Effects of Gasoline on Aerobic Methyl *tert*-Butyl Ether Biodegradation in Fluidized-Bed Bioreactors

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Abstract Since the1990 Clean Air Act, methyl *tert*-butyl ether (MTBE) has been used commonly as a gasoline oxygenate to increase fuel efficiency and lower vehicle emissions. It has become one of the most common pollutants of ground water and surface water. Because of its undesirable effects on drinking water and ecologically harmful effects, MTBE removal has become a public health and environmental concern. Pure MTBE has been found to be biodegradable in *ex-situ* treatments, but in pollution sites, it is found with gasoline. This study examines the effects of gasoline's presence on MTBE degradation. In this study, two up-flow fluidized-bed reactors were fed pure MTBE as a sole carbon source for 124 days, and then gasoline was added for 13 days. MTBE concentration was measured for influent and effluent samples of each reactor by gas chromatography. Degradation was determined by MTBE removal. MTBE was degraded to non-detectable levels without gasoline present. After gasoline was added, MTBE degradation was significantly impeded. Nine days after gasoline was no longer added, the reactors began degrading MTBE at pre-gasoline levels. Results suggest that treatment of MTBE contamination is possible under controlled environmental conditions.

Introduction

Methyl tert-butyl ether (MTBE, CAS no. 1634-04-4) was introduced in the 1970s to replace lead and other toxic fuel components that were used to increase engine efficiency. More recently, the 1990 Clean Air Act Amendments required the addition of fuel oxygenates in order to lower carbon monoxide emissions. Compared to the most common fuel oxygenates, which include ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME), benzene, toluene, ethyl-benzene, and xylene (BTEX), MTBE is the most cost-effective. As a result, MTBE is currently added to over 30% of gasoline sold in the U.S. (Steffan 1997). It makes up 11% of gasoline by volume. Oxygenates are potentially beneficial to the environment. Gasoline with MTBE has significantly helped reduce vehicle emissions and increase engine combustion efficiency (Deeb 2000).

Although gasoline oxygenates are used to lessen environmental damage and improve public health, they pose indirect human and environmental health risks. MTBE has become the second most common water pollutant in the US (Stringfellow 2001). Widespread gasoline spills and storage tank leaks have contaminated groundwater systems. These water sources supply 60% of American drinking water (Bradley 1999). The undesirable taste and odor of MTBE contaminated water is detectable by consumers at low concentrations. The US Environmental Protection Agency (EPA) has established a drinking water advisory level of 20-40μg/L (Fayolle 2001). Fuel oxygenates pose a particularly difficult environmental problem because of their high water solubility, allowing them to move quickly and easily through the water column and makes them difficult to remove. Once these contaminants move below the surface, they can have even longer life spans due to anaerobic conditions (Zogorsky 1999).

Gasoline oxygenate contamination is a cause for concern due to various human health risks. The long-term and short-term effects of human exposure to MTBE are still uncertain. Introduction of MTBE in various regions of the U.S. has coincided with health complaints including symptoms included headache, nasal, throat, or ocular irritation, nausea or vomiting, dizziness, and sensations of “spaciness” or disorientation (University of California Toxic Substances Research and Teaching Program 1998). However, a study by Prah et al. (1994) that exposed humans to MTBE at 1.39 ppm (typical exposure during vehicle refueling) for one hour showed that MTBE inhalation and dermal absorption had no immediate adverse effects on humans. Evidence remains anecdotal. MTBE is recognized as an animal carcinogen. The body metabolizes MTBE to another carcinogen, tert-butyl alcohol (TBA, CAS no. 75-65-0), which

remains in the body longer than MTBE (National Toxicology Program 1995). Studies of MTBE exposure in rats showed that incidences of tumors, lymphomas, and leukemia increased (International Agency for Research on Cancer 2000). One experiment tested the inhalation of MTBE by mice and rats. It increased the incidence of hepatocellular adenomas in female mice and that of renal tubular tumors in male rats at 8000 ppm (University of California Toxic Substances Research and Teaching Program 1998). Such studies have led the U.S. EPA to classify MTBE as a possible human carcinogen. The Material Safety Data Sheet (MDL 2000) lists MTBE as a moderate central nervous system toxin by ingestion and inhalation. Ecologically, MTBE is toxic to aquatic organisms only at high concentrations. 50% of fish exposed to MTBE at 1,000,000 μg/L for 96 hours died. At the same concentration, exposure was fatal to 50% of aquatic invertebrates.

