Effect of BTEX on Degradation of MTBE and TBA by Mixed Bacterial Consortium

Marie A. Sedran[1]; Amy Pruden[2]; Gregory J. Wilson[3]; Makram T. Suidan[4]; and Albert D. Venosa[5]

Abstract: Methyl *tert*-butyl ether (MTBE) contamination in groundwater often coexists with benzene, toluene, ethylbenzene, and xylene (BTEX) near the source of the plume. Tertiary butyl alcohol (TBA) is a prevalent intermediate of MTBE degradation. Therefore, there is a significant potential for interference of MTBE and TBA degradation by the presence of BTEX whether treatment is in situ or ex situ. In this study, the effect of BTEX on the degradation of MTBE and TBA was examined using a mixed bacterial culture enriched on MTBE and BTEX. In batch studies, the presence of BTEX did not have a significant effect on MTBE degradation, but did have a slight effect on TBA degradation. Under continuous flow conditions, all compounds degraded simultaneously. Normalizing rates to the MTBE loading to the reactor indicates that BTEX may assist in the development of the biomass for TBA and overall MTBE degradation. Using denaturing gradient gel electrophoresis, several diverse organisms were identified, two of which showed very high similarity with PM1, a known MTBE degrader.

DOI: 10.1061/(ASCE)0733-9372(2002)128:9(830)

CE Database keywords: Biodegradation; Ground water; Water treatment; Gasoline.

Introduction

Methyl *tert*-butyl ether (MTBE) is a gasoline additive used to reduce knocking and boost octane levels. Upon implementation of the 1990 Clean Air Act, production and use of MTBE boomed. Ten years later, the United States Environmental Protection Agency (U.S. EPA) recommended that the use of MTBE begin to be phased out. MTBE is extremely water soluble, and leakage from underground fuel storage tanks has contaminated the drinking water of several communities throughout the U.S. Nevertheless, MTBE is still the most commonly used fuel additive and is present in 85% of all reformulated fuels (U.S. EPA 1999).

Biological remediation of MTBE contaminated aquifers can be approached both in situ and ex situ. While in situ treatments are generally preferred in terms of cost and less intrusion on the site, ex situ treatment strategies allow for faster remediation under more controlled conditions. In particular, hydrogen ion concentration (pH), dissolved oxygen (DO), and high concentrations of biomass have been found to be key factors for efficient MTBE biodegradation (Salanitro et al. 1994; Wilson et al. 2000; Wilson et al. 2002).

Near the source of contamination, MTBE usually exists commingled with benzene, toluene, ethylbenzene, and xylene (BTEX). The presence of BTEX in a gasoline-contaminated aquifer can pose a challenge to both in situ and ex situ bioremediation of the site. Because the migration rate of BTEX is much slower than that of MTBE, it is especially problematic near the source of the plume. For in situ treatment, BTEX has been observed to be a problem because it adds to the chemical oxygen demand (COD) already imposed by the MTBE. If native microorganisms show preference to BTEX degradation, then insufficient dissolved oxygen (DO) will remain for degradation of the MTBE. Documentation of anaerobic mineralization of MTBE is sparse, and many studies have observed only partial decomposition of MTBE under anaerobic conditions (Mormile et al. 1994; Yeh and Novak 1994). Even more problematic, one study with pure cultures has shown BTEX to be metabolically inhibitive to MTBE biodegradation (Deeb et al. 2001). Other studies have also shown MTBE degradation to be inhibited by alternative substrates (Yeh and Novak 1994; Mo et al. 1997). This poses a problem to ex situ treatment as well. Although contaminated water can be pumped to the surface and adequately aerated, BTEX may still directly inhibit MTBE degradation.

The effect of BTEX on the degradation of *tert*-butyl alcohol (TBA), a major degradation intermediate of MTBE, is also an important factor for study and consideration in the design of MTBE remediation techniques. Successful bioremediation strategies must be able to achieve complete mineralization of MTBE in the presence of BTEX, not simply convert it to TBA, another potentially dangerous compound.

This study addresses the effect of BTEX on the degradation of MTBE and TBA in batch and continuous-feed modes. Degradation rates are compared with those of a recent study (Wilson et al. 2002) using a culture enriched with MTBE as the sole carbon

[1]Dept. of Civil and Environmental Engineering, ML 0071, Univ. of Cincinnati, Cincinnati, OH 45221-0071.

[2]Dept. of Civil and Environmental Engineering, ML 0071, Univ. of Cincinnati, Cincinnati, OH 45221-0071.

[3]Dept. of Civil and Environmental Engineering, ML 0071, Univ. of Cincinnati, Cincinnati, OH 45221-0071.

