

Aerobic biodegradation of gasoline oxygenates MTBE and TBA

G.J. Wilson^a, A.P. Richter^a, M.T. Suidan^{a*}, and A.D. Venosa^b

^aDepartment of Civil and Environmental Engineering, University of Cincinnati, Cincinnati, Ohio 45221, USA

^bU.S. Environmental Protection Agency National Risk Management Research Laboratory, Cincinnati, Ohio, 45268, USA

Abstract MTBE degradation was investigated using a continuously stirred tank reactor (CSTR) with biomass retention (porous pot reactor) operated under aerobic conditions. MTBE was fed to the reactor at an influent concentration of 150 mg/l (1.70 mmol/l). A second identical reactor was operated as a control under the same conditions with the addition of 2.66 g/l of sodium azide, to kill any biological activity. Results from these experiments suggest that biomass retention is critical to the degradation of MTBE. The rate of MTBE removal was shown to be related to the VSS concentration. MTBE removal exceeded 99.99% when the VSS concentration in the reactor was over 600 mg/l. Results obtained from batch experiments conducted on mixed liquor samples from the porous pot reactor indicate that the individual rates of biodegradation of MTBE and TBA were higher for initial concentrations of 15 mg/l than for concentrations of 5 mg/l. The presence of TBA at lower concentrations did not effect the rate of MTBE degradation, however higher concentrations of TBA did reduce the rate of biodegradation of MTBE. Denaturing Gradient Gel Electrophoresis (DGGE) analysis reveals that the culture consisted of a community of bacterial organisms of about 6 species.

Keywords MTBE, TBA, oxygenate, gasoline, aerobic biodegradation

Introduction

When gasoline from underground storage tanks (USTs) is spilled into the environment, it represents a potential source of groundwater contamination. The aromatic compounds of most concern are the monoaromatic hydrocarbons benzene, ethylbenzene, toluene, and xylenes (BTEX), which are the most soluble and mobile of the gasoline constituents. Since the late 1970s, oxygenates such as alcohols and ethers have been added to gasoline to replace lead and to boost the octane rating. In 1990, the Clean Air Act Amendments required the use of oxygenates in gasoline to reduce vehicle emissions from the combusted fuel. Thus, in addition to the potential exposure of individuals to the toxic monoaromatic hydrocarbons from gasoline spillage, further exposure to these oxygenates compounds the problem.

An oxygenate that has grown substantially as the additive of choice in gasoline is methyl-*t*-butyl ether (MTBE). MTBE (as well as the other oxygenates) are polar compounds and thus have higher water solubilities than most gasoline hydrocarbons. For that reason, they can be anticipated to occur in higher concentrations than the BTEXs contaminating groundwater from UST spills. Polar compounds adsorb poorly to soil organic matter, adding to the mobility of oxygenates in a spreading plume. In addition, MTBE has the potential to decrease the sorptive retardation of BTEXs via cosolvent effects, which would further enhance the mobility of BTEXs in groundwater. Finally, the presence of high concentrations of MTBE could reduce the biodegradability of BTEXs if MTBE is preferentially attacked by the degrading microbial populations present in groundwater or if it is toxic to the degraders.

Previously, researchers have studied MTBE biodegradation under both anaerobic and aerobic conditions (Steffan *et al.*, 1997; Salinitro *et al.*, 1994; Suflita and Mormile, 1993;

Mormile *et al.*, 1994; Hardison *et al.*, 1997; Yeh and Novak, 1994 and 1995). Generally, the findings have shown that MTBE degrades either slowly or not at all, although some differences have been noted. Thus, current knowledge is controversial due in part to the fact that perhaps insufficient time has been allotted by some workers for microbial enrichment to occur before studies were initiated.

Experimental section

Experimental procedures

Two chemostat reactors were used in this study. Each chemostat was constructed of 304 stainless steel with an internal diameter of 21.6 cm and a height of 30.5 cm. Each reactor was jacketed for temperature control and the temperature maintained at 20°C. A porous pot (Atlas Minerals & Chemicals, Mertztown, PA) was placed inside each chemostat for biomass retention and control. The porous pot was constructed as a cylinder using 0.48 cm thick filter grade porous polyethylene. The cylinder was 19.1 cm in internal diameter and 29.2 cm in overall height. It was welded to a base plate 21.6 cm in diameter. To prevent floating, each pot was secured in place within the chemostat using a 19.7 cm internal diameter 304 stainless steel ring. The reactor contents were maintained completely mixed using magnetically coupled variable speed mixers (Autoclave Engineers, Erie, PA).

