michaelis-Menton kinetic model describes enzyme mediated reaction kinetics when the behavior of the reaction rate is dominated by a single substrate such as oxygen. Figure 1.1 is a schematic of the characteristics of the Michaelis-Menton model. Michaelis-Menton plots contain three distinct regions that correspond to different reaction orders. At low substrate concentrations, the reaction accelerates as more substrate is added, reflecting first order kinetics. At high substrate concentrations, the concentration of enzyme becomes limiting, and additional substrate will not accelerate the reaction. This is known as zero order kinetics. The third region is a transition period between first order and zero order where kinetics are mixed.

The Michaelis-Menton model is given as follows:

$$\mu = \frac{\mu_{\max} \cdot [S]}{K_m + [S]}$$

where μ = reaction rate

μ_{\max} = maximum reaction rate

$[S]$ = substrate concentration

K_m = half saturation coefficient

The term K_m represents the amount of substrate required to bind one half of the available enzyme. If K_m is small, then tight binding occurs and the enzyme has a high affinity for the substrate. A large K_m represents weak binding and low substrate affinity.

For the purpose of fitting the data in for the experiments described in this thesis, the model parameters are described as follows:

μ = rate of MTBE degradation [mg L^{-1} MTBE days^{-1}] or
adjusted MTBE degradation rate [days^{-1}]

μ_{\max} = maximum rate of MTBE degradation [mg L^{-1} MTBE days^{-1}] or
adjusted maximum rate of MTBE degradation [days^{-1}]

$[S]$ = concentration of dissolved oxygen, [mg L^{-1}]

K_m = half saturation coefficient

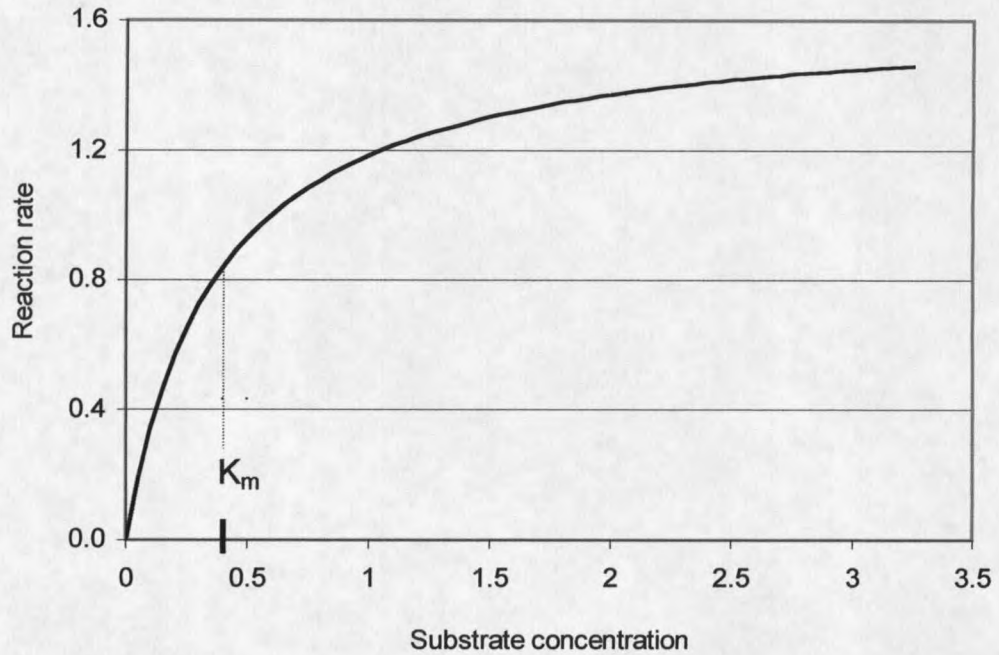


Figure 1.1 Schematic of the Michaelis-Menton model.
 $\mu_{max} = 1.628$, $K_m = 0.373$.

The Hill model describes enzyme mediated reaction kinetics in which cooperativity occurs. Cooperativity refers to the phenomenon by which an enzyme can bind several substrate molecules at any given time. The binding of substrate at a given site leads to a conformational change in shape of the enzyme as a whole, so that the catalytic activity of other sites is changed. Thus, initial enzyme activity increases the activity of subsequent enzymes.

Hill kinetics produce characteristically sigmoidal curves that include a lag time for enzyme activation. A schematic of the Hill model is shown in Figure 1.2. After this lag period, the Hill model displays an initial region of first order kinetics followed by a region of zero order kinetics, similar to the Michaelis-Menton model. This model is given as:

$$\mu = \frac{\mu_{\max} \cdot [S]^n}{(K_m)^n + [S]^n}$$

The parameter n in the Hill equation can be thought of mechanistically as the number of molecules that bind to a receptor. Recall that the Hill model describes enzyme activity in which one enzyme can bind several substrate molecules. Values of the parameter n greater than one indicate positive cooperativity, in which initial enzyme activity increases subsequent activity. Similarly, values of n less than one indicate negative cooperativity, in which initial enzyme activity decreases subsequent activity.