[4]Dept. of Civil and Environmental Engineering, ML 0071, Univ. of Cincinnati, Cincinnati, OH 45221-0071. E-mail: makram.suidan@uc.edu

[5]U.S. Environmental Protection Agency National Risk Management Research Laboratory, 26 W. Martin Luther King Drive, Cincinnati, OH 45268.

Note. Associate Editor: George A. Sorial. Discussion open until February 1, 2003. Separate discussions must be submitted for individual papers. To extend the closing date by one month, a written request must be filed with the ASCE Managing Editor. The manuscript for this paper was submitted for review and possible publication on February 13, 2002; approved on March 19, 2002. This paper is part of the ***Journal of Environmental Engineering***, Vol. 128, No. 9, September 1, 2002. ©ASCE, ISSN 0733-9372/2002/9-830–835/$8.00+$.50 per page.

substrate. For further insight, the culture was profiled and characterized using denaturing gradient gel electrophoresis (DGGE).

Methods

Bench-Scale System

The MTBE and BTEX degrading reactor was a well-mixed chemostat constructed of 304 stainless steel with an internal diameter of 21.6 cm and a height of 30.5 cm. The chemostat was modified to contain a 0.48-cm-thick filter grade polyethylene porous pot (Atlas Minerals & Chemicals, Mertztown, Pa.) with a pore size of 0.25 μm for the retention of biomass (Wilson et al. 2000; Wilson et al. 2001). The reactor was seeded with a combination of cultures enriched on MTBE and other oxygenated compounds (Richter et al. 2000). Wasting from the reactor only took place for sampling purposes, and thus a sludge age of greater than 100 days was maintained. The reactor was mixed continuously with a magnetically driven mixer. The total flow rate to the reactor was 2.70 L/day, which accounted for a hydraulic retention time of 3.75 days. An acidified nutrient solution (see Pruden et al. 2001 for nutrient composition) containing all essential salts and nutrients comprised one fifth of the flow, and a pH adjusting buffer in distilled water made up the remainder of the flow. Initially the pH was maintained with sodium hydroxide, but better performance was achieved upon switching to a phosphate buffer. The phosphate buffer was an adjusted ratio of sodium phosphate monobasic and sodium phosphate dibasic. The pH was maintained in a range of 7.4–7.7. Influent MTBE concentration was 75 mg/L with an equivalent COD of BTEX, or approximately 17 mg/L for each of the four BTEX compounds. All substrates were injected directly into the buffer line using a Model 11 high-precision syringe infusion pump (Harvard Apparatus, Inc., South Natick, Mass.) with a 2.5 mL fixed needle syringe (Hamilton Co., Reno, Nev.).

Analysis

Liquid and gaseous concentrations of MTBE, TBA, and BTEX were determined using a Hewlett Packard 5890 Series II gas chromatograph (GC) (Hewlett Packard, Palo Alto, Calif.) equipped with a flame ionization detector and 60/80 Carbopack B/5% Carbowax 20 M glass column (column number 2-1641 packed with 11766) (Sulpelco, Bellefonte, Pa.). Liquid concentrations of MTBE, TBA, and BTEX when present at low concentrations were measured using a Tekmar Dohrmann (Cincinnati) 3100 sample concentrator with a Hewlett Packard 6890 Series Plus GC and a DB-1 column (J&W Scientific, Folsom, Calif.). Flow rates to the reactor as well as pH (Model 720A pH meter, Orion Research Co., Boston) were measured daily. Analysis of dissolved oxygen was monitored with a Corning Checkmate II dissolved oxygen sensor (Corning, N.Y.) and COD was monitored using Hach (Loveland, Colo.) ultralow range digestion vials. Dissolved organic carbon was measured using the difference between the total carbon and inorganic carbon or using nonpurgeable organic carbon analysis when the effluent concentration of MTBE and BTEX was below 0.1 mg/L, each measured on a Shimadzu TOC-5000 Analyzer (Shimadzu, Kyoto, Japan). Volatile suspended solids (VSS) were measured by drying a Whatman (Clifton, N.J.) 934-AH Glass Microfibre filter at 550°C for 45 min, filtering the sample, drying at 105°C for 90 min, and taking the difference in mass after baking at 550°C for 120 min.

Batch Studies

Batch tests were carried out in 160 mL serum bottles containing 90 mL of reactor effluent, 10 mL of biomass from the reactor, and 60 mL of headspace. The first set of batch studies (TBA batch) took place on day 471 of reactor operation, and the second set (MTBE batch) took place on day 479. The bottles were spiked with the following combinations: MTBE alone, MTBE with BTEX, TBA alone, and TBA with BTEX. Batch studies were carried out at three initial concentrations of MTBE or TBA: 0.07, 0.2, and 0.5 mM. BTEX was added at a COD equivalent of the MTBE or TBA. Bottles were placed on a tumbler with rotational speed of 20 rpm, and were sacrificed for sampling in triplicate. Mercuric chloride (2.72 g/L) killed controls were monitored as well. Samples were measured for liquid MTBE, TBA, and BTEX concentrations by GC (packed column method, described above). Samples were also monitored for DO and pH. VSS measurements were done in quadruplicate at the time of the study.