Although MTBE was once considered recalcitrant, recent studies have shown that it is biodegradable by an assortment of aerobic microbial cultures (Deeb 2000, Salintro 1994, Wilson 2001). Both *in-situ* and *ex-situ* treatments have been explored. *In-situ* treatment consists of injection of an engineered MTBE-degrading strain into the contaminated site, then augmenting conditions to favor the desired strain (Salintro 2000). Subsurface *in-situ* treatments have been ineffective because degrading cultures are sensitive to a variety of unpredictable environmental conditions including nutrient and substrate availability and a lack of oxygen (Finneran 2001). Studies have shown that *ex-situ* treatment in fluidized-bed reactors (FBR) is effective in biological degradation of MTBE. In a laboratory-scale system, biofilm-bound microorganisms were been observed to convert 97% of influent MTBE to CO_2 (Fortin 1999). Kharoune et al. (1998) observed 99% removal efficiency in a laboratory scale fluidized-bed reactor. The FBR system uses a granular activated carbon (GAC) substrate for culture growth to simulate the attached microbial growth that is predominant in soil and groundwater (Kharoune 2001). This study uses fluidized-bed reactors because they accurately replicate field-scale reactors while providing controlled environments for monitoring degradation.

While numerous past studies have observed degradation of pure MTBE in controlled environments, recent studies have begun to examine the effects of other gasoline components on MTBE degradation. In contamination sites, other gasoline components, including toluene, iso-pentane, benzene, xylene, and ethyl-benzene, are likely to be encountered along with MTBE. Stringfellow and Oh (2001) examined the effects of gasoline hydrocarbons on MTBE

degradation in fluidized-bed reactors where toluene was found to be a strong inhibitor of degradation. The present study examines the effects of commercial gasoline in an MTBE-degrading fluidized-bed reactor. Based on previous observations, an impediment in MTBE degradation was expected after gasoline addition. The reactors were expected to degrade virtually all influent MTBE in the absence of gasoline. The objective of this study is to examine degradation of MTBE in an environment that simulates the chemicals present in contamination sites. Another objective is to shed light on future possibilities for larger-scale bioremediation.

Methods

Laboratory studies were performed over 174 days in two identical fluidized-bed reactors (reactors 1 and 2) at Lawrence Berkeley National Laboratory (LBNL). Each reactor was equipped with an influent flow control pump, an oxygen supply, and a reaction column (Figure 1). The columns held a volume of 1.6 liters each and contained granular activated carbon (GAC) packed to approximately 60 cm in height. One reactor had an average flow rate of approximately 4.5 liters per day and an MTBE loading rate of 114 mg/L/day. The other reactor was set at 4 liters per day and had a loading rate of 102 mg/L/day. The reactors operated at ambient pressure and temperature (average 28ºC).