The feed to the reactors was prepared in two separate glass reservoirs. One of the reservoirs contained a solution of sodium hydroxide prepared in deionized distilled water. The nutrient solution had the salts and vitamins needed to support biological growth and was prepared in deionized distilled water. Flow of the two solutions was delivered to the reactor via 0.64 ID 316 stainless steel tubing metered with constant speed (2 rpm) Masterflex pumps (Cole Palmer, Chicago, Ill.). Power to the feed pumps was channeled through electronic timers (Lindberg Enterprises Inc., San Diego, Calif.). These on/off timers were used to adjust the flows and obtain the desired hydraulic residence time. The total flow rate delivered to each chemostat was 2.37 litres per day. The buffer solution represented 0.8 of the total flow or 1.90 litres per day while the remaining flow was primarily from the nutrient solution. MTBE was introduced into the buffer feed line in neat form using a Model 11 high precision syringe infusion pump (Harvard Apparatus, Inc., South Natick, MA) with a 5.0-mL fixed needle syringe (Hamilton Co., Reno, NV).

The sludge age was initially maintained at 30 days while the hydraulic residence time was controlled at 4.2 days. The pH of both reactors was maintained between 7.5 and 8.2 with the temperature controlled at 20°C (68°F). The temperature control system consisted of a chiller pumping a propylene glycol/water mixture to a reservoir. The mixture was pumped from the reservoir to the chemostat via a recirculation pump.

The biologically active reactor was originally seeded with a combination of 2 litres of mixed liquor from the Cincinnati Metropolitan Sewer District, 0.6 litres of MTBE acclimated mixed liquor provided by J. Salinitro, Shell Oil Corp., Houston, Texas, and 0.14 litres of water collected from washing MTBE contaminated aquifer material from Port Hueneme, California.

The effluent from the CSTR served as the background solution for the batch experiments ensuring sufficient nutrients and buffering capacity. Before MTBE and/or TBA addition, the background solution was measured for pH, dissolved oxygen (DO), total carbon (TC), inorganic carbon (IC), and MTBE and TBA concentrations. After the MTBE and/or TBA were added to a stock solution and sufficient mixing had taken place, the pH, DO, TC, IC, and MTBE and TBA concentrations were measured again. The solution was split in two with the one part used for the biologically active experiment and the second part used for the controls. Mercuric chloride and sodium azide were added to the control solution to inhibit any biological activity at concentrations of 2.72 g/l (10 mmol/l) and 2.66 g/l

(40 mmol/l), respectively. Each batch experiment consisted of 27 – 160 ml serum bottles. Seven sampling events were conducted in triplicate for the biologically active test while the controls required three events in duplicate. Each bottle received 90 ml of MTBE and/or TBA solution and 10 ml of biomass from the CSTR. After the addition of biomass, each bottle was sealed using a butyl rubber stopper and an aluminium seal. All bottles were placed in a tumbler and withdrawn when analyzed. All analysis occurred immediately after sample withdrawal.

Materials and analyses

Daily monitoring of the chemostat reactor included buffer and nutrient flow rates, syringe flow rates, and pH. The pH was measured using an Orion Model 720A pH meter (Orion Research Co., Boston, MA). For the two reactors, weekly samples were analyzed for aqueous effluent concentration of MTBE and its degradation products, gas phase concentrations of MTBE and its daughter products, chemical oxygen demand (COD), TC, and IC. For the batch experiments, samples were analyzed for the aqueous and gas phase concentrations of MTBE and biodegradation products, the gas phase was also analyzed for CO₂ and O₂, while the aqueous phase was monitored for pH, TC, IC, and DO. All samples were filtered using 0.45- μ m MAGNA nylon membrane filters (Micron Separations, Inc., Westboro, Massachusetts). The concentrations of MTBE and its daughter products were analyzed on a Hewlett Packard 5890 Series II gas chromatograph (GC) (Hewlett Packard, Palo Alto, CA) using a flame ionization detector (FID) with 60/80 Carbowax B/5% Carbowax 20 M glass column (Supleco, Bellefonte, PA). Further analysis was conducted on a Model ALS 2016 purge and trap (Telmar, Cincinnati, OH) followed by a Hewlett Packard 5890 Series II GC using a flame ionization detector (FID) with PTA-5 column (Supleco, Bellefonte, PA). CODs were determined using the Hach low range digestion vials (0–150 mg/L) and a Hach COD reactor Model 45600. The digested vials were measured for transmittance on a Hach 2000 spectrophotometer (Hach Co., Loveland, CO). The TC and IC of the samples was measured using a Shimadzu TOC-5000 Analyzer (Shimadzu, Kyoto, Japan). The DO was measured with a DO meter (Corning, New York).