Three rates were calculated from the batch studies: the MTBE degradation rate, the TBA degradation rate, and the overall degradation rate, which accounted for loss of both MTBE and TBA. The rates were determined as the linear portion of the curve (mmol/min.) normalized to the volatile suspended solids (VSS) giving final units of (mmol/min)/kg VSS. To determine the overall rate, the moles of (MTBE+TBA) lost with time was normalized to the VSS. Because conversion of MTBE to TBA is equimolar, the rates can be compared and added in this way.

An alternative rate calculation was also explored that took the rate determined above, multiplied it by the actual mass of VSS in the reactor, and normalized it to MTBE loading to the reactor

$$\frac{\text{m mol(MTBE, TBA, or MTBE+TBA)}_{\text{batch}}}{\text{kg VSS}_{\text{batch}} \times \text{min}} \times \frac{\text{kg VSS}_{\text{reactor}} \times \text{min}}{\text{m mol MTBE}_{\text{reactor feed}}}$$

The resulting units of this rate calculation are dimensionless. Calculating the rate in this way accounts for the fact that the microbial resources in this reactor were divided among many substrates, and describes what the rate is relative to the actual MTBE feed to the reactor. It also allows for direct comparison with other reactors with different MTBE loadings. In this case, the MTBE and TBA degradation rates of this culture will be compared to a culture that received MTBE as the only carbon substrate.

Chemicals Used

The following chemicals were used as supplied: MTBE (M_w = 88.15, 99%, Aldrich, Milwaukee), TBA (M_w = 74.12, 99%, Aldrich, Milwaukee), benzene (M_w = 78.11, 99%, Fisher Scientific, Pittsburgh), toluene (M_w = 92.14, 99%, Fisher Scientific, Pittsburgh), ethyl-benzene (M_w = 106.17, 99%, Fisher Scientific, Pittsburgh), and *p*-xylene (M_w = 106.17, 99%, Fisher Scientific, Pittsburgh).

Denaturing Gradient Gel Electrophoresis

Microbial community structure of the culture was profiled using denaturing gradient gel electrophoresis (DGGE). Samples of the culture were taken in duplicate at the time of the batch studies. DNA extractions were done using a Fast DNA and a FastPrep sample homogenizer kit provided by Bio101 (Vista, Calif.). Polymerase chain reaction (PCR) was used to amplify a 192 base pair

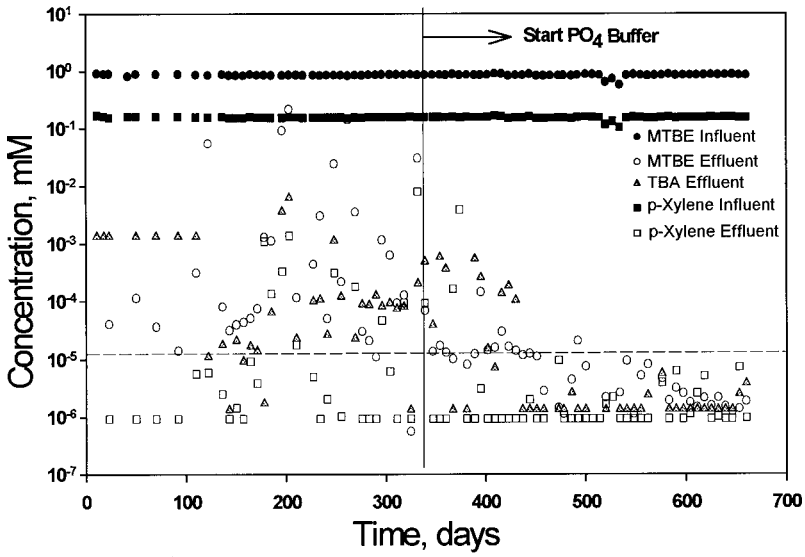

Fig. 1. Performance of bench-scale MTBE and BTEX porous pot reactor plotted on a log scale. For simplicity, only *p*-xylene is plotted of the BTEX compounds, as this was consistently measured to be the highest of the four BTEX compounds in the effluent. Limit of detection for TBA decreased from 0.1 mg/L to 0.1 μg/L on day 100 of the study. Dashed line indicates 1 μg/L MTBE concentration level.

portion of the V3 region of the 16S rDNA. Primers 534R (*E. coli* numbering system) and 341F (containing a GC clamp), described in Muyzer et al. (1993) were chosen based on their higher sensitivity and the superior ability of the resultant product to be resolved on a DGGE gel when compared to other universal primers (Chang et al. 2000). Primers were synthesized by Stratagene, Inc. (La Jolla, Calif.). PCR reactions were carried out in 25 μL volume using 1.25 units of Expand Hi Fidelity DNA polymerase (Roche, Indianapolis) with a temperature program of 93°C for 2 min (initial denaturing), followed by 35 cycles of 92°C for 1 min, 55°C for 1 min 68°C for 45 s, followed by 72°C for 2 min (final extension).