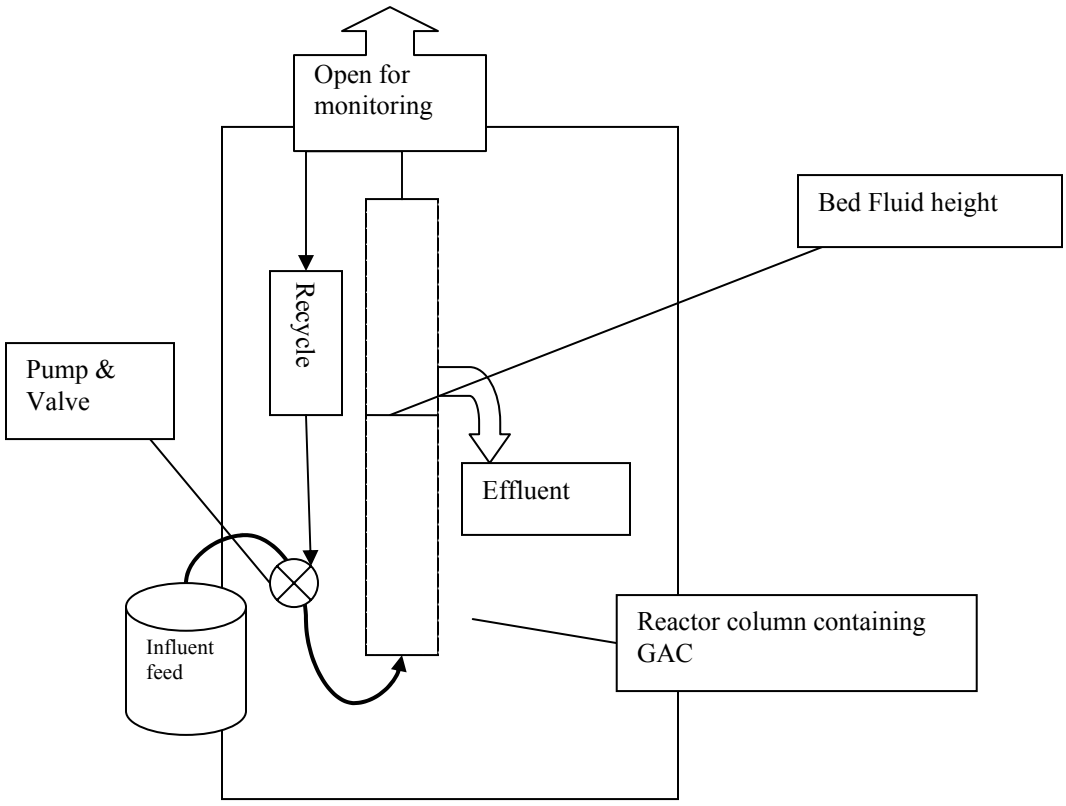


Figure 1: Laboratory Fluidized-bed reactor set-up

One reactor was inoculated with a 2-methylbutane (*iso*-pentane) degrading enrichment culture from a field-scale fluidized-bed reactor. The second reactor began degrading MTBE without any inoculation. It may have incorporated airborne microbes from the laboratory or from the other reactor. A biofilm formed on the GAC, which acted as a substrate for culture growth. The reactors were continuously fed a minimal salts media containing MTBE at 29.6 mg/L, prepared in industrial grade water. The media consisted of 40 mg/L of potassium phosphate monobasic (EM Science), 168 mg/L of potassium phosphate dibasic (JT Baker), and 20 mg/L of ammonium chloride (Sigma). The reactors were fed MTBE in solution for 123 days. MTBE-free gasoline was added to the media solution and fed to both reactors from day 124 of operation through day 137. Gasoline concentrations were 59 mg/L. On day 137, the influent feed was returned to the original MTBE solution for the rest of the experiment.

Samples of the bioreactor's effluent and influent were analyzed and compared for MTBE concentration 26 times throughout the study. Triplicate influent and effluent samples were analyzed twice per week by a Varian Star 3400 gas chromatograph equipped with a flame-

ionization detector. Samples were injected isothermally at 150°C. The column was 30m long and 250 microns in diameter. The carrier gas was helium. The lowest detection level was 0.1 mg/L.

Effluent and influent samples were preserved with 2 drops of 6 N HCl in 40 mL EPA certified VOA vials and analyzed within 2 weeks by gas chromatograph-solid phase micro extraction. These samples were taken from day 126 to 154 because this was the time period of gasoline's effects. A Varian 3400 gas chromatograph coupled with a Saturn 2000.40 solid phase micro-extractor (GC-SPME) analyzed headspace MTBE concentration in 0.5 mL samples. MTBE was detected with an isothermal injector at 240˚C and the detector at 225˚C. The GC column was set at 40˚C, 30m long and 250 microns in diameter, and used Helium as a carrier gas. The lowest detection level for the GC-SPME was 70 parts per billion. The column temperature increased 10.0˚C/minute with a hold time of 1.00 minute. Dissolved oxygen concentration, pH, GAC bed fluidization height, temperature, and flow rate of each reactor were also recorded twice per week.

Results

The addition of gasoline to the MTBE-degrading bioreactors impeded treatment efficiency after a lag time of 2 weeks. Data collected from day 0 through day 123 of treatment revealed that the reactors were an effective treatment for MTBE in water. During this period, a biofilm was established that grew on MTBE as a sole carbon source. As hypothesized, both reactors removed over 99% of influent MTBE. Figures 2 and 3 show results from gas chromatograph-flame ionization detector analysis. Percent removal was calculated by comparing effluent MTBE in parts per million with influent MTBE. Effective treatment continued until day 137, 14 days after gasoline was added, as shown by GC-FID data in figures 1 and 2. Then the percent of MTBE removed dropped to 68% in reactor 1 and 1% in reactor 2.

