

Biodegradation Kinetics of MTBE in Laboratory Batch and Continuous Flow Reactors

G. J. Wilson¹; A. Pruden²; M. T. Suidan³; and A. D. Venosa⁴

Abstract: Methyl *tert*-butyl ether (MTBE) biodegradation was investigated using a continuously stirred tank reactor 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 mM). An identical reactor was operated as a killed control under the same conditions. Operation of these reactors demonstrated that removal of MTBE was biological and suggests that biomass retention is critical for effective degradation. MTBE removal exceeded 99.99% when the volatile suspended solids concentration in the reactor was above 600 mg/L. Batch experiments conducted using mixed liquor from the porous pot reactor indicated that the individual rates of biodegradation of MTBE and *tert*-butyl alcohol (TBA) increase with increasing initial concentration. When batch tests were later repeated, the MTBE degradation rates were found to have increased while the TBA degradation rates remained constant. All batch tests confirmed that the degradation rate of TBA governed the overall degradation rate (degradation rate of both MTBE and TBA). The presence of TBA at lower concentrations did not affect the rate of MTBE degradation; however, higher concentrations of TBA did reduce the rate of MTBE biodegradation.

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Introduction

When gasoline from underground storage tanks (USTs) is spilled into the environment, it presents a potential source of groundwater contamination. Historically, the compounds of most concern are the monoaromatic hydrocarbons: benzene, toluene, ethylbenzene, 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 boost the octane rating. In 1990, the Clean Air Act Amendments required the use of oxygenates in gasoline in order to reduce vehicle emissions from the combusted fuel. These fuel oxygenates have now possibly become an even greater threat to groundwater supplies.

The oxygenate that has grown substantially as the additive of choice in gasoline is methyl-*tert*-butyl ether (MTBE). Methyl-*tert*-butyl ether is a polar compound and thus has a higher-water solubility than most gasoline hydrocarbons and can be anticipated to have more widespread contamination in groundwater than

BTEX when originating from UST spills. Polar compounds adsorb poorly to soil organic matter, adding to the mobility of MTBE in a spreading plume. In addition, MTBE has the potential to decrease the sorptive retardation of BTEX via cosolvent effects, which would further enhance the mobility of BTEX in groundwater. Finally, the presence of high concentrations of MTBE could reduce the biodegradation of BTEX if MTBE is preferentially attacked by the degrading microbial populations present in groundwater or if it is toxic to the microbial populations.

Previously, researchers have studied MTBE biodegradation under both aerobic and anaerobic conditions (Suflita and Mormile 1993; Mormile et al. 1994; Salanitro et al. 1994; Yeh and Novak 1994, 1995; Hardison et al. 1997; Steffan et al. 1997). Generally, the findings have shown that MTBE degrades either slowly or not at all. Current knowledge may be inconclusive due to insufficient time for microbial enrichment prior to initiation of studies. There is reasonable evidence that a high-biomass concentration is necessary for effective degradation of MTBE (Salanitro et al. 1994), and this may be a major factor in the conflicting results of previous studies. In addition, a significant number of studies report the necessity of a cosubstrate in order for MTBE biodegradation to proceed. Degradation of MTBE without a cosubstrate would be more desirable in terms of bioremediation cost.

Still another issue that remains unresolved is the role of *tert*-butyl alcohol (TBA) in MTBE biodegradation. TBA is a prominent MTBE biodegradation intermediate that itself is often applied as a fuel oxygenate. Because of potentially dangerous health effects of TBA, it is important that MTBE be completely mineralized, and not simply converted to TBA. It has not been determined whether there is a microbial preference for TBA or MTBE.

This paper describes the operation of a bench-scale biomass retaining reactor receiving MTBE as the sole carbon substrate. Batch studies applying the mixed liquor from this reactor provide insight of MTBE and TBA degradation kinetics.

¹Graduate Research Assistant, Dept. of Civil and Environmental Engineering, Univ. of Cincinnati, Cincinnati, OH 45221-0071.