The following chemicals were used: methyl *tert*-butyl ether (MTBE, M_w =88.15, 99%, Aldrich, Milwaukee, WI), *tert*-butyl alcohol (TBA, M_w =74.12, 99%, Aldrich, Milwaukee, WI), diethyl ether (M_w =74.12, 99%, Fisher), diisopropyl ether (M_w =102.18, 99%, Aldrich, Milwaukee, WI), and ethanol (95 proof, Midwest Grain Products).

Results and discussion

Biodegradation of MTBE in the porous pot reactors

To demonstrate that MTBE was not lost to aeration and mixing, a control chemostat was operated exactly as the chemostat treating MTBE as the only carbon source. Air was delivered to the biotic system to maintain a dissolved oxygen level above 3 mg/l at all times. The control reactor was aerated using the same air flow rate. Sodium azide was added to the buffer solution to prevent any biological activity. In Figure 1, MTBE was recovered in the effluent at a concentration similar to the influent concentration indicating no significant loss in the gas phase. Gas samples of the chemostat's head space further support the finding that MTBE was not lost due to stripping. Results from Figure 1 also show TBA was only present at the beginning of the experiment when active biomass was added to the reactor but quickly fell below the detection limit after increasing the sodium azide to a final concentration of 2.66 g/l. Since that increase, TBA did not appear in the effluent above the detection limit.

Results from the biologically active porous pot reactor indicate that biomass retention was critical to MTBE biodegradation. Initially, a 30 day sludge age was maintained.

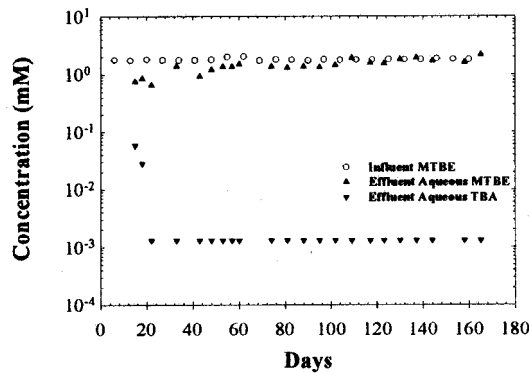


Figure 1 Control porous pot reactor

However, the VSS concentration fell from higher than 560 mg/l to 240 mg/l in the first 112 days of operation. While the influent MTBE concentration remained constant at 150 mg/l (1.70 mmol/l), the effluent MTBE concentration erratically fluctuated between 0.004 and 0.4 mmol/l indicating unstable operation as seen in Figure 2. TBA was also detected in the effluent at the beginning of the study but fell below the detection limit by the 60th day of operation. After the 112th day of operation, biomass was removed only for VSS analysis, giving the reactor time to recover. The MTBE concentration quickly fell below the detection limit for GC analysis (0.1 mg/l), requiring further measurement by a purge and trap/GC. The effluent MTBE concentration continued to fall from 0.07 mmol/l to below 1×10^{-4} mmol/l. The VSS concentration increased to 900 mg/l by the 196th day of operation. After that time, biomass was withdrawn, on average, at a rate of 52 ml/day for kinetic experiments. This resulted in a reduction in the maintenance of a volatile suspended solids concentration in the reactor of 900 to 1100 mg/l. The MTBE concentration remained below 1×10^{-4} mmol/l, rising only once to 1×10^{-3} mmol/l due to a fluctuation in pH above 8.6. TBA remained below the detection limit of 0.1 mg/l rising slightly above the detection limit only during the period of pH fluctuation.

Figure 3 shows the relationship between the VSS concentration in the chemostat and the effluent MTBE concentration. The MTBE removal efficiency was approximately 90% at VSS concentrations between 150 and 400 mg/l. However, when the biomass concentration increased to greater than 600 mg/l, MTBE removal improved to an excess of 99.99%. Only once did the MTBE effluent concentration increase to 0.1 mg/l, while the VSS concentration remained above 600 mg/l. As stated earlier, this was attributed to a fluctuation in pH. The VSS concentration remained below 1100 mg/l due to biomass removal for sampling.

