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Biotreatment of groundwater contaminated with MTBE: interaction of common environmental co-contaminants

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Abstract

Contamination of groundwater with the gasoline additive methyl *tert*-butyl ether (MTBE) is often accompanied by many aromatic components such as benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene and *p*-xylene (BTEX). In this study, a laboratory-scale biotrickling filter for groundwater treatment inoculated with a microbial consortium degrading MTBE was studied. Individual or mixtures of BTEX compounds were transiently loaded in combination with MTBE. The results indicated that single BTEX compound or BTEX mixtures inhibited MTBE degradation to varying degrees, but none of them completely repressed the metabolic degradation in the biotrickling filter. *Tert*-butyl alcohol (TBA), a frequent co-contaminant of MTBE had no inhibitory effect on MTBE degradation. The bacterial consortium was stable and showed promising capabilities to remove TBA, ethylbenzene and toluene, and partially degraded benzene and xylenes without significant lag time. The study suggests that it is feasible to deploy a mixed bacterial consortia to degrade MTBE, BTEX and TBA at the same time.

Abbreviations: BTEX compounds – benzene, toluene, ethylbenzene, *o*-xylene, *m*-xylene and *p*-xylene; MTBE – methyl *tert*-butyl ether; TBA – *tert*-butyl alcohol

Introduction

Methyl *tert*-butyl ether (MTBE) and its biodegradation daughter product, *tert*-butyl alcohol (TBA) have been heavily used as gasoline additives during the past decade to increase combustion efficiency and to reduce air pollution. However, this has lead to widespread groundwater contaminations. The relative high water solubility has resulted in frequent occurrences of MTBE and TBA in groundwater and raised public health concerns (Squillace et al. 1996; Landmeyer et al. 1998; EPA 1999). As a consequence, MTBE is being phased out in the United States.

In addition to numerous studies on biodegradability of MTBE, considerable effort has been directed to develop both conventional and innovative methods for bioremediation of MTBE-impacted groundwater (Stocking et al. 2000; Fiorenza & Rifai Hanadi 2003). MTBE in groundwater is often found together with other gasoline contaminants, the most prevalent being benzene, toluene, ethylbenzene, and xylenes (BTEX), and TBA (Happel et al. 1998). Therefore, research is needed to determine the effect of simultaneously treating MTBE and these co-contaminants in groundwater.

Deeb et al. in a study on a selected toluene-adapted cultures found that the presence of MTBE at concentrations comparable to those detected in contaminated groundwater had no effect on BTEX degradation rates (Deeb & Alvarez-Cohen 2000). Recently in another study with pure MTBE degrading culture, *Rubrivivax* sp. PM1, Deeb et al. found that PM1 enriched on MTBE was unable to

degrade ethylbenzene, *m*- and *p*-xylene at concentrations of 20 mg L^{-1} (Deeb et al. 2001). The presence of xylene completely inhibited MTBE degradation. When benzene and toluene were fed, they were degraded rapidly after an initial lag of several hours, but the rate of MTBE degradation slowed significantly and did not recover until benzene and toluene were entirely degraded. Based on these results, Deeb et al. suggested that BTEX and MTBE degradation occurred primarily via two independent and inducible pathways (Deeb et al. 2001).

More recently, experiments conducted in microcosms containing sediment and groundwater from four MTBE-contaminated leaking underground fuel tank (LUFT) sites showed that MTBE metabolism was markedly inhibited (Kane et al. 2001). TBA accumulation was more pronounced, presumably as a result of the presence of BTEX. In contrast, BTEX degradation was rapid and was not affected by the presence of MTBE. This may explain why many MTBE contaminated sites also often test positive for the presence of TBA.

Similar results were also found in engineered systems and field trials. A study using laboratory columns packed with aquifer material from four gasoline-contaminated sites showed that MTBE was degraded in the columns only in the absence of BTEX compounds (Church et al. 1999). Similarly, at a field site where the activity of aquifer microorganisms was stimulated using oxygen release compounds (ORC), MTBE degradation was shown to occur only after BTEX concentrations were significantly reduced (Koenigsberg et al. 1999). In parallel microcosm studies, the presence of xylene caused a 43% reduction in MTBE biodegradation (Koenigsberg et al. 1999).