DGGE was performed to separate the PCR products using a D-Code 16/16 cm acrylamide gel system (BioRad, Hercules, Calif.). Gradient was formed between 15 and 55% denaturant (100% denaturant defined as 7 M urea plus 40% v/v formamide). The PCR products were loaded onto the DGGE gel and a charge of 35 V was run across the gel for 20 h. The buffer, which consisted of 0.5 X TAE (20 mM Tris-acetate, 0.5 mM ethylenediaminetetra acetic acid pH 8.0) was maintained at a constant temperature of 60°C. The central 1-mm^2 portions of the visible bands were excised with razor blades, reamplified using the same primer and conditions, and the products were purified using Gene Clean Spin columns (Bio-101, Vista, Calif.) for DNA sequencing, which was done off site by Davis Sequencing (Davis, Calif.) using an ABI Prism 377 DNA Sequencer (Perkin-Elmer, Foster City, Calif.).

Sequences were screened for possible chimeric origin by use of the RDP ''*CHECK CHIMERA*'' program (Maidak et al. 2001). Also, due to the presence of multiple PCR products migrating to the same point in the DGGE gel, not all DGGE bands generated high-quality sequence data. Illegible sequence data was discarded. Sequences were compared to the organisms in the RDP using the ''sequence match'' tool and reference sequences from the most similar organisms were thus obtained for subsequent phylogenetic comparison (Maidak et al. 2001). Sequences were aligned using ClustalX (Thompson et al. 1997) followed by manual adjustment. A phylogenetic tree was constructed using maximum likelihood analysis as implemented by *Paup** version 4.0b8 (Sinauer Associates, Inc. Sunderland, Mass.). Bootstrap values were determined using the same program with 1000 replicates.

Results and Discussion

Reactor performance is plotted in Fig. 1. Similar performance was observed in a reactor treating MTBE as the sole carbon substrate, ''MTBE only'' (Wilson et al. 2002). Reactor performance was fair but erratic during the first 300+ days and was observed to be very sensitive to pH. Performance improved greatly upon switching to a phosphate buffer on day 330 (Fig. 1), and effluent concentrations of all substrates gradually achieved levels of about 1 μg/L. TBA was observed in the effluent upon start-up and perturbations of the reactor, but also attained concentrations in the range of 1 μg/L during stable periods of operation. The presence of BTEX had no apparent detrimental effect on the performance of the reactor. Effluent levels of MTBE and TBA were comparable for both reactors, and even slightly lower in the MTBE and BTEX reactor towards the end of operation.

The results of the DGGE community profiling are shown in Fig. 2. The culture contained a wide diversity of microorganisms including members most closely related to the *Flavobacteria-Cytophaga*, α- and β-*Proteobacteria*, and Gram positive bacteria. We have observed the prevalence of *Flavobacteria-Cytophaga*-related organisms in several other MTBE degrading cultures (Richter et al. 2000; Pruden et al. 2001). Of particular interest are two bands (MTBEBTX band 3, MTBEBTX band 7), which showed notably high similarity with PM1, a well-characterized MTBE degrader that has also been studied for its effects on BTEX on MTBE degradation (Hanson et al. 1999; Deeb et al. 2001). This allows for some degree of comparison between our study and those done with PM1.

Fig. 3 shows representative degradation curves for both MTBE and TBA with and without BTEX. TBA was not observed to accumulate as an intermediate, as it was in the MTBE only culture (Wilson et al. 2001). Fig. 4 shows a plot of MTBE and TBA degradation rates versus initial concentration for the MTBE and BTEX culture. The rates calculated were observed to increase