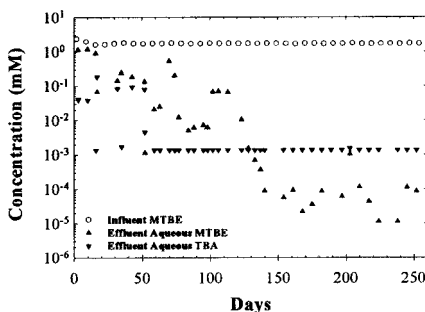


Figure 2 MTBE biodegrading porous pot reactor

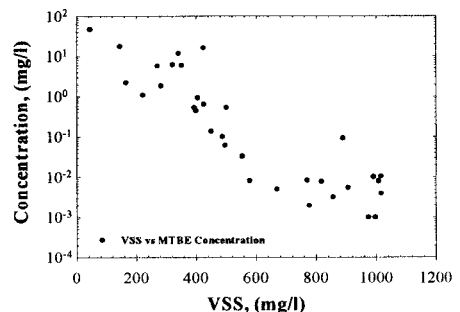


Figure 3 Effluent MTBE concentration as a function of biomass concentration

Results from the head space analysis confirm insignificant loss of MTBE due to aeration and mixing. Results from COD and TC analysis indicate the absence of MTBE and its intermediates when the VSS concentration remained above 600 mg/l (data not presented). Often, COD measurements remained at or slightly above detection limits while TC results showed that virtually all the carbon present in the effluent was in the form of inorganic carbon.

Retaining biomass is a critical factor when designing an MTBE treatment process. Systems with high levels of biomass control will have a better chance of success than systems which do not possess such control. As seen in this study, MTBE removal can approach the standards now being considered for drinking water levels.

MTBE and TBA biodegradation rate experiment

Experiments with only MTBE Addition. The overall pattern on biodegradation was similar between the 15 mg/l and 5 mg/l spike experiments. For the sake of brevity, only the results from the 15 mg/l batch study are included. The data shown in Figure 4 represent the time varying total MTBE and TBA concentration. Analysis showed that gas phase MTBE concentrations were approximately 2% of the liquid phase concentration which is consistent with estimates derived using Henry's Law. The gas phase concentration of TBA was negligible. With an initial MTBE concentration of 15 mg/l (0.17 mmol/l), MTBE was not degraded in the samples poisoned with mercuric chloride and sodium azide and its concentration remained constant. However, in the biologically active samples, MTBE was degraded completely within 23 hours. TBA was detected as an intermediate but did not appear as an equimolar conversion of MTBE, rising to its highest concentration of 0.032 mmol/l midway through the experiment.

Figure 5 presents a carbon balance on the experiment when MTBE is the only carbon source. Total carbon in the system represents the total carbon in the aqueous phase as well as any CO_2 in the gas phase. Inorganic carbon represents the inorganic carbon measured in the aqueous phase and the CO_2 in the gas phase. The difference between the total carbon and the inorganic carbon represents the dissolved organic carbon (DOC) in the aqueous phase. The aqueous phase carbon represents the carbon mass equivalent of the measured MTBE and/or TBA concentration. As seen in Figure 5, total carbon remained relatively constant throughout the experiment. Measurements of the background solution revealed inorganic carbon comprises virtually all of the total carbon in the aqueous phase. After MTBE was added to the solution, analysis showed that the DOC mass was mainly composed of MTBE. As the experiment progressed, inorganic carbon in the aqueous and gas phases increased. The resulting decrease in DOC concentration was paralleled by a decrease in the equivalent carbon mass of the measured MTBE and TBA in the aqueous phase.