All the above-mentioned studies conclude that the co-contamination with BTEX inhibits the biodegradation of MTBE, at least to some degree. This poses a challenge for onsite MTBE bioremediation, since in most cases, MTBE and BTEX are commingled. On the other hand, Hatzinger et al. (2001) found that MTBE biodegradation occurred constitutively in a pure culture ENV735. This finding, and the proposal of an independent pathway for BTEX and MTBE biodegradation (Deeb et al. 2001), strongly suggests that it is possible to degrade BTEX and MTBE simultaneously by using mixed cultures.

This paper is intended to demonstrate the feasibility of biotrickling filters inoculated with mixed bacterial cultures for simultaneously treating MTBE, TBA and BTEX contaminated groundwater under field simulated conditions. The objectives were as follows: (1) to examine the impact of co-contaminants such as BTEX and TBA on MTBE biodegradation in biotrickling filters; and (2) to determine the biodegradation of BTEX by the mixed bacterial consortium.

Materials and methods

Microorganisms and mineral medium

The microbial consortium was initially enriched from MTBE contaminated materials (Fortin & Deshusses 1999a, 1999b). It has been maintained in a Bioflo I reactor (New Brunswick Scientific, Edison, NJ, USA) by naturally attaching it onto silicon tubing through which air was passed to ensure oxygen supply without stripping the MTBE. MTBE was added weekly to the reactor. The mineral medium consisting of the following components (in g L^{-1}): $MgSO_4 \cdot 7H_2O$, 0.25; KNO_3, 0.5; $CaCl_2 \cdot 2H_2O$, 0.009; KH_2PO_4, 0.5; K_2HPO_4, 0.5; NaCl, 1.0; and 1.0 mL L^{-1} of trace elements solution. The trace elements solution (Pfennig et al. 1981) (pH 4.2) contained (in g L^{-1}): $FeCl_2 \cdot 4H_2O$, 1.5; $CuCl_2 \cdot 2H_2O$, 0.015; $NiCl_2 \cdot 6H_2O$, 0.025; $MnCl_2 \cdot 4H_2O$, 0.1; $CoCl_2 \cdot 6H_2O$, 0.12; $ZnCl_2$, 0.07; $NaMoO_4 \cdot 2H_2O$, 0.025; H_3BO_3, 0.06; EDTA $\cdot 4H_2O$, 5.2.

Laboratory-scale biotrickling filter and operating conditions

Figure 1 shows the configuration of the biotrickling filter. A glass column was packed with commercially available perlite (Uni-Gro®, L&L Nursery Supply Inc., Chino, CA, USA) with a mean particle diameter of 3.2 ± 1.5 mm and inoculated with 1 g wet biomass of the consortium. The liquid flow was supplied and drained by a single peristaltic pump fitted with two pump heads (model 7553–80, Cole-Parmer, Vernon Hills, IL, USA). Different size tubing were used to introduce and drain the liquid. In doing so, a co-current airflow was induced through a needle placed at the

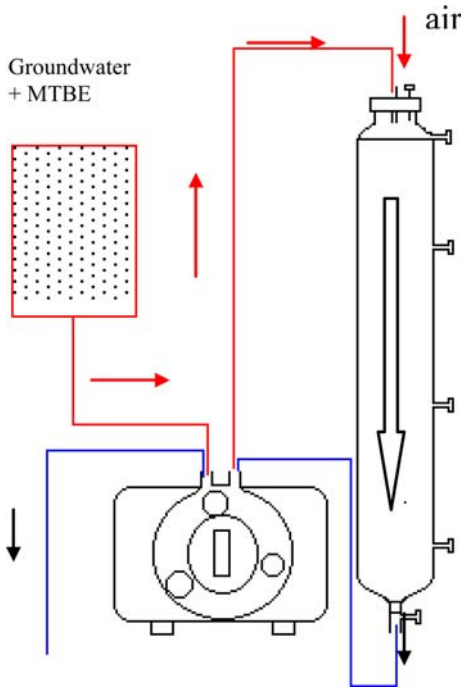


Figure 1. Schematic of the laboratory-scale biotrickling filter (height: 47 cm; ID: 4.5 cm; volume: 747 mL; inoculum: 1 g wet biomass).

top of the column. The liquid to air flow ratio was constant at 1:2.0 to 1:2.5 throughout the experiment. Preliminary experiments indicated MTBE loss by air stripping was always less than 0.2% of the total load and negligible in regular experiments (Wang 2003).