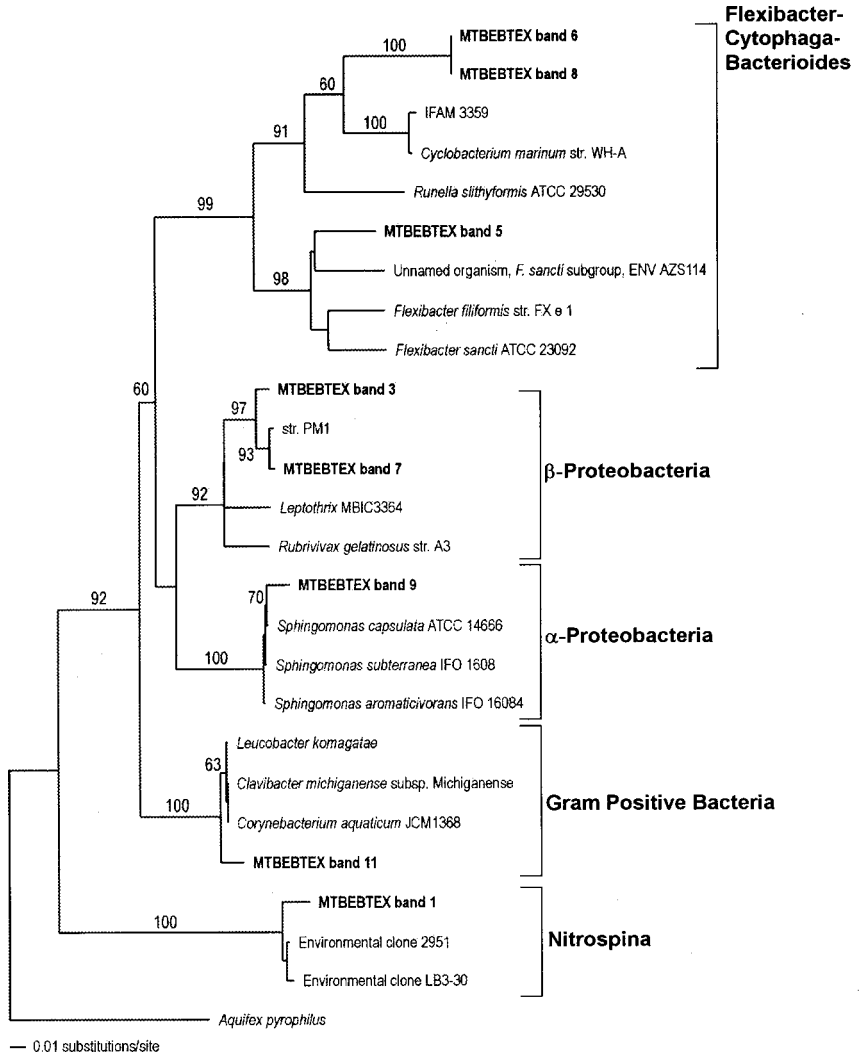

Fig. 2. Phylogenetic analysis of DGGE bands identified in MTBE and BTEX culture (shown in bold), compared with closest matches from the RDP. Bootstrap values at major nodes are based on 1000 replicates.

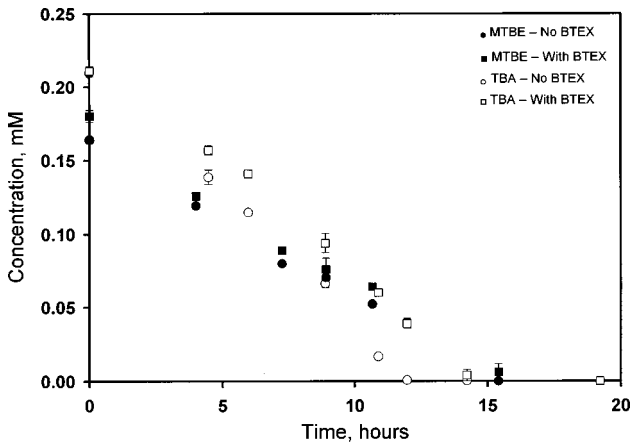

Fig. 3. Representative plots of MTBE and TBA degradation with time, with and without BTEX. TBA as an intermediate of MTBE degradation was negligible.

with increasing initial concentration. Also included in Fig. 4 are the rates from the MTBE only enrichment culture. The overall rate of MTBE degradation (which accounted for complete mineralization of MTBE and TBA) in the MTBE/BTEX culture was equal to the MTBE rate, since TBA did not accumulate as an intermediate. The degradation curves in Fig. 3 show that TBA did not accumulate as an intermediate, which was true for MTBE degradation at all concentration levels. Also, Fig. 4 shows that the rate of TBA degradation was significantly higher than the rate of MTBE degradation at all concentration levels. This indicates that TBA degradation is not a rate-limiting step for this culture, as it was observed to be for the MTBE-only culture in Wilson et al. (2002). In the MTBE-only culture, the overall rate was equal to the TBA rate (Fig. 4), indicating that TBA degradation was the rate-limiting step for this culture. When normalized to VSS, the MTBE, TBA, and overall rates are all much faster in the MTBE-only culture than in the MTBE and BTEX culture.

BTEX degraded much faster than MTBE or TBA (data not shown). Rates of BTEX degradation were too fast to be quantified, but degraded within the first sampling event of each study. This was 1–2 h for the low-concentration level, 4–5 h for the middle concentration level, and 6–7 h for the high-concentration level.