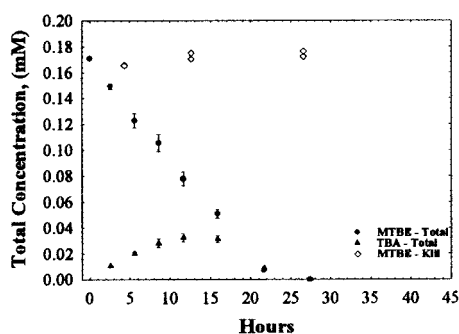


Figure 4 Total concentration during the MTBE spiked batch study

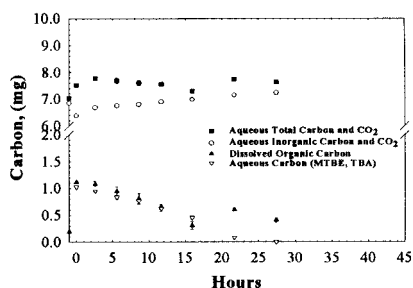


Figure 5 Carbon Balance for the MTBE spiked batch study

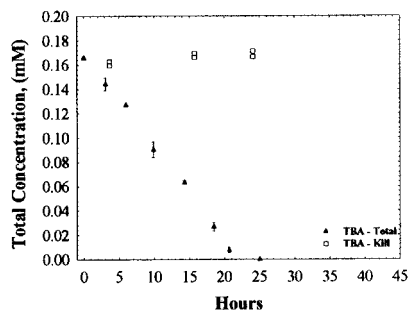


Figure 6 Total concentration during the TBA spiked batch study

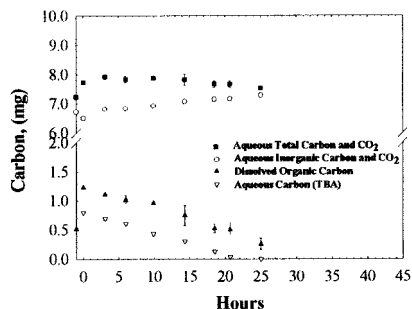


Figure 7 Carbon balance for the TBA spiked batch study

Experiments with only TBA Addition. Figure 6 shows results obtained from the degradation of TBA using a culture grown only on MTBE. TBA was added at an initial concentration of 12.5 mg/l (0.169 mmol/l) and was recovered from the killed samples at similar concentrations. In the biologically active samples, TBA was below the GC detection limit after 22 hours. The TBA concentration in the gas phase was insignificant compared with its concentration in the aqueous phase.

The total mass of carbon remained constant during this experiment as seen in Figure 7. Initially, the inorganic carbon (6.7 mg) comprises the majority of the total carbon (7.2 mg) in the background solution, leaving the DOC mass in the solution at 0.5 mg of carbon. After TBA addition, the DOC mass in the solution increased to 1.23 mg of carbon, a difference of 0.83 mg. The mass of carbon attributed to the measured TBA concentration was calculated to be 0.80 mg. Therefore, the increase in the mass of carbon was due solely to the TBA addition. Figure 8 reveals that as the inorganic carbon increases over time, the DOC decreases. The carbon mass equivalent of the TBA also decreases over time at a similar rate indicating the decrease in DOC was due to the mineralization of TBA. No evidence of undetected intermediates appeared since all of the carbon was accounted for during the experiment.

Experiments with addition of MTBE and TBA. MTBE and TBA were added together at concentrations of 11.1 mg/l (0.126 mmol/l) and 12.7 mg/l (0.171), respectively. Figure 8 shows MTBE was degraded significantly prior to the biodegradation of TBA. The data show no additional TBA conversion due to MTBE degradation. The rate of TBA degradation increased significantly once MTBE was completely degraded. Data from the poisoned bottles shows no MTBE or TBA loss. Results from the 5 mg/l of MTBE and TBA addition experiment (data not presented) showed a slight increase in TBA due to MTBE degradation, but not an equimolar conversion. In both cases, MTBE was degraded significantly before TBA degradation commenced. Complete mineralization of MTBE and TBA occurred after 36 hours.

Figure 9 shows no loss of total carbon in the system during the experiment. As in the previous two experiments presented, the total carbon (7.11 mg) consisted initially of inorganic carbon (6.66 mg). With the addition of MTBE and TBA, the DOC mass increased to 1.83 mg of carbon. The equivalent carbon conversion of the MTBE and TBA equalled 1.57 mg of carbon indicating the DOC was in the form of MTBE and TBA. Similar to what was observed from the other experiments, the DOC and the equivalent carbon conversion of MTBE and TBA decreased at an equal rate suggesting no accumulation of undetected intermediates. By the end of the experiment, the DOC mass was similar to that of the initial background solution.