²Graduate Research Assistant, Dept. of Civil and Environmental Engineering, Univ. of Cincinnati, Cincinnati, OH 45221-0071.

³Professor, Environmental Engineering, Dept. of Civil and Environmental Engineering, 741 Baldwin Hall (ML 0071), Cincinnati, OH 45221-0071 (corresponding author). E-mail: makram.suidan@uc.edu

⁴Program Manager, U.S. EPA Oil Spill Research Program, U.S. EPA National Risk Management Research Laboratory, Cincinnati, OH 45268.

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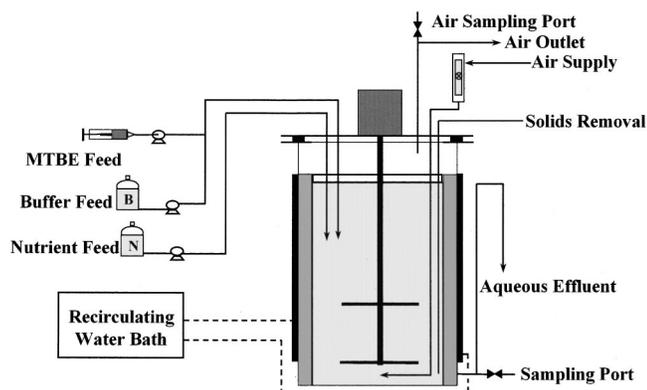


Fig. 1. Schematic of methyl *tert*-butyl ether (MTBE) porous pot reactor

Experiment

Two chemostats were constructed and operated under similar conditions, except one was the control reactor (abiotic) and one was a biological reactor. In order to demonstrate that MTBE was not lost by volatilization due to reactor operation (i.e., aeration and mixing), a sterile control porous pot reactor was operated exactly as the biologically active reactor. Both reactors were fed MTBE at a concentration of 1.7 mM (150 mg/L). Air was delivered to the biotic system to maintain a dissolved oxygen concentration above 3 mg/L at all times. The control reactor was aerated using the same airflow rate. Each reactor was constructed of 304 stainless steel with an internal diameter (i.d.) of 21.6 cm and a height of 30.5 cm as shown in Fig. 1. The reactors were jacketed for temperature control and the temperature maintained at 20°C. The temperature control system consisted of a thermostatically controlled chiller with a recirculating propylene glycol/water mixture. A porous liner (Atlas Minerals & Chemicals, Mertztown, Pa.) was placed inside each chemostat for biomass retention and control. The porous liner 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. The buffer solution contained sodium hydroxide prepared in deionized distilled water and, in the case of the control reactor, sodium azide. 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 cm i.d. 316 stainless steel tubing metered with constant speed (2 rpm) Masterflex pumps (Cole Palmer, Chicago). Power to the feed pumps was channeled through electronic timers (Lindberg Enterprises Inc., San Diego). These on/off timers were used to adjust the flows and obtain the desired hydraulic residence time of 4.2 days. The total flow rate delivered to each chemostat was 2.37 L per day. The buffer solution represented 80% of the total flow or 1.90 L per day while the remaining flow was provided by 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, Mass.) with a 5.0-mL fixed needle syringe (Hamilton Co., Reno,

Nev.). The sludge age was initially maintained at 20 days. The hydrogen ion concentration (pH) of both reactors was maintained between 7.5 and 8.2.

The biologically active reactor was originally seeded with a combination of 2 L of mixed liquor from the Cincinnati Metropolitan Sewer District, 2 L of backwash water from a biofilter treating diethyl ether, 0.6 L of MTBE acclimated mixed liquor provided by J. Salanitro, Equilon Enterprises LLC, Houston, Tex. and 0.14 L of water collected from washing MTBE contaminated aquifer material from Port Hueneme, California.