Throughout the entire set of experiments, the flow rates of the air and the liquid were maintained, in the range of 7.2–8.0 and 3.6–3.7 mL/min, respectively. This corresponds to a hydraulic residence time of the groundwater in the system of about 1.1 h. The oxygen provided by the airflow was approximate five times of the stoichiometric amount required for complete mineralization of all contaminants fed to the system.

Chemicals

MTBE (HPLC grade), TBA (certified grade), benzene, toluene, and ethylbenzene were obtained from Fisher Scientific Co. (Fair Lawn, NJ); *p*-xylene and *m*-xylene (>99%, anhydrous) were obtained from Acros® Inc.; *o*-xylene was from ICN Biomedicals Inc. All other chemicals were of the highest quality commercially available and were purchased from Fisher Scientific or Sigma.

Investigation of interaction between MTBE and BTEX or TBA biodegradation

The protocol included an array of experiments that all followed the same time format. The biotrickling filter at steady-state was first monitored for 2 h to determine initial performance when fed mineral medium with 10 mg L^{-1} MTBE. Then, the inlet feed was switched to another tank that contained a mixture of 10 mg L^{-1} MTBE and either TBA or the selected BTEX compound(s) while all other operating conditions remained unchanged. After 5 h the inlet was switched back to the initial feed, with 10 mg L^{-1} MTBE only, and the system was monitored for another 3 h. During the entire experiment, the liquid and the gas effluent were sampled every 30 min and analyzed by gas chromatography (see below). For benzene, toluene, and ethylbenzene, and TBA, two concentration step levels were tested separately, i.e., 10 and 25 mg L^{-1}. For xylene, two mixtures of xylene isomers were used: one was made of 5 mg L^{-1} of each isomer (*m*-, *o*-, and *p*-xylene); another one had 10 mg L^{-1} of each isomer. Finally, two BTEX mixtures containing 2.5 or 6 mg L^{-1} of each compound (for xylenes, 0.8 and 2 mg L^{-1} of each isomer) were used in order to determine the impact of a complex blend of co-contaminants.

The results included calculation of the mass of co-contaminant biodegraded during the experiment, examination of the carbon dioxide and MTBE effluent concentration patterns. In order to calculate the inhibition of MTBE biodegradation due to the presence of co-contaminants, the additional mass of MTBE leaving the system as a result of bacteria inhibition was calculated by integrating the effluent MTBE concentration over time and correcting for the steady-state baseline condition from before and after the step input of co-contaminant. Several measures were taken to avoid erroneous overestimation of performance. First of all, all removal were based on the cumulative masses in and out determined by integration of the concentration vs. time patterns. When effluent concentrations peaked after the experiment (e.g., for TBA) the maximum value of the mass out was used to calculate the removal.

Analysis

Liquid samples were analyzed by direct injection into a Hewlett-Packard model 6890 GC equipped with an HP-FFAP column (50 m × 320 μm × 0.5 μm) and a FID detector. The GC was calibrated using MTBE, TBA and BTEX standards in deionised water and the detection limit was approximately 100 ppb. Simultaneous analysis of CO_2 and MTBE or other volatiles in gas samples was achieved by injection of grab samples into a Hewlett-Packard model 5890 GC fitted with a packed column (80/100, 8′ × 1/4″, Supelco, Bellefonte, PA) and a thermal conductivity detector, and with a capillary column (Supelcowax 10, 30 m × 0.53 mm × 1 m) and a FID detector, respectively.

Results and discussion

Dynamic responses of the biotrickling filter to exposure to TBA and individual BTEX compounds

Under standard and steady-state conditions, the biotrickling filter was able to remove over 90% of the influent 10 mg L^{-1} of MTBE. This corresponds to an elimination capacity (EC = (inlet – outlet) × flow/bed volume) of about 4 g m^{-3} h^{-1}. The actual hydraulic residence time of the groundwater in the system was 30 min.