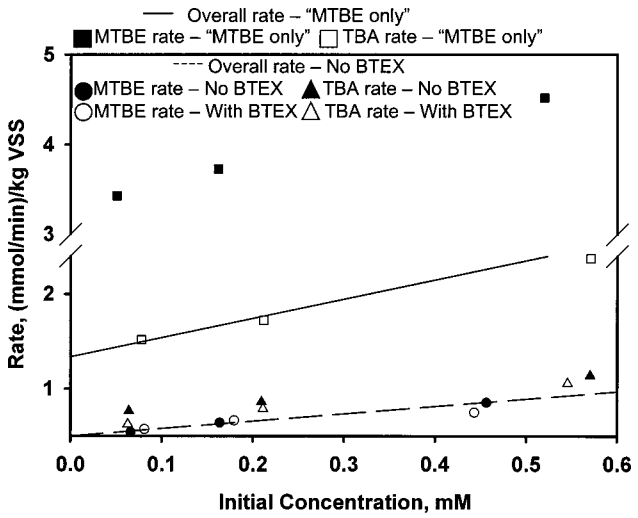

Fig. 4. Plots of VSS normalized rates versus initial concentration, comparing MTBE and BTEX culture with MTBE only culture, enriched with MTBE as sole substrate (Wilson et al. 2002). Regression lines represent overall degradation rate of MTBE and TBA intermediate for these two cultures. Range between 2.4 and 3.0 has been omitted from *y* axis.

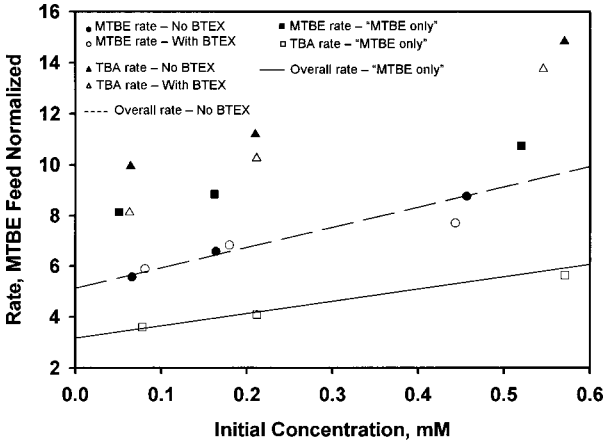

Fig. 5. Plot of MTBE feed normalized rates versus initial concentration. Feed normalized rates from MTBE only culture (Wilson et al., 2002) are shown for comparison.

BTEX did not have a noticeable effect on MTBE degradation, but did have an effect on TBA degradation. In Fig. 3, there is a slight degradation lag in TBA degradation when BTEX is present, as observed if the linear portion of the degradation curve intersects the *y* axis (concentration) above the initial concentration point. No degradation lag was observed in MTBE degradation when BTEX was present. TBA degradation did not exhibit a lag period when BTEX was absent, indicating that the culture was adapted to TBA.

A slowing effect of BTEX on the TBA degradation rate was observed even after the BTEX was degraded. In Fig. 4, it is seen that the TBA degradation rates at all three initial concentration levels are higher when BTEX is absent. These degradation rates plotted in Fig. 4 are calculated from the linear portion of the curve, at which time the BTEX has already degraded. Therefore, the negative effect of BTEX on TBA degradation extended beyond the initial period when BTEX was still present. This effect was observed at all concentration levels.

Fig. 5 shows a plot of rate versus initial concentration using the dimensionless MTBE feed normalized rate. Calculating the rate in this way accounts for the division of microbial resources among five substrates as opposed to one substrate in the MTBE-only reactor. The MTBE-only reactor received the same influent COD as the MTBE and BTEX reactor, but twice the MTBE loading (Wilson et al. 2002). The feed normalized rate determination quantifies the MTBE degradation potential of the biomass as it has developed inside the reactor. Here a very interesting observation emerges: when considering the relative MTBE feed to the reactor, the MTBE and BTEX reactor actually outperforms the MTBE-only reactor in terms of the TBA degradation rate and the overall degradation rate. The TBA rates and the overall rates are higher in the BTEX culture than in the MTBE-only culture at all initial concentration levels (Fig. 5). The MTBE rates, however, are still higher in the MTBE-only culture than in the BTEX culture, but the TBA rates are lower than the BTEX culture (Fig. 5). It is possible that BTEX assists TBA and thus overall MTBE degradation through the mechanism of biomass diversification, as was observed from the DGGE results. The MTBE-only culture contained only one group of dominant organisms related to the *Flavobacteria-Cytophaga* group (Richter et al. 2000), while the BTEX culture contained several groups. Another possibility is that if the same or similar enzymes are capable of TBA and BTEX degradation, then addition of BTEX to the feed is contributing to the enrichment of TBA degraders as well. This may explain the lag period that was observed when BTEX was fed with TBA, indicating that the same enzymes degrade both TBA and BTEX, but have a preference for BTEX. The fact that BTEX may assist in the development of biomass for overall MTBE degradation is a promising finding for the operation of ex situ bioreactors.