The effluent from the biologically active 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. Results from this analysis indicated low concentrations of MTBE and TBA. 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. MTBE and/or TBA were added at target concentrations of either 5, 15, or 43 mg/L. 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 at concentrations of 2.72 g/L (10 mM) and 2.66 g/L (40 mM), respectively, to inhibit any biological activity. Each batch experiment consisted of 27–160 mL serum bottles (Wheaton, Millville, N.J.). Seven sampling events were conducted in triplicate for the biologically active test while the controls were analyzed at three events in duplicate. Each bottle received 90 mL of MTBE and/or TBA solution and 10 mL of biomass from the porous pot reactor. After the addition of biomass, each bottle was sealed using a butyl rubber stopper and an aluminum seal (Wheaton, Millville, N.J.). All bottles were placed on a 20 rpm tumbler and withdrawn when analyzed. All analyses 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). 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, Mass.). The concentrations of MTBE and its daughter products were analyzed on a Hewlett Packard 5890 Series II gas chromatograph (GC) (Hewlett Packard, Palo Alto, Calif.) using a flame ionization detector (FID) with a 60/80 Carbowax B/5% Carbowax 20 M glass column (Supelco, Bellefonte, Pa.). Further analysis was conducted on a Model ALS 2016 purge and trap (Tekmar, Cincinnati, Ohio) followed by a Hewlett Packard 6890 Plus GC using a FID with a DB-1 column (J & W Scientific, Folsom, Calif.). COD was 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

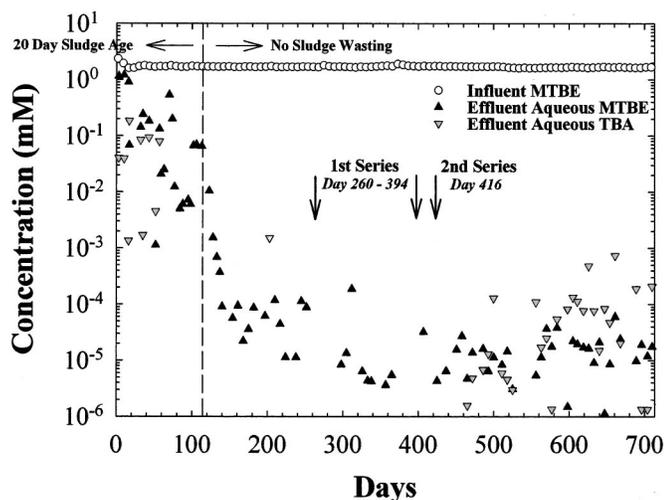


Fig. 2. MTBE porous pot influent and effluent data

transmittance on a Hach 2000 spectrophotometer (Hach Co., Loveland, Colo.). The TC and IC of the samples were measured using a Shimadzu TOC-5000 Analyzer (Shimadzu, Kyoto, Japan). The DO was measured with a DO meter (Corning, N.Y.).

The following chemicals were used: MTBE ($M_w = 88.15$, 99%, Aldrich, Milwaukee), TBA ($M_w = 74.12$, 99%, Aldrich, Milwaukee), diethyl ether ($M_w = 74.12$, 99%, Fisher), diisopropyl ether ($M_w = 102.18$, 99%, Aldrich, Milwaukee), and ethanol (95%, Midwest Grain Products).

Results and Discussion

Biodegradation of Methyl *tert*-Butyl Ether in Porous Pot Reactor

For the control reactor, MTBE was recovered in the effluent at a concentration similar to the influent concentration indicating no significant volatilization losses in the gas phase in the control reactor (data not presented). Gas samples of the head space further support the finding that MTBE was not lost due to stripping in the abiotic reactor.