On day 146, treatment efficiency returned to its normal 99% removal, 9 days after the influent feed was returned to an MTBE solution without gasoline. Bed fluidization height, pH, dissolved oxygen concentration, and temperature remained constant. They were analyzed and showed no correlation to changes in MTBE removal.

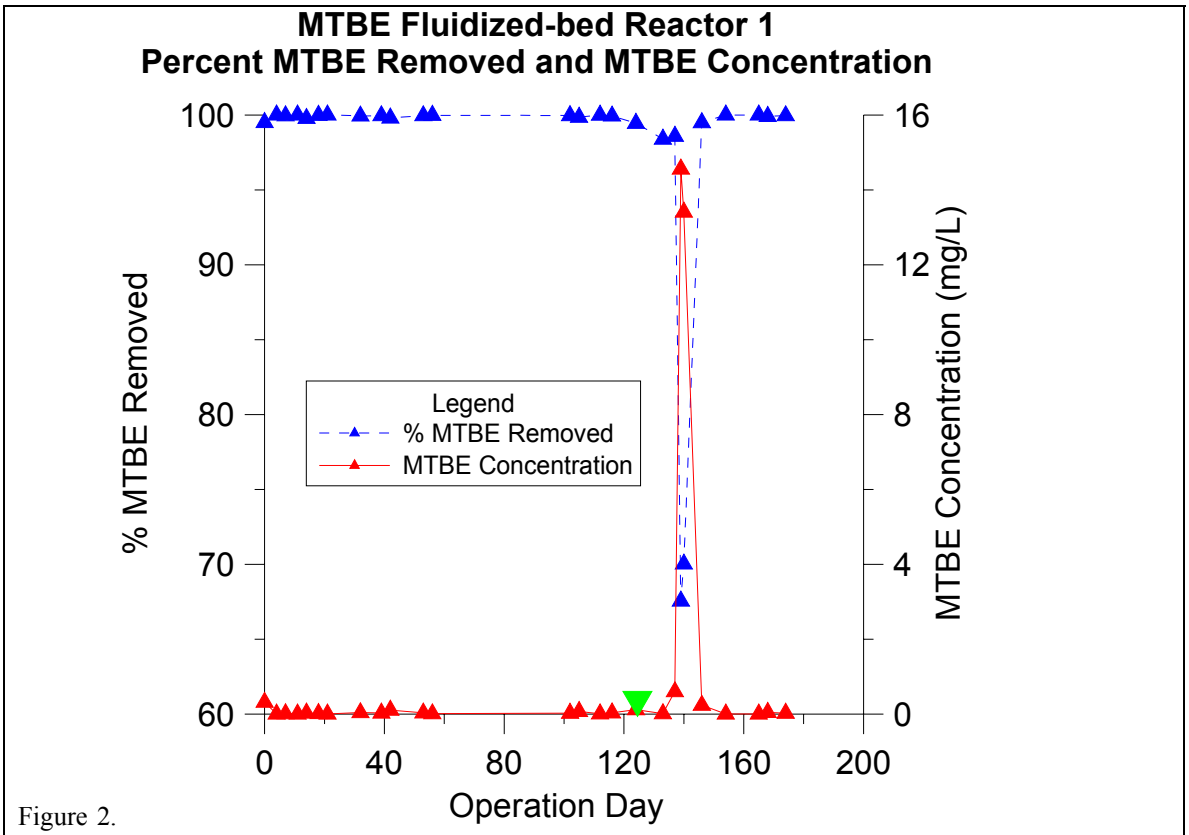
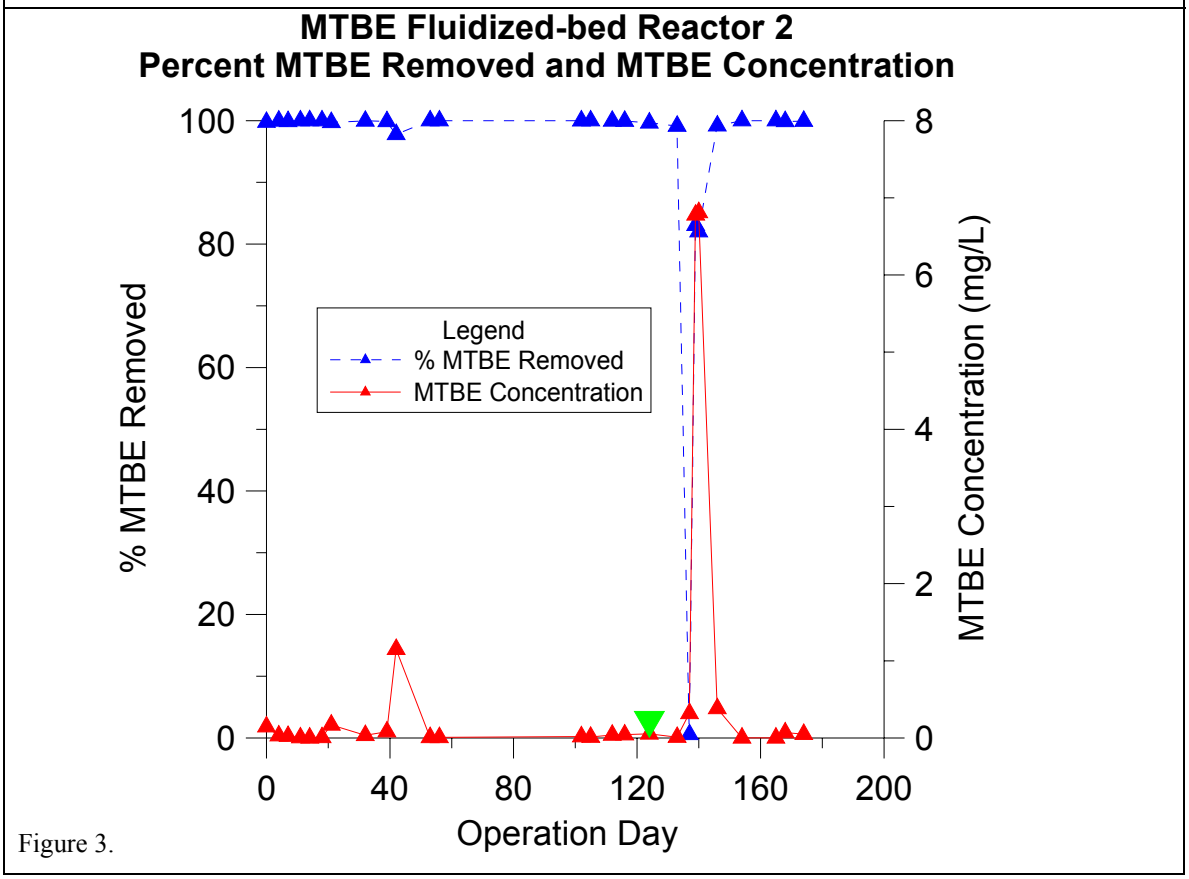