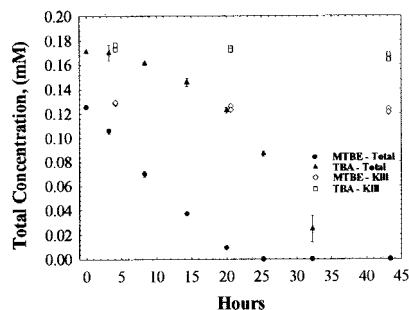


Figure 8 Total concentration during the MTBE and TBA spiked batch study

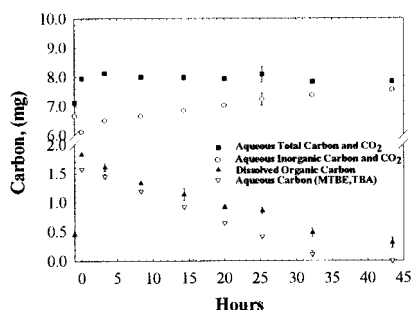


Figure 9 Carbon balance for the MTBE and TBA spiked batch study

Comparison of rate data

The rates of biodegradation of MTBE and TBA for the six experiments were normalized for the VSS concentration and are presented in Table 1.

Table 1 Experimental rate comparison

	MTBE 5 mg/l	TBA 5 mg/l	MTBE and TBA 5 mg/l	MTBE 5 mg/l	MTBE 15 mg/l	MTBE and TBA 15 mg/l 15 mg/l
MTBE (mmol / min / mg-VSS)	1.17×10^{-6}	na	1.13×10^{-6}	1.88×10^{-6}	na	1.18×10^{-6}
TBA (mmol / min / mg-VSS)	na	1.16×10^{-6}	1.00×10^{-6}	na	1.52×10^{-6}	1.61×10^{-6}

na: not available

For the MTBE and TBA combination experiment, the rates of TBA biodegradation were obtained after MTBE was completely transformed. Results from these experiments show that the rates of biodegradation for the higher concentrations of MTBE and TBA were higher than those observed for the lower concentrations. The data also suggest that the MTBE biodegradation rate was higher than that observed for TBA, especially for the higher concentrations. The presence of TBA at a lower concentration did not affect the rate of MTBE degradation, however at higher concentrations, TBA slowed the rate of MTBE degradation from 1.88×10^{-6} mmol/min/mg-VSS to 1.18×10^{-6} mmol/min/mg-VSS.

Since it is well documented that TBA is an intermediate of MTBE degradation, a relationship between the change in the total sum of MTBE and TBA was established in Figure 10 for the higher concentrations. Figure 11 establishes the same relationship for the lower

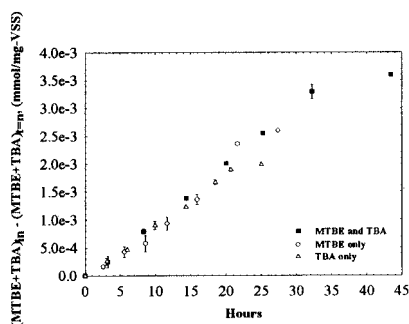


Figure 10 Overall TBA biodegradation rate for higher concentration studies

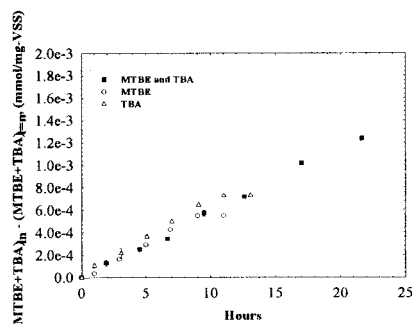


Figure 11 Overall TBA biodegradation rate for lower concentration studies

concentrations of MTBE and TBA. All data in these figures are normalized to the VSS concentration in each experiment. The data suggest similar removal rates for the TBA regardless of whether only TBA was spiked into the bottles, or whether it was introduced in combination with MTBE or only as MTBE. The overall rate of TBA biodegradation was higher for the higher spike concentrations.

Conclusions

MTBE was biodegraded at an efficiency that exceeded 99.99% in a porous pot reactor when the VSS concentration in that reactor was higher than 600 mg/l. COD and TC analysis confirmed the absence of MTBE and its intermediates with the effluent carbon virtually all in the inorganic form. Results show the individual rates for the higher concentrations of MTBE and TBA were higher than for the lower concentrations. The presence of TBA at a lower concentration did not affect the rate of MTBE degradation, however at higher concentrations, TBA slowed the rate of MTBE degradation. Carbon balances performed on the data from the rate experiments show an increase in inorganic carbon throughout the study while DOC decreased at rates similar to the carbon mass equivalent of MTBE and TBA. The overall rate of TBA biodegradation was higher for the higher spike concentrations.

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