In contrast to the control reactor, the biologically active reactor demonstrated significant removal of MTBE. Methyl *tert*-butyl ether removal by the biologically active reactor is shown in Fig. 2. Results from the operation of this reactor confirm previous reports that biomass retention is critical to MTBE biodegradation. Prior to day 114, a 20-day sludge age was maintained. During this time period, the influent MTBE concentration remained constant at 150 mg/L (1.70 mM), while the effluent MTBE concentration erratically fluctuated between 0.004 and 0.4 mM indicating unstable operation. TBA was also detected in the effluent at the beginning of the study but fell below the direct GC analysis detection limit of 0.0013 mM (0.1 mg/L) by the 60th day of operation. The volatile suspended solids (VSS) concentration fell from more than 560 to 240 mg/L during the first 114 days of operation. After the 114th day of operation, biomass was removed only for VSS analysis, which increased the sludge age. The MTBE concentration quickly fell below the detection limit for direct GC analysis (0.0011 mM or 0.1 mg/L), requiring further measurement by a purge and trap/GC. The effluent MTBE concentration continued to fall from 0.07 mM to below 1×10^{-4} mM. 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 batch kinetic experiments. This resulted in a reduction in the VSS concentration in the reactor of approximately 100 mg/L. The MTBE concentration remained below 1×10^{-4} mM, rising only once to 1×10^{-3} mM due to a pH excursion above 8.6. When sludge wasting was stopped, TBA remained below the detection limit rising slightly above the detection limit only during the period of pH fluctuation. After day 465, a newly developed in-house purge and trap GC method lowered the detection limits for MTBE and TBA to 1.35×10^{-6} mM (0.1 $\mu\text{g/L}$). During this time period, effluent MTBE concentrations never exceeded 7×10^{-5} mM, but effluent TBA concentrations fluctuated between 2×10^{-6} and 8×10^{-4} mM. These fluctuations may be attributed to occasional biomass removal from the porous pot reactor to seed other reactors for different experiments and the biodegradation of TBA as a rate-limiting step in the biodegradation of MTBE. Prior to day 644, VSS concentrations steadily increased to 2,500 mg/L, but fell to 1,000 mg/L after biomass was removed for batch testing. While MTBE degradation was not impaired, an order of magnitude transient increase in effluent TBA concentration was noted. Results from the head space analysis confirm insignificant loss of MTBE due to aeration and mixing (data not shown) and agreed with the control reactor. Results from COD and TC analyses indicate the absence of MTBE and its intermediates when the VSS concentration remained above 600 mg/L (data not shown). 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. The pH was maintained between 7.5 and 8.2 throughout the experiment, except during the reactor upset period when the pH surged to 8.6. Results from the biologically active reactor provide compelling evidence that MTBE is being mineralized, in the absence of any direct cosubstrate addition.

Methyl *tert*-Butyl Ether and *tert*-Butyl Alcohol Batch Rate Experiment

Batch experiments were conducted for three MTBE or TBA concentration ranges of 5, 15, and 43 mg/L. Each experiment consisted of either MTBE, TBA, or a combination of MTBE and TBA spiked-serum bottles sealed with a butyl rubber stopper and crimped with an aluminum seal. Each biotic experiment consisted of seven triplicate sampling events, while the abiotic controls were only sampled in duplicate at three sampling events. Each serum bottle was seeded with biomass withdrawn from the porous pot reactor during the time period shown in Fig. 2 between days 260 and 394. Carbon balance, pH profile, oxygen uptake, and MTBE and TBA mass balances were established during this first set of batch studies. On day 416 the entire experiment was repeated for all three concentrations of MTBE, TBA, and combination MTBE and TBA. This was done in order to confirm previous results and to determine whether significant changes occurred in the culture with time.

Experiments with Only Methyl *tert*-Butyl Ether Addition

The overall biodegradation pattern for MTBE biodegradation was similar among the three MTBE concentrations studied. For the sake of brevity, only the results from the 43 mg/L batch study are presented in Fig. 3. The data shown represent the time varying total MTBE and TBA concentration normalized to the VSS concentration from the 43 mg/L MTBE spiked study. In all cases,