Figure 2.



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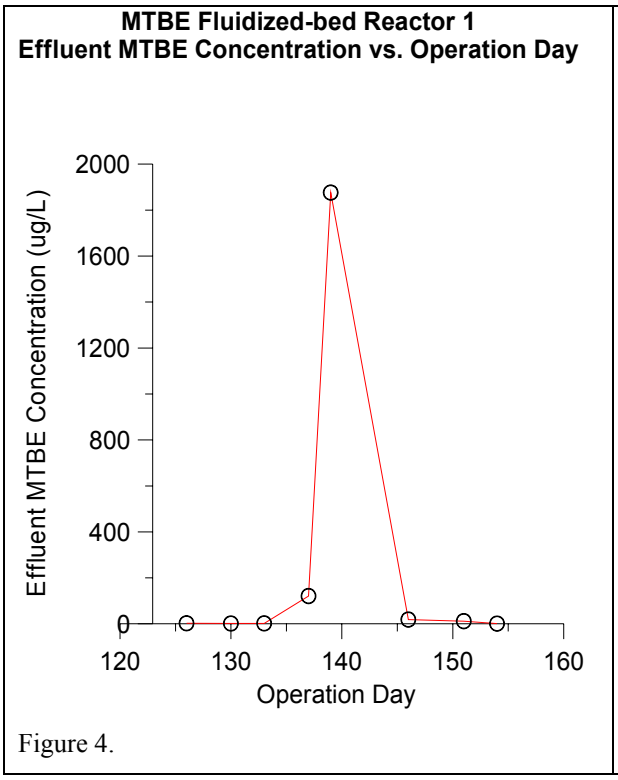