Figure 2 illustrates the dynamic response of the biotrickling filter to the simultaneous feeding of MTBE and benzene. When 10 mg L^{-1} of benzene were added along with 10 mg L^{-1} of MTBE, an increase of MTBE concentration both in the gas and liquid effluents was observed about 10 min after starting the experiment. However, the increase of MTBE in the outlet did not continue over time. After 90 min, MTBE reached its highest concentration in the outlet, and then started to decline. The same pattern was observed with benzene, which had a similar increase until about 3.5 h, followed by a moderate decrease, until benzene was removed from the feed, after which its effluent markedly decreased as a result of the combined effect of washout and biodegradation. These results indicate that after a very short exposure to benzene, the enrichment culture either synthesized or already had the proper enzymes needed for benzene degradation, so that significant

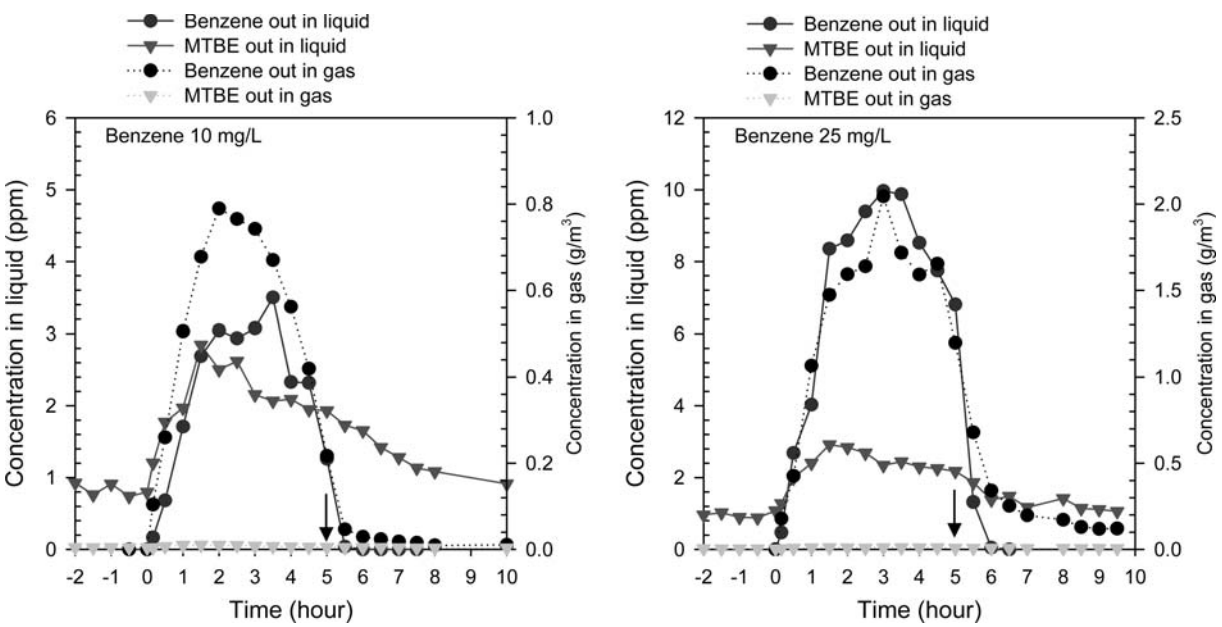


Figure 2. Response of the biotrickling filter degrading MTBE to a 5-h exposure to benzene. Experimental condition as in methods: MTBE continuously fed at 10 mg L^{-1}; benzene feeding duration: 5 h (0–5 h, end of benzene feeding shown by arrows), left: 10 mg L^{-1}, right: 25 mg L^{-1}. Note that the experiment with 10 mg L^{-1} of benzene was done prior to the experiment with 25 mg L^{-1}.

removal of benzene occurred. At the same time, benzene had a negative impact on MTBE biodegradation, but the inhibitory effect decreased over time, as indicated from the MTBE outlet concentration pattern. After stopping the feeding of benzene, MTBE elimination capacity returned to its previous value within about 5 h. Interestingly, traces of benzene (0.003 g m^{-3}) were detected in the effluent gas for over 2 days.

The subsequent addition of 25 mg L^{-1} benzene was conducted 3 days later (Figure 3, right). Again, the addition of benzene resulted in a temporary increase of benzene in the outlet, but the degree of its inhibition on MTBE degradation was almost the same as during the experiment with 10 mg L^{-1} benzene. The fact that a higher concentration of benzene did not result in greater inhibition of MTBE degradation indicates that the microorganisms in the reactor had acclimated to the presence of benzene during the initial exposure to benzene. Because of the short time scale of exposure to the pollutant, the acclimation was mostly probably for the induction of the proper enzymes rather than cell growth.

Figure 3 shows the behavior of the biotrickling filter during the transient feeding of a mixture of MTBE and toluene. As indicated above with benzene, the sequence of the experiments should be kept in mind when looking at the results. Here the 25 mg L^{-1} experiment was conducted first. Toluene at both concentrations negatively impacted MTBE degradation, as seen by the immediate increase of MTBE concentration in the outlet, after which MTBE outlet seemed to reach some kind of pseudo steady-state. The pattern of toluene was very different from that observed for benzene. At 25 mg L^{-1} of toluene, the effluent concentration of toluene kept increasing until toluene feeding was shut off. While it is unclear to what level the effluent concentration would have increased if the toluene feeding had continued, the relatively poor removal of toluene indicates that the microorganisms in the reactor did not handle well the sudden addition of toluene. The 10 mg L^{-1} experiment (Figure 3, left) shows some acclimation and improvement of toluene biodegradation during the 5-h period. It resulted in a lesser inhibition of MTBE biodegradation (see e.g., MTBE gas outlet).

Next, the effect of ethylbenzene was tested. As shown in Figure 4, ethylbenzene additions were largely biodegraded during the experiment. Ethylbenzene was found to have a significant

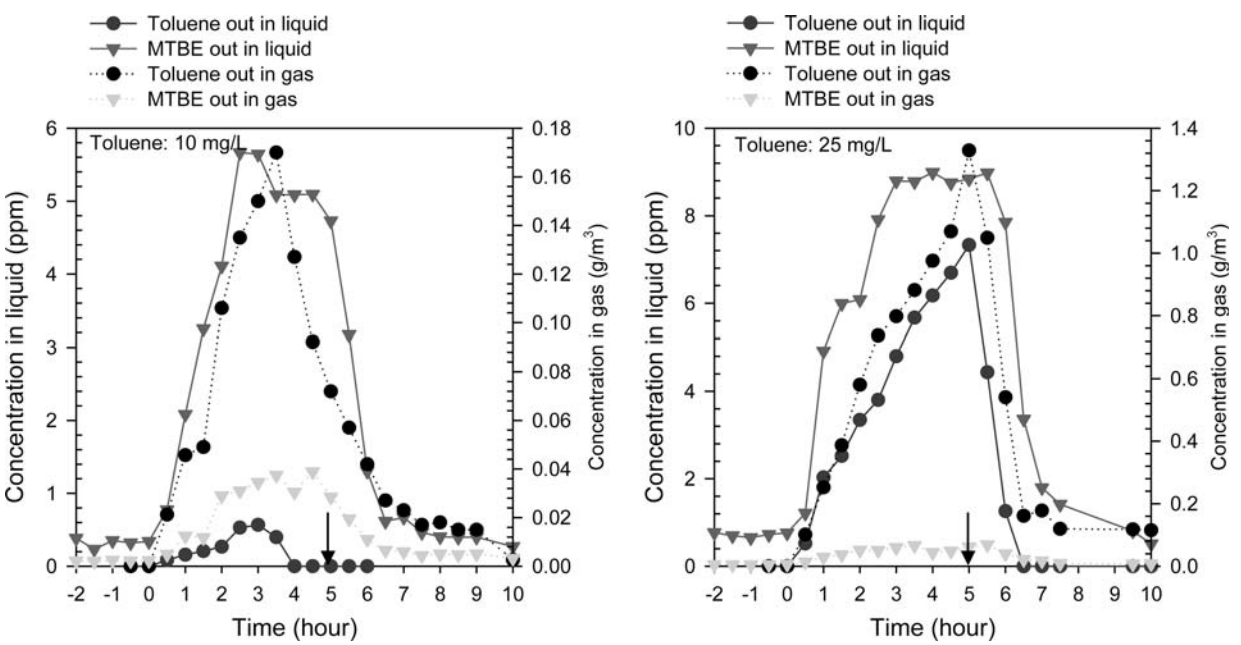


Figure 3. Response of the biotrickling filter degrading MTBE to a 5-h exposure to toluene. Experimental condition: MTBE continuously fed at 10 mg L^{-1}; toluene feeding duration: 5 h (0–5 h, end of toluene feeding shown by arrows), left: 10 mg L^{-1}, right: 25 mg L^{-1}. Note that the experiment with 25 mg L^{-1} of toluene was done prior to the experiment with 10 mg L^{-1}.

inhibitory effect on MTBE biodegradation, with higher concentration of ethylbenzene causing a greater effect. The adaptation of the enrichment culture to benzene and toluene seen earlier was not apparent with ethylbenzene. This suggests that biodegradation of ethylbenzene interferes with biodegradation of MTBE in a different way than the biodegradation of benzene and toluene.

The experiment with xylenes was conducted next and results are reported in Figure 5. Since, *m*- and *p*-xylene peaks overlap during GC analysis, an exact pattern for each isomer could not be obtained. Figure 5 shows the dynamic response of the reactor to the addition of xylenes. A higher concentration of xylenes had a stronger effect on the biodegradation of MTBE, though the difference was not as large as expected from the difference in xylenes feed concentration. With the limitation that *m*- and *p*-xylene could not be resolved, *m*- and *p*-xylene isomers were removed to a different extent than *o*-xylene in the biotrickling filter. *o*-Xylene was the most difficult to remove. In none of the cases, was any adaptation to xylenes observed, and effluent xylene concentrations peaked at the 5-h mark.

In addition to the experiments conducted with BTEX, the interaction between MTBE and TBA biodegradation was evaluated. Figure 6 shows the dynamic response of biotrickling filter with concurrent feeding of TBA. MTBE effluent concentrations did not exhibit any noticeable changes when transiently exposed either 10 or 25 mg L^{-1} of TBA. TBA was rapidly detected in the outlet, and reached a pseudo steady-state after 2–3 h at about 5 when 10 mg L^{-1} were fed, and at 15 when 25 mg L^{-1} were fed. This indicates that the TBA biodegradation enzymes were present at the start of the experiment, or that it was initiated very rapidly. This is consistent with CO_2 emission patterns discussed further in the paper.

Dynamic changes of CO_2 production and contaminant mass integration

As discussed above, TBA and BTEX compounds were partially removed by the biotrickling filter during the 5-h shock loading experiments. The removal was due to biodegradation as abiotic experiments (not shown) indicated that sorption of MTBE, TBA and BTEX to the packing matrix was negligible. A detailed examination of the patterns of CO_2 emission, and pollutants mass flows in and out of the biotrickling filter is warranted.

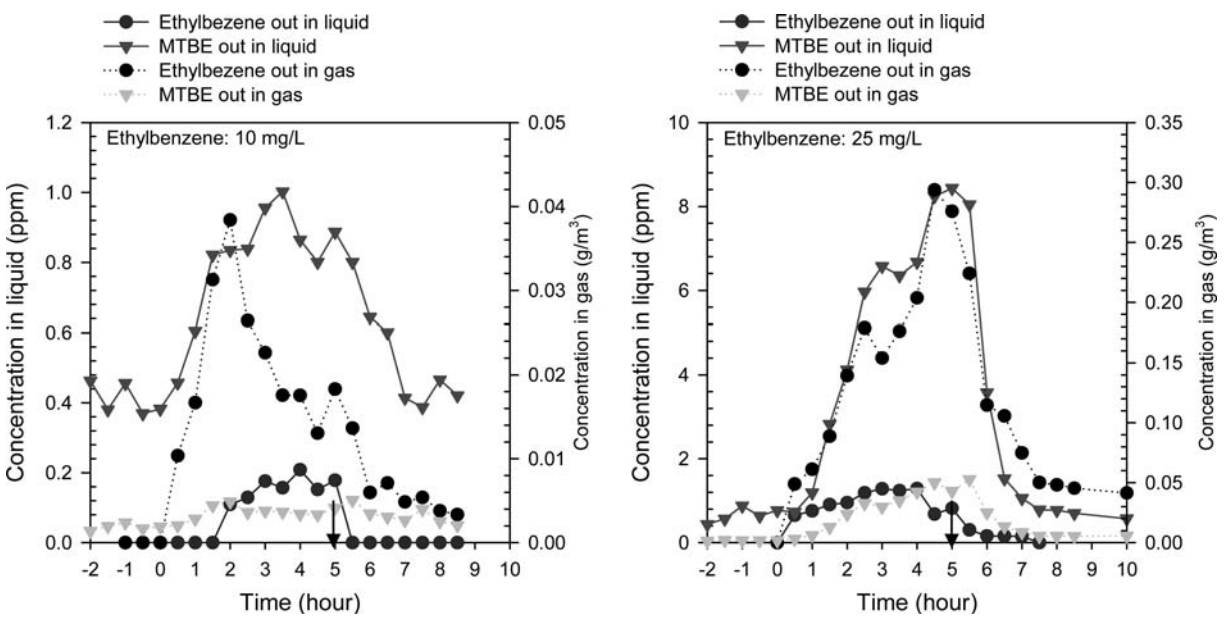


Figure 4. Response of the biotrickling filter degrading MTBE to a 5-h exposure to ethylbenzene. Experimental condition: MTBE fed at 10 mg L^{-1}; ethylbenzene feeding duration: 5 h (0–5 h, shown by arrows), left: 10 mg L^{-1}, right: 25 mg L^{-1}. Experiment at 10 mg L^{-1} was conducted first.

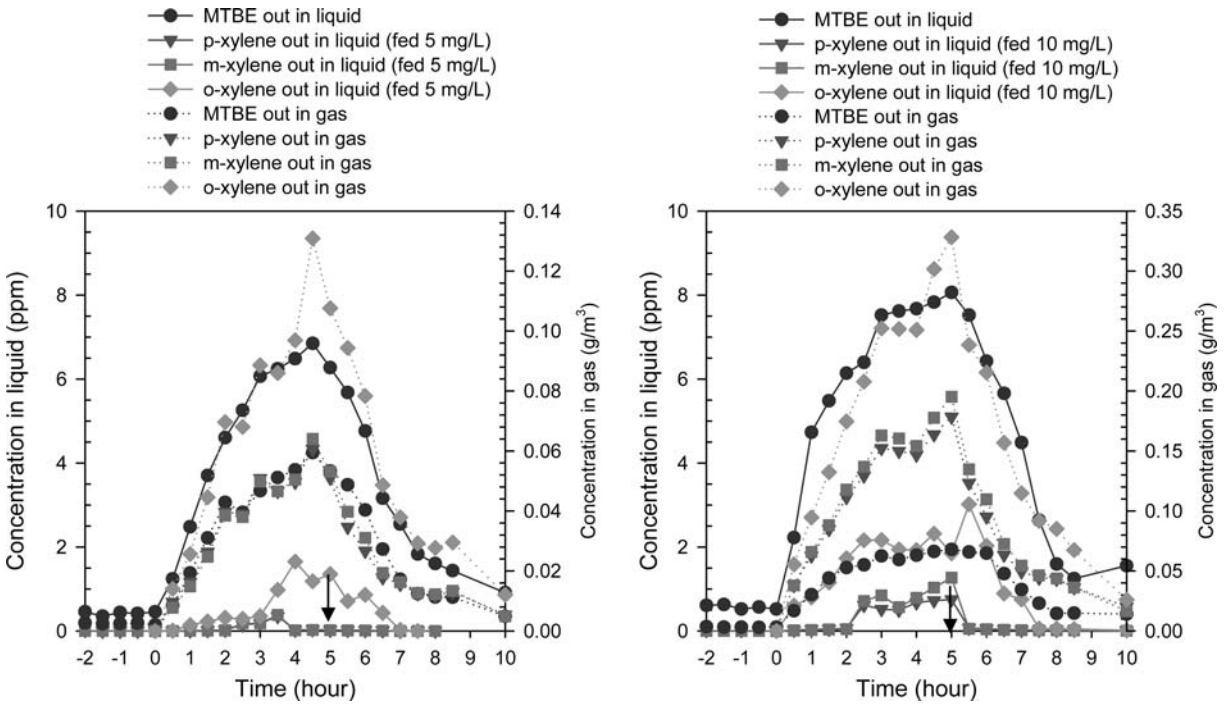


Figure 5. Response of the biotrickling filter degrading MTBE to a 5-h exposure to xylene. MTBE in the feed: 10 mg L^{-1}. Xylene feeding duration: 5 h (0–5 h, shown by arrows), left: 3 mg L^{-1} each xylenes isomer, right 10 mg L^{-1} each isomer.

To avoid multiple similar figures, only one set of results is shown for each compound. All results are summarized in Table 1.

Figure 7 shows the dynamic profile of CO_2 production and elimination rate of MTBE when 10 mg L^{-1} of benzene was fed. The elimination