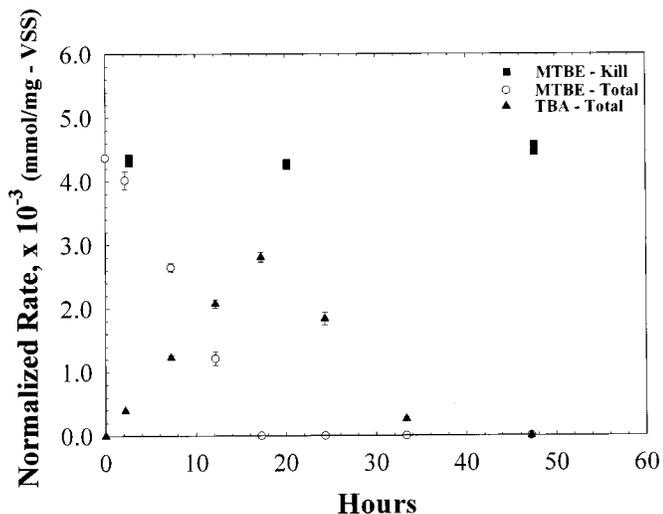


Fig. 3. Batch experiment using 43 mg/L methyl *tert*-butyl ether (MTBE) as sole carbon source

MTBE degradation proceeded with an intermediate buildup of TBA. MTBE degraded to completion, followed by the disappearance of the intermediate TBA. 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 (data not shown). The gas phase concentration of TBA was negligible. With an initial MTBE concentration of 43 mg/L (0.49 mM), MTBE was not degraded in the samples poisoned with mercuric chloride and sodium azide. However, in the biologically active samples, MTBE was degraded completely within 47 h. TBA was detected as an intermediate but did not appear as an equimolar conversion of MTBE, rising to its highest concentration of 2.8×10^{-3} mmol/mg-VSS midway through the experiment.

Fig. 4 presents a carbon balance from the experiment with MTBE as the only carbon source. The carbon balance of the batch studies provided key evidence that the MTBE and TBA were being mineralized by this culture. Total carbon in the system is defined as the total carbon in the aqueous phase as well as any

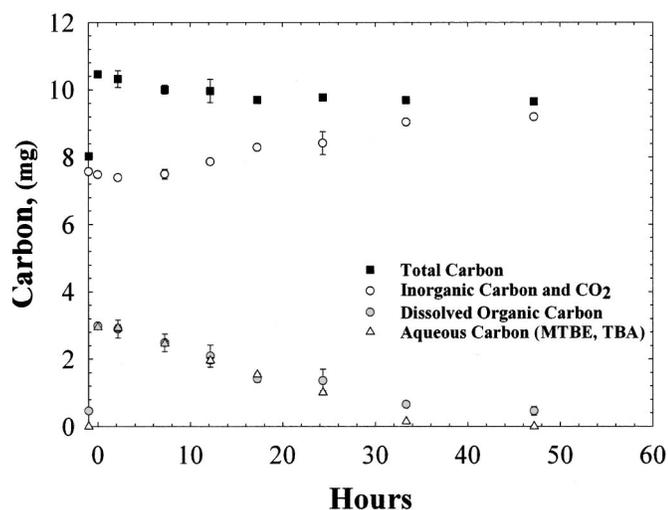


Fig. 4. Carbon balance for the batch experiment fed 43 mg/L methyl *tert*-butyl ether (MTBE) as sole carbon source

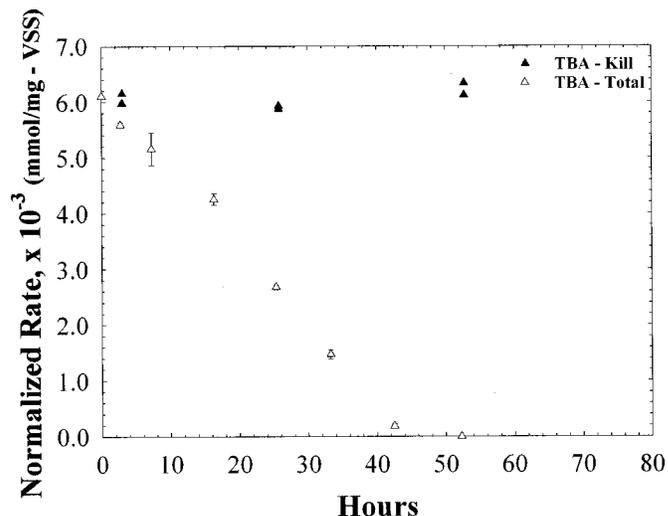


Fig. 5. Batch experiment using 48 mg/L *tert*-butyl alcohol (TBA) as sole carbon source

CO₂ in the gas phase. The inorganic carbon includes the inorganic carbon measured in the aqueous phase and the CO₂ 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 Fig. 4, 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. By the end of the experiment, DOC and equivalent carbon mass of the measured MTBE and TBA in the aqueous phase returned to levels observed before MTBE addition, indicating complete mineralization of MTBE.