The behavior of this culture in the batch studies gives insight into what may be expected in an in situ remediation approach, while the continuous flow conditions simulate the ex situ approach. In the batch studies, there was clearly a microbial preference for BTEX over MTBE or TBA. Therefore, BTEX is likely to exhaust the available DO native to a contaminated aquifer. While there are some reports of anaerobic degradation of MTBE, degradation rates are very slow, and require acclimation periods in the range of 300 days (Suflita and Mormile 1993; Finneran and Lovley 2001). Because MTBE migrates in groundwater at such a rapid rate, 300+ days is too long to wait when downstream receptors are of concern. In addition, while BTEX had no apparent negative effect on MTBE degradation, BTEX affected TBA in batch mode by inducing a lag period in addition to retarding the subsequent TBA degradation rate. This indicates that BTEX may interfere metabolically with TBA degradation, possibly through a mechanism such as competitive inhibition, as was mentioned above. In situ the effects of this may be more severe, as was observed in the batch study in comparison with the continuous flow study. However, TBA degradation was still much faster than MTBE degradation, even in the presence of BTEX. For this reason, MTBE degraded to completion at the same rate with or without BTEX. Therefore, BTEX may only have a detrimental effect in areas where TBA has accumulated due to other factors.

In contrast to the results presented by Deeb et al. (2001), no BTEX effect on MTBE degradation was observed. In the prior study, MTBE did not degrade at all in the presence of ethylbenzene and xylene, while degradation was severely retarded in the presence of benzene and toluene. In the present study, MTBE degraded without a lag phase and at the same rate regardless of the presence of BTEX. This discrepancy is most likely due to the

diversity of the culture used in this study. While PM1-like microorganisms were detected in the MTBE/BTEX culture, so were a whole host of other bacteria, including: α- and β-*Proteobacteria*, Gram positive bacteria, *Nitrospina*, and *Flavobacteria-Cytophaga*-related organisms. These diverse organisms apparently can overcome the metabolic inhibition observed in pure culture. Similarly, Schroeder et al. (2000) found that, in a mixed-culture biofilter containing the original PM1 seed culture, toluene and MTBE could both be removed effectively in mixture. However, while the Deeb et al. (2001) study did not address TBA degradation, the present study indicated that even in an adapted, biologically diverse culture, BTEX still slows TBA degradation. Although Deeb et al. found that BTEX completely prevented the biodegradation of MTBE, we demonstrated that TBA degradation still proceeds faster than MTBE degradation even in the presence of BTEX. Herein lies the advantage of using mixed cultures as opposed to pure cultures. A mixed culture rather than a pure culture will be the most effective strategy whether treatment is in situ or ex situ.

Finally, it can be concluded from this study that the ex situ treatment approach may be the best option for MTBE and BTEX cocontamination. First of all, from the operation of the bioreactors BTEX did not affect steady-state performance of the reactor. At steady state, effluent quality was excellent, in the range of 0.1–1.5 μg/L for both MTBE and BTEX compounds. Ex situ bioreactor technology is also advantageous because bioreactors can be designed for the retention of biomass. Most studies indicate that exceptionally high biomass concentrations are required for efficient MTBE degradation (Salanitro et al. 1994; Wilson et al. 2000, 2002; Morrison et al. 2002). Such high concentrations have actually clogged aquifers when in situ attempts have been made. An ex situ bioreactor may also be desirable considering the ease of aeration upon bringing the water to the surface. If BTEX acts in boosting the TBA and overall MTBE biodegradation potential of the biomass, then an ex situ bioremediation approach will be ideal.

Conclusions

Contrary to the current conception of the effect of BTEX on MTBE degradation, this study clearly shows not only that MTBE degradation can take place at the same rate regardless of whether BTEX is present or not, but also gives evidence that BTEX may significantly enhance the effective overall MTBE degrading capability of the culture. This will be a great advantage for the success of ex-situ treatment systems near the source of the plume where MTBE and BTEX coexist. However, in all cases, BTEX degraded orders of magnitude faster than MTBE or TBA in the batch studies. This suggests that in situ MTBE treatment efforts will be hampered by the oxygen demand imposed by BTEX.