A significant study by Park and Cowan (1997) found that the biodegradation rate of MTBE was highly dependent on dissolved oxygen concentration when degraded by an aerobic mixed culture. Specifically, MTBE degradation was inhibited at oxygen concentrations below 2 mg L^{-1} . Furthermore, the study found the half saturation coefficient, K_m , to be 0.9 mg DO L^{-1} . Typical K_m concentrations for heterotrophs are on the order of 0.1 to 0.2 mg DO L^{-1} . The authors state that this reveals that the MTBE degrading mixed culture which they studied is more sensitive to the concentration of DO than normal heterotrophs.

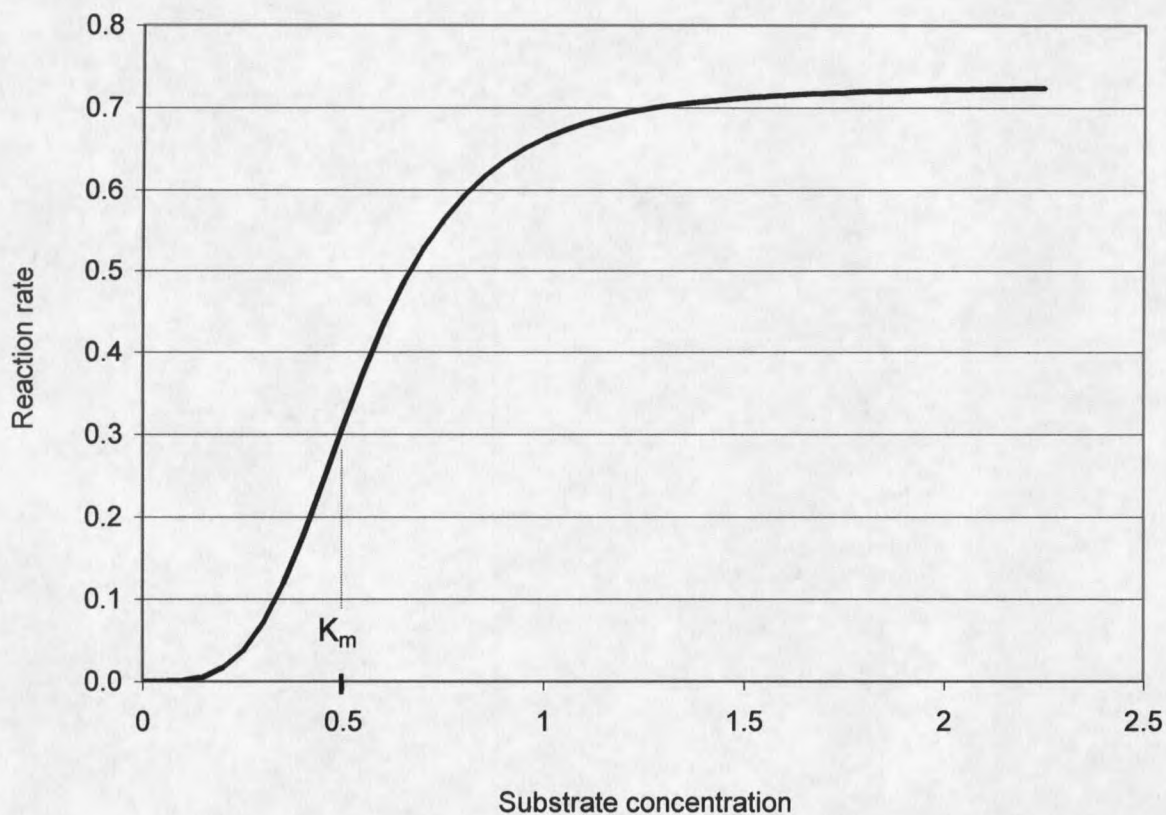


Figure 1.2 Schematic of the Hill model.
 $\mu_{\max} = 0.73$, $K_m = 0.54$, $n = 3.78$.

This study investigates the dependence of MTBE biodegradation rate on dissolved oxygen concentration when degraded by an aerobic bacterial isolate. The Michaelis-Menton and Hill model are fit to the data to determine the best model for predicting the behavior of this system. These findings are compared to the findings of Park and Cowan in which an aerobic mixed culture was investigated.

CHAPTER 2

LITERATURE REVIEW

Chemical Characteristics

MTBE is an aliphatic ether with the chemical formula $C_5H_{12}O$ (Figure 2.1). Table 2.1 shows the physical and chemical characteristics of MTBE and the BTEX compounds. MTBE has a molecular weight of $88.15 \text{ g mole}^{-1}$. It is a flammable colorless liquid at room temperature. The solubility of MTBE is approximately 50 g L^{-1} at 25°C . The Henry's Law Constant of MTBE ranges from 0.018 to 0.24 in the reported literature. MTBE has a log octanol-water partitioning coefficient ($\log K_{ow}$) of 1.20.

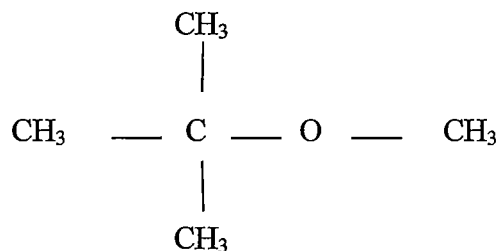


Figure 2.1 Chemical Structure of MTBE

MTBE is the most widely used fuel oxygenate. It is commonly found in the environment with the BTEX (benzene, toluene, ethyl-benzene, xylene) compounds.

Table 2.1 Physical and Chemical Properties of MTBE, Benzene, Toluene, Ethyl-benzene, and O-xylene.

Physical and chemical properties	Benzene	Toluene	Ethyl-benzene	O-xylene	MTBE
Molecular weight [g mole ⁻¹]	78.11	92.14	106.17	106.17	88.15
Vapor Density @ 1 atm; 10°C (Air = 1)	3.36	3.97	4.57	4.57	3.8
Specific gravity @ 25°C	0.88 (1)	0.8669 (1)	0.867 (1)	0.8802 (1)	0.744 (1)
Water solubility [mg L ⁻¹]	1730 (1)	534.8 (1)	161 (1)	175 (1)	43,000-54,300 50,000 (1)
Henry's Law constant	0.23 (2)	0.272 (2)	0.336 (2)	0.212 (2)	0.2399 (1) 0.04496(1) 0.05722(1) 0.1226 (1) 0.026 (1) 0.018 @ 20°C (1)
Vapor pressure [mm Hg] (@ 25°C)	76, 95.19 (1)	28.4 (1)	9.53 (1)	6.6 (1)	245-276 (1)
Log K _{ow}	2.36 (2)	2.73 (2)	3.24 (2)	3.10 (2)	1.20 (1)

(1) OSTP, 1997.. (2) Crittenden et al, 1997.

Compared to the BTEX compounds, MTBE is more soluble, less volatile, and less sorptive (Table 2.1). Although MTBE is classified as a volatile organic compound, MTBE will partition more strongly than benzene from the gas phase to the water phase. Based on its chemical and physical characteristics, MTBE moves virtually at the speed of groundwater.

MTBE Detection in the Environment

During the past decade, MTBE has been detected with increasing frequency in US water sources. Three studies are discussed in the following section. The first two studies, conducted by the United States Geological Survey (USGS), look at both urban and agricultural well water contamination by MTBE as well as MTBE detection in urban storm water runoff. A third study investigates the occurrence of MTBE in a multiple use lake in the Sierra Nevada Mountains. These three studies highlight the affinity of MTBE for partitioning into the water phase as well as the high occurrence of MTBE in these water sources.

As part of the National Water Quality Assessment Program of the USGS, an assessment of 60 volatile organic compounds (VOCs) in untreated, ambient groundwater in the US was conducted from 1985 to 1995. MTBE was the second most frequently detected chemical, contaminating 27% of the 210 urban wells sampled, 1.3% of the 549 agricultural wells sampled, and 1% of the 412 wells in deeper groundwater from major aquifers. No MTBE was detected in drinking water wells. Only 3% of the shallow wells sampled in urban areas had concentrations of MTBE that exceeded $20 \mu\text{g L}^{-1}$. The

reporting level for MTBE was $0.2 \mu\text{g L}^{-1}$. In shallow urban groundwater, MTBE was generally not found with the BTEX compounds, likely due to its high mobility and low sorption coefficient (Squillace et al. 1996).

Another study conducted by the USGS in 1991 through 1995 sampled 16 cities with populations of over 100,000 persons that were required by the Clean Water Act to obtain permits for stormwater discharged from municipal separate storm sewer systems into surface waters. The study encompassed 11 states. MTBE was the seventh most frequently detected VOC in urban storm water, following toluene, total xylene, chloroform, total trimethylbenzene, tetrachloroethene, and naphthalene. MTBE was detected in 6.9% of the 592 storm water samples collected. When detected, MTBE concentrations ranged from 0.2 to $8.7 \mu\text{g L}^{-1}$. Sixty-six percent of all occurrences of MTBE detection occurred with BTEX compounds, with a proportionate increase in concentrations when these compounds occurred together. Eighty-three percent of all detections of MTBE were in samples collected during October through March of each year, which corresponds with the expected seasonal use of oxygenated gasoline (Delzer et al. 1996).

A study conducted by Reuter et al. (1998) sampled Donner Lake in California to determine, among other things, the relative contribution of motorized watercraft as a source of MTBE. Donner Lake is located in the Sierra Nevada Mountains and is classified as a multiple-use lake. MTBE measurements were made at 9 individual depths from surface to bottom on 16 dates. The study found that 86% of the change in MTBE concentration between the respective dates was explained by motorized watercraft use.