Experiments with Only *tert*-Butyl Alcohol Addition

Fig. 5 shows results obtained from the degradation of TBA using the MTBE grown culture. *tert*-butyl alcohol was added at an initial concentration of 48 mg/L (0.65 mM) and was recovered from the killed samples at similar concentrations. In the biologically active samples, TBA was below the GC detection limit after 52 h. The TBA concentration in the gas phase was insignificant compared to its concentration in the aqueous phase. These experiments clearly indicated that the culture degraded TBA at a slower rate than MTBE.

Experiments with Addition of Methyl *tert*-Butyl Ether and *tert*-Butyl Alcohol

Methyl *tert*-butyl ether and TBA were added together at concentrations of 43 mg/L (0.49 mM) and 46 mg/L (0.62 mM), respectively. Fig. 6 shows MTBE was degraded significantly prior to the biodegradation of TBA. The data also showed 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 and 15 mg/L of MTBE and TBA addition

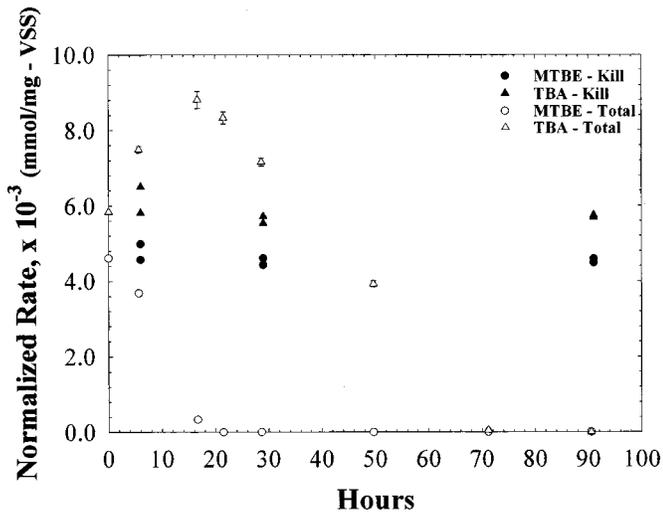


Fig. 6. Batch experiment using 43 mg/L methyl *tert*-butyl ether (MTBE) and 46 mg/L *tert*-butyl alcohol (TBA) as carbon source

experiments (data not presented) also showed a slight increase in TBA due MTBE degradation, but not an equimolar conversion. In all cases, MTBE was degraded significantly before TBA degradation commenced. Complete mineralization of MTBE and TBA occurred after 71 h.

Comparison of Rate Data with Experimental Repeat

A comparison of the rates for the batch experiments involving either MTBE or TBA spiked bottles is presented in Fig. 7. Experiments using the lower concentrations of MTBE or TBA were conducted first followed by the higher-concentration experiments. Biomass was removed from the porous pot reactors between days 260 and 394 (first series) to seed the batch serum bottle. Results from these experiments showed 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 demonstrate that the MTBE biodegradation rate was higher than that observed for TBA, especially at the higher concentrations. When the experiment was repeated on day 416 (second series) using the