References

Chang, Y.-J., et al. (2000). ''Phylogenetic analysis of aerobic freshwater and marine enrichment cultures efficient in hydrocarbon degradation: Effect of profiling method.'' *J. Microbiol. Methods* 40, 19–31.

Deeb, R. A., Hu, H.-Y., Hanson, J. R., Scow, K. M., and Alvarez-Cohen, L. (2001). ''Substrate interactions in BTEX and MTBE mixtures by an MTBE-degrading isolate.'' *Environ. Sci. Technol.,* 35, 312–317.

Finneran, K. T., and Lovley, D. R. (2001). ''Anaerobic degradation of methyl tert-butyl ether (MTBE) and tert-butyl alcohol (TBA).'' *Environ. Sci. Technol.,* 35, 1785–1790.

Hanson, J. R., Ackerman, C. E., and Scow, K. M. (1999). ''Biodegradation of methyl tert-butyl ether by a bacterial pure culture.'' *Appl. Environ. Microbiol.,* 65, 4788–4792.

Maidak, B. L., et al. (2001). ''The RDP-II (Ribosomal Database Project).'' *Nucleic Acids Res.,* 29, 173–174.

Mo, K., Lora, C. O., Wanken, A. E., Javanmardian, M., Yang, X., and Kulpa, C. F. (1997). ''Biodegradation of methyl t-butyl ether by pure bacterial cultures.'' *Appl. Microbiol. Biotechnol.,* 47, 69–72.

Mormile, M. R., Liu, S., and Suflita, J. M. (1994). ''Anaerobic biodegradation of gasoline oxygenates: Extrapolation of information to multiple sites and redox conditions.'' *Environ. Sci. Technol.,* 28, 1727–1732.

Morrison, J. R., Suidan, M. J., and Venosa, A. D. (2002). ''Use of membrane bioreactor for biodegradation of MTBE in contaminated water.'' *J. Environ. Eng.,* 128, 836–841.

Muyzer, G., de Waal, E. C., and Uitterlinden, A. G. (1993). ''Profiling of microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction amplified genes coding for 16 s rDNA.'' *Appl. Environ. Microbiol.,* 59, 695–700.

Pruden, A., Wilson, G. J., Suidan, M. T., and Venosa, A. D. (2001). ''Biodegradation of methyl *tert*-butyl ether under various substrate conditions.'' *Environ. Sci. Technol.,* 35, 4235–4251.

Richter, A. P., Wilson, G. J., Suidan, M. T., and Venosa, A. D. (2000). ''Identification and monitoring of MTBE degraders by DGGE analysis.'' *Proc., Water Environment Federation 73rd Annual Technical Exhibition and Conf.,* Anaheim, Calif.

Salanitro, J. P., Diaz, L. A., Williams, M. P., and Wisniewski, H. L. (1994). ''Isolation of a bacterial culture that degrades methyl tert-butyl ether.'' *Appl. Environ. Microbiol.,* 60, 2539–2596.

Schroeder, E. D., Eweis, J. B., Chang, D. P. Y., and Veir, J. K. (2000). ''Biodegradation of recalcitrant components of organic mixtures.'' *Water, Air, Soil Pollut.,* 123, 133–146.

Suflita, J. M., and Mormile, M. R. (1993). ''Anaerobic biodegradation of known and potential gasoline oxygenates in the terrestrial subsurface.'' *Environ. Sci. Technol.,* 27, 976–978.

Thompson, J. D., Gibson, T. J., Plewniak, F., Jeanmougin, F., and Higgins, D. G. (1997). ''The ClustalX windows interface: Flexible strategies for multiple sequence alignment aided by quality analysis tool.'' *Nucleic Acids Res.,* 24, 976–978.

United States Environmental Protection Agency (U.S. EPA) (1999). ''Achieving clean air *and* clean water: The report of the blue ribbon panel on oxygenates in gasoline.'' *Final Report No. EPA420-R-99-021.*

Wilson, G. J., Pruden, A., Suidan, M. T., and Venosa, A. D. (2002). ''Biodegradation kinetics of MTBE in laboratory batch and continuous flow reactors.'' *J. Environ. Eng.,* 128, 824–829.

Wilson, G. J., Richter, A. P., Suidan, M. T., and Venosa, A. D. (2000). ''Mineralization of MTBE with various primary substrates.'' *Battelle 2nd Int. Conf. on Remediation of Chlorinated and Recalcitrant Compounds,* Monterey, Calif.

Wilson, G. J., Richter, A. P., Suidan, M. T., and Venosa, A. D. (2001). ''Aerobic biodegradation of gasoline oxygenates MTBE and TBA.'' *Water Sci Technol.,* 43, 277–284.

Yeh, C. K., and Novak, J. T. (1994). ''Anaerobic biodegradation of gasoline oxygenates in soils.'' *Water Environ. Res.,* 66, 744–752.