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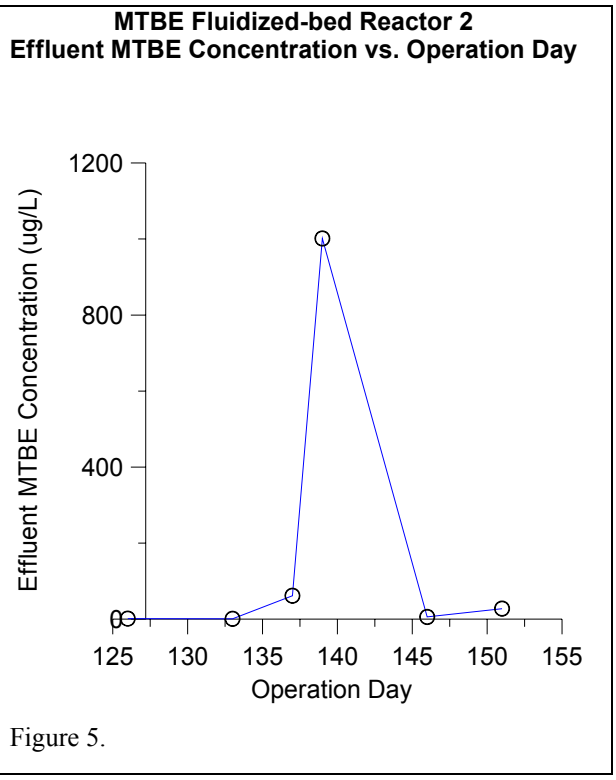

Figure 5.

Discussion

Results showed that treatment of MTBE in the fluidized bed reactors was successful in degrading MTBE to non-detectable levels. However, after gasoline was added, treatment efficiency was significantly lowered. There are several possible reasons that gasoline interfered with MTBE biodegradation. First, it is possible that the gasoline contained substances that were toxic to the microbial community that degraded MTBE in the first 123 days of the study. Toluene is suspected to be directly toxic to MTBE degrading microbes. Since MTBE degradation returned to pre-gasoline removal rate, a major death of the microbe community was unlikely. Also, GAC bed fluidization height stayed relatively constant throughout the study, suggesting the biofilm remained intact.

A plausible explanation lies in inhibitory compounds. MTBE degrading microbes may have degraded other gasoline hydrocarbons because they were more readily metabolized. 2-methylbutane (*iso*-pentane), which is present in gasoline, is a known co-metablolite of MTBE

biodegradation (Stringfellow 2001, Ju 2001). Microbes that rely on 2-methylbutane as a primary carbon source degrade MTBE as a side effect. Therefore, 2-mthylbutane is a competitive inhibitor of MTBE. Toluene has also been shown to inhibit MTBE degradation, but this mechanism is still unclear.

In addition, ecological relationships between various microbial communities may have affected MTBE biotreatment. The addition of gasoline to the reactor system served as a selective environmental factor for the microbial communities. In the reactor column, the MTBE-degrading microorganisms may have been out-competed by microbes that rely on other gasoline hydrocarbons as a carbon source. There was a limited GAC surface area that microbes competed for. The problems that gasoline posed for treatment of MTBE contaminated water in the reactors represent challenges in *in-situ* treatment attempts. In spill-site environments, different microbial strains in competition for nutrients and substrate for growth are ubiquitous.

After gasoline was added to the reactor's influent solution, data shows a 14-day period where no change in treatment efficiency occurred. This lag time is most likely due to properties of the granular activated carbon in the reactor columns. The GAC was able to adsorb influent gasoline for about 14 days until it reached a breakthrough capacity and gasoline was detectable in the effluent. While GAC is an ideal substrate for treatment and microbial growth in a reactor, future studies may use sand in the reactor column to reduce the delay in effluent MTBE detection. Sand does not absorb materials as well, so a lag time until breakthrough would not occur.

In conclusion, this study showed that *ex-situ* treatment of MTBE is possible if inhibiting compounds are removed. Future studies on the mechanisms of inhibiting compounds are necessary before effective field-scale treatments can occur. The challenge for future treatments of MTBE will be to find cost-effective solutions to prevent MTBE contamination of drinking water supplies while treating existing contaminations from leaking underground storage tanks and pipelines. Studies by the University of California's Toxic Substances Research and Teaching Program (1998) reports that there is no significant air quality benefit to the use of MTBE in reformulated gasoline, relative to alternative non-oxygenated formulations in new vehicles. While MTBE is likely to be phased out of use in various regions in the near future, its contamination of drinking water will remain a significant environmental issue.

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