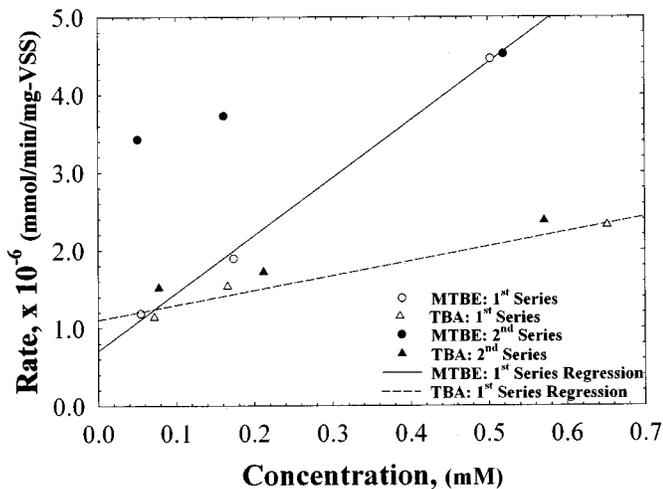


Fig. 7. Comparison of rates for batch experiments involving methyl *tert*-butyl ether (MTBE) and *tert*-butyl alcohol (TBA) between days 260 and 416

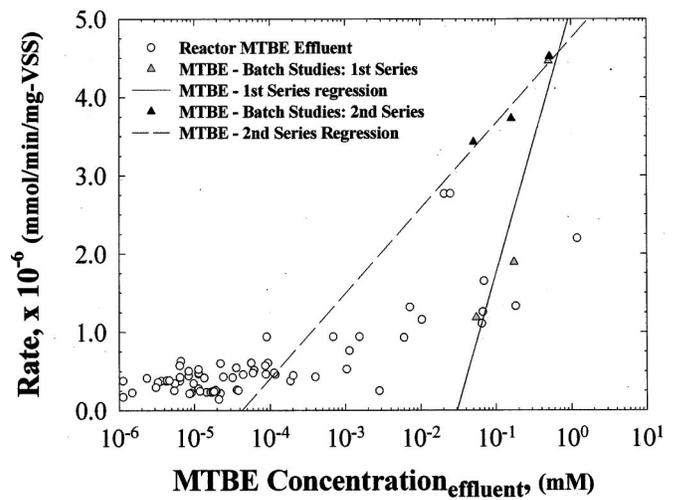


Fig. 8. Porous pot reactor and batch experiment methyl *tert*-butyl ether (MTBE) rate comparison

same biomass simultaneously for all experiments, the overall rate, governed by the TBA degradation rate, remained unchanged. However, the MTBE degradation rate increased, especially at the lower-MTBE concentrations, suggesting an enhanced MTBE degrading culture. Recently published results (Pruden et al. 2001) confirm with molecular analysis that the microbial composition of the culture was indeed changing during the time period that these studies were conducted.

MTBE degradation rates obtained from batch studies were compared to the MTBE degradation rate from the porous pot reactor. Results are shown in Fig. 8. MTBE reactor effluent results show data clustered at MTBE effluent concentrations below 1×10^{-4} mM and 5×10^{-7} m mol/min/mg-VSS where the majority of reactor operation occurred. Data obtained at higher-MTBE effluent concentrations occurred mainly at the beginning of the reactor operation when conditions were still unstable. Biodegradation rates calculated at the 5 (0.056 mM) and 15 mg/L (0.17 mM) MTBE concentrations from the first series of batch studies fall within the data range calculated from the reactor. However, the study rate of 4.6×10^{-6} m mol/min/mg-VSS at the 45 mg/L (0.51 mM) MTBE concentration is twice as high as the rates calculated from the first series of lower MTBE concentration batch experiments. When the MTBE batch experiment was repeated, the degradation rates increased suggesting a change in the reactor culture which improved the MTBE degrading capability. Batch studies conducted with 0.056 mM (5 mg/L) MTBE showed an increased rate from 1.2×10^{-6} to 3.4×10^{-6} m mol/min/mg-VSS, while the rate from the 0.168 mM (15 mg/L) MTBE batch test increased from 1.9×10^{-6} to 3.7×10^{-6} m mol/min/mg-VSS. These data suggest an underperformance in the porous pot reactor relative to the batch studies. However, this kind of comparison between the continuous flow and the batch studies may not be justified. In the continuous flow study, MTBE is being degraded essentially at the same rate that it is being fed to the reactor, but because the reactor design is a CSTR, the culture is actually operating at an extremely low concentration, in the range of a few micrograms per liter. The batch studies exposed the cultures to concentrations orders of magnitude higher than those ever encountered in the continuous flow reactor.

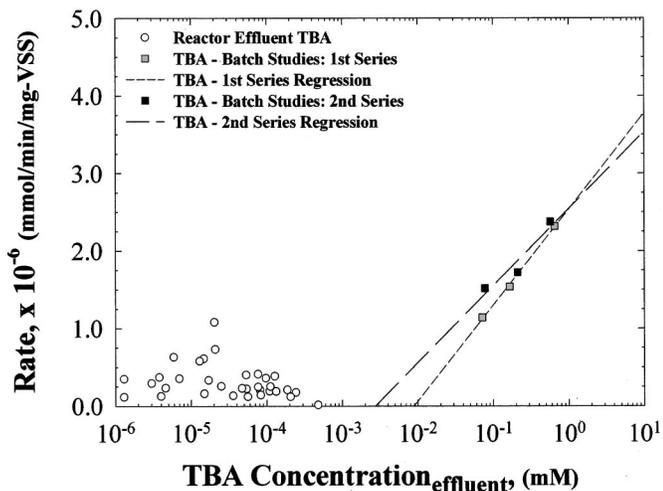


Fig. 9. Porous pot reactor and batch experiment *tert*-butyl alcohol (TBA) rate comparisons

Similar comparisons were made between the TBA degradation rate in the porous pot reactor and the rates obtained from the batch studies shown in Fig. 9. Due to the higher-TBA detection limit early on in the study, the TBA data set is smaller than the MTBE data set, and TBA occurred at concentrations lower than 5×10^{-4} mM with rates never exceeding 1×10^{-6} m mol/min/mg-VSS. The TBA degradation rates calculated from the batch studies were generated from higher-TBA concentrations and yielded increasing rates with increasing initial concentration. No relationship could be developed between the porous pot reactor rates and the rates calculated from the batch experiments due to a lack of data points at higher-TBA concentrations. Little difference was noted between the first and second series of experiments, indicating that, in contrast to the MTBE degrading populations, the microbial populations degrading TBA did not change significantly between batch studies. This may also be evidence that the TBA degradation rate is “fixed” in comparison to the MTBE degradation rate. Since TBA degradation rates were lower than the MTBE degradation rates, the degradation of TBA was determined to be the rate-limiting step. *tert*-butyl alcohol degradation rates ranged linearly from 1.5×10^{-6} to 2.4×10^{-6} m mol/min/mg-VSS for TBA concentrations of 5.8 mg/L (0.078 mM) to 42 mg/L (0.57 mM). The rate-limiting phenomenon of TBA observed in this study may prove to be a significant finding for MTBE bioremediation. If TBA is indeed the rate-limiting compound, and not MTBE, then that would suggest that future bioremediation efforts should focus on the enhancement of TBA degradation.

Conclusions

This paper provides evidence that MTBE and TBA can be mineralized in an aerobic continuous flow biomass retaining reactor with MTBE as the sole carbon substrate. In addition, it details the biodegradation kinetics of MTBE and TBA in batch studies, implying that TBA is the rate-limiting step of MTBE degradation, at least with this culture. MTBE was biodegraded at an efficiency that exceeded 99.99% in a porous pot reactor when the volatile

suspended solids concentration was higher than 600 mg/L. COD and TC analysis confirmed the absence of MTBE and its intermediates, with virtually all of the effluent carbon in the inorganic form. Carbon balances performed on the data from batch studies also supports the conclusion that MTBE and TBA are being mineralized, with an increase in inorganic carbon observed throughout the studies with DOC decreasing at rates similar to the carbon mass equivalent of MTBE and TBA. Results from batch rate studies demonstrated that the individual rates of degradation of MTBE and TBA increased with increasing initial concentration of these compounds. When the batch test was repeated, the MTBE degradation rates increased at lower initial MTBE concentrations suggesting a change in the reactor culture. However, no change in the TBA degradation rate was noted when the TBA batch studies were repeated. 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. Results of this study suggest that enhancement of TBA degradation may be a key factor in improving the mineralization of MTBE in future bioremediation strategies.

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