Influence of gasoline hydrocarbons on methyl *tert*-butyl ether biotreatment in fluidized bed bioreactors

W.T. Stringfellow and K.-C. Oh

Center for Environmental Biotechnology, Lawrence Berkeley National Laboratory, MS 70A–3317, 1 Cyclotron Road, Berkeley, CA 94720, USA. (E-mail: *Wstringfellow@lbl.gov; KCOh@lbl.gov*)

Abstract Fluidized bed bioreactors (FBRs) are frequently used for the treatment of gasoline contaminated groundwater. Due to changes in gasoline formulations in the last decade, gasoline contaminated groundwater may also contain significant quantities of methyl *tert*-butyl ether (MTBE), a recalcitrant gasoline additive. It has been shown that MTBE is biodegradable and there is interest in determining if MTBE contaminated groundwater can be biologically treated using FBR technology. We examined the biodegradation of MTBE in FBRs treating contaminated groundwater and establish that there was an inverse correlation between total petroleum hydrocarbon (TPH) loading and MTBE treatment efficiency. Follow-up laboratory studies demonstrated that toluene, a component of TPH, is a strong inhibitor of MTBE biotreatment in FBRs.

Keywords Biodegradation; biological treatment; *iso*-pentane; methyl *tert*-butyl ether; MTBE

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Introduction

Methyl-tert butyl ether (MTBE) is a synthetic compound added to gasoline to improve combustion efficiency. Originally utilized in gasoline as an anti-knock compound, MTBE is now widely used in the United States (US) as a fuel-oxygenate to meet gasoline oxygen-content specifications established by provisions of the Clean Air Act Amendments of 1990. Under programs mandated by the United States Environmental Protection Agency (USEPA) to improve air quality, gasoline in some regions of the US can contain up to 15% MTBE by volume (U. S. Environmental Protection Agency, 1998). In oxygenated gasoline, MTBE can be the single most abundant ingredient, exceeding the weight percent of both toluene (8.1%) and *iso*-pentane (7.9%), the most abundant single hydrocarbons in standard gasoline (Potter and Simmons, 1998).

As a result of its use in gasoline, MTBE has now become the second most commonly detected groundwater pollutant in the US (Squillace *et al.*, 1999). The major source of MTBE contamination to groundwater is leaking underground storage tanks, but surface runoff and even rainwater can be sources of groundwater contamination in urban areas (Squillace *et al.*, 1996). Although MTBE is relatively non-toxic, it imparts an unpleasant taste and odor. The USEPA has issued a drinking water advisory of 25 to 35 μg/L based on studies of the taste and odor threshold for MTBE in water (U. S. Environmental Protection Agency, 1997). A few states have adopted regulatory limits of 35 μg/L or above for MTBE in treated effluents or drinking water, but many states have instituted stricter limits and California is proposing a limit that may be as low as 5 μg/L (Martin, 2001).

MTBE contamination of groundwater was not recognized as an emerging problem until the mid-1990s, when it was discovered that important drinking water supplies in California were contaminated with MTBE. Since that time, there has been a concerted effort to develop technology and methods to treat MTBE contaminated groundwater. Although it was originally thought that MTBE was not biodegradable, it has now been demonstrated that a variety of microorganisms can degrade MTBE (Deeb *et al.*, 2000), and there is new interest in applying biological treatment for MTBE contaminated groundwater

(Stocking *et al.*, 2000). In this paper we investigate how the presence of gasoline hydrocarbons can negatively influence the treatment of MTBE in aerobic biological treatment systems.

Methods

Field data was collected over a one-year period from a 930 litre fluidized-bed reactor (FBR) that was installed for the treatment of groundwater contaminated with gasoline. The reactor consisted of a 4.6 metre tall reaction tower, an influent flow control pump, a pump to fluidize the bed material with recycled reactor water, a biomass control system, and an oxygen contactor designed to maintain high oxygen concentrations without stripping volatile organic compounds. The tower was loaded with a granular activated carbon (GAC), upon which bacteria were grown as a biofilm. The recycle flow was fixed at a rate sufficient to maintain fluidization of the bed material. The oxygen delivery was on a feedback control to maintain an effluent concentration of 2.5 mg/L. For most of the study period, the influent flow to the FBR was set at 15 litres per minute (Lpm) and was limited to that rate by available ground water flow. The average hydraulic residence time in the reactor was 0.8 hours. The temperature of the reactor was dependent on the ambient air temperature, influent water temperature and flow, and heating that occurs from compressed oxygen injection and recycle flow. The average temperature of the reactor during this study was 22°C. In this study there was no attempt to control reactor temperature, except to avoid over-heating during periods when influent flow was halted.

Laboratory reactor studies were conducted in 1.6 litre fluidized-bed bioreactors designed to model the field operational reactor. GAC bed-material was collected from the field FBR and loaded into two identical laboratory bioreactors. The laboratory reactors were fed an influent of mineral media supplemented with approximately 10 mg/L MTBE and 23 mg/L of MTBE-free gasoline. Loading rates of the laboratory reactor were set to mimic loading rates observed in the field reactor, but flow rates were, of necessity, lower. The hydraulic residence time of the laboratory reactor averaged 7.2 hours. The reactor was operated at ambient temperature (average 29°C).

In the field study, influent and effluent water samples from the FBR were collected twice per week and sent to a contract laboratory for analysis (Alpha Analytical, Reno, NV). In the laboratory study, influent and effluent samples were collected three times per week and analyzed at Lawrence Berkeley National Laboratory (LBNL). Volatile organic compounds (including benzene, toluene, ethyl-benzene, xylenes, and MTBE) were measured by EPA Method 8260B. Extractable and purgable total petroleum hydrocarbons (TPH) were measured by EPA Method 8015B. TPH was calibrated to a gasoline standard.

Biofilm on the GAC bed material was measured using a protein assay at LBNL. For biofilm analysis, one tenth of a gram of GAC (wet weight) was placed in a 2.5 mL centrifuge tube, 200 μL of deionized water was added to each test tube, followed by 1.0 mL of Modified Lowery Protein Assay Reagent (Pierce, Rockford, IL) and, after 10 minutes, 100 μL of diluted (1:1) Folin-Ciocalteu Reagent. The reaction was allowed to go to completion (30 min) and the absorbance of each sample was measured at 750 nm versus the blank sample. Results were quantitated using bovine serum albumin and expressed as μg protein per gram dry weight GAC.

Loading rate was determined by calculating total daily loads (mg/L influent concentration times daily flow in litres) and dividing by either the reactor volume or the total protein in the reactor. Removal rate was calculated from the mg of each constituent removed per day (influent mg/L minus effluent mg/L, times flow) divided by the reactor volume. Volumetric loading and rate are expressed as mg of compound per litre reactor volume per day. Protein loading is expressed as mg of compound per mg of protein per day. For loading

based on protein, the protein concentration of the GAC was multiplied by the total GAC originally added to the reactor to estimate total protein in the reactor.

Results

Data was collected from a field FBR and examined to evaluate the ability of the FBR to treat gasoline hydrocarbons and MTBE. Specific removal rates of purgable total petroleum hydrocarbons (TPH) and MTBE as a function of specific loading rates for the bioreactor are shown in Figure 1 and 2, respectively. The bioreactor was very efficient at treating petroleum hydrocarbons (measured as TPH) and there was no indication that the loading capacity of the reactor has been reached. Benzene, toluene, ethyl-benzene, and o-, m- and p-xylenes (BTEX) were treated to non-detect levels (< 1 μg/L) during most of the period under investigation. Gasoline hydrocarbon removal is a linear function of hydrocarbon loading rate.

It is apparent from the results in Figure 2 that MTBE biodegradation is not following the classic pattern observed for the biodegradation of gasoline hydrocarbons seen in Figure 1. Loading and MTBE removal are more poorly related ($r^2 = 0.771$, $n = 61$) in comparison to the relationship observed with the gasoline hydrocarbons (e.g. TPH, $r^2 = 0.985$, $n = 50$). Treatment was not significantly correlated with MTBE loading on a per gram protein basis and treatment was better when the hydrocarbon/protein ratio is lower (data not shown), suggesting that treatment appears to be responding to hydrocarbon loading rather than MTBE loading.

In order to investigate the variables influencing the biodegradation of MTBE and the treatment efficiency of the bioreactor, we examined the field data for correlation between periods of poor MTBE treatment (expressed as percent removal) and a number of independent operational variables. We found that higher TPH loading was associated with poor reactor MTBE removal (Figure 3). Treatment was also negatively correlated with the toluene loading per gram protein (Figure 4), but not ethyl-benzene, benzene, or xylene loading. There was a significant correlation between TPH and toluene, so it was not possible, solely from field data analysis, to determine if toluene was the major inhibitor of MTBE treatment or if other gasoline hydrocarbons, such as *iso*-pentane, were also negatively affecting MTBE biotreatment.

To better determine the influence of individual gasoline hydrocarbons on MTBE treatment efficiency, we conducted a laboratory study using GAC collected from the field reactors. When the GAC was transferred from the field to the laboratory FBRs, MTBE treatment stabilized at a high treatment efficiency, suggesting that the increased hydraulic retention time of the laboratory reactor was beneficial to treatment.

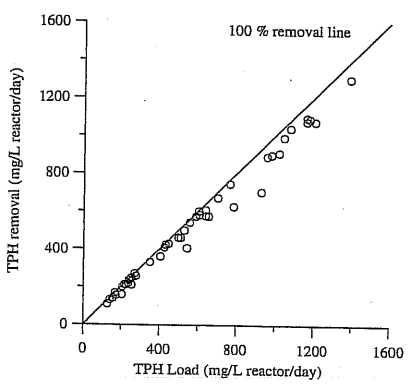

Figure 1 Total petroleum hydrocarbon (TPH) treatment in a fluidized bed bioreactor treating contaminated groundwater

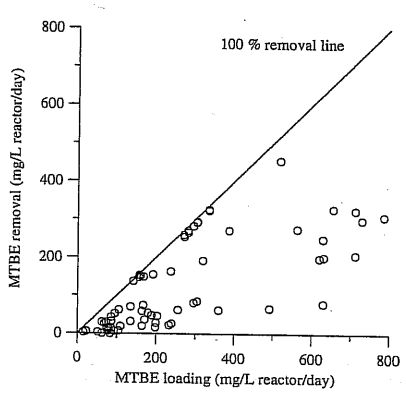

Figure 2 MTBE treatment in the same fluidized bed bioreactor treating contaminated groundwater

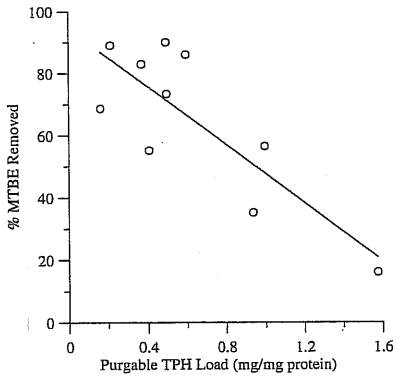

Figure 3 Increasing TPH loading is negatively correlated with MTBE biotreatment efficiency

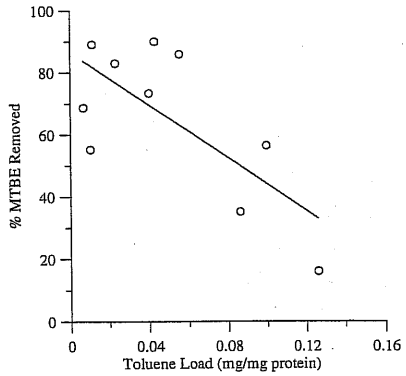

Figure 4 Increasing toluene loading is negatively correlated with MTBE biotreatment efficiency

Two experiments were carried out to determine if the two major components of gasoline, toluene and *iso*-pentane, when added individually could inhibit MTBE treatment efficiency. The first experiment involved the addition of *iso*-pentane to the laboratory reactor and the second experiment involved the addition of toluene. These compounds were added in addition to the normal gasoline loading fed to the reactors. Results from the test reactors were compared to a control reactor. Through the entire experiment, the control reactor maintained stable MTBE treatment (data not shown).

The addition of *iso*-pentane was used to increase the *iso*-pentane loading from approximately 5-g/litre-reactor-volume/day to 9-g/litre-reactor-volume/day. Breakthrough of *iso*-pentane was observed at the higher loading, but there was little apparent effect on MTBE treatment efficiency (Figure 5). The experiment was repeated using toluene with a much more dramatic effect. Toluene loading was increased from approximately 5-g/litre-reactor-volume/day to 9-g/litre-reactor-volume/day. Breakthrough of toluene was observed at the higher loading and increasing effluent toluene concentration had a proportional effect on MTBE treatment efficiency (Figure 6). This effect was not due to a reduction in the bulk oxygen concentration.

Discussion and conclusions

Statistical analysis of data collected from a 930-litre FBR treating gasoline contaminated groundwater indicated that gasoline hydrocarbons were interfering with the biotreatment

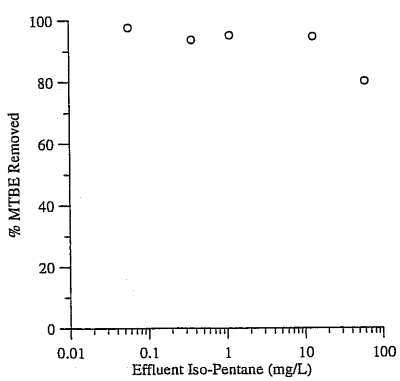

Figure 5 Break-through of *iso*-pentane in the effluent of the laboratory reactor had little effect on MTBE treatment efficiency

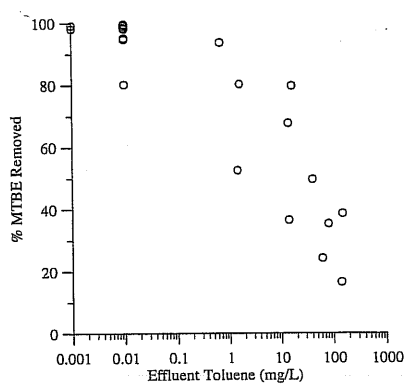

Figure 6 Break-through of toluene in the effluent of the laboratory reactor had an inhibitory effect on MTBE treatment efficiency

of MTBE. In the field, there was a strong correlation between individual gasoline components and total gasoline hydrocarbons (measured as TPH), so it was not possible to determine from the field study alone which individual hydrocarbons in gasoline were inhibiting MTBE treatment. A previous study had indicated that higher toluene loading (on a volumetric basis) was inhibiting MTBE treatment efficiency in a 17,600 litre FBR (Stringfellow *et al.*, 2000). However, in that study TPH was not measured, so it was not known if there was a correlation between toluene and other gasoline hydrocarbons in that system.

It was hypothesized that the most likely candidates as inhibitory compounds in aerobic biological treatment were toluene and *iso*-pentane, both of which are measured in the TPH analysis and occur in high concentrations in gasoline (Potter and Simmons, 1998). These compounds were identified as possible inhibitors for several reasons. The results of this field study, and of a previous study (Stringfellow *et al.*, 2000), demonstrated an inverse correlation between toluene loading and MTBE treatment efficiency. Kinetic experiments demonstrated that toluene was a non-specific inhibitor of MTBE biodegradation in biofilms (Stringfellow *et al.*, 2000). *Iso*-pentane is a co-metabolite for MTBE biodegradation (Stringfellow, 2001), and therefore will act as a competitive inhibitor for MTBE biodegradation (Stringfellow and Aitken, 1995).

Laboratory reactor studies demonstrated that toluene was a more potent inhibitor of MTBE treatment than *iso*-pentane. This was a somewhat surprising result, as *iso*-pentane is a known co-metabolite in this system and is therefore by definition a competitive inhibitor of MTBE metabolism (Stringfellow, 2001). The mechanism of toluene inhibition is not certain. It is possible that toluene is directly toxic to MTBE degrading microorganisms or that toluene is a competitive inhibitor for MTBE degrading enzymes. However, our current results, in combination with kinetic experiments reported previously (Stringfellow *et al.*, 2000), lead us to propose that inhibition occurs when toluene degraders outcompete MTBE degraders for oxygen in the biofilm. This hypothesis is currently under investigation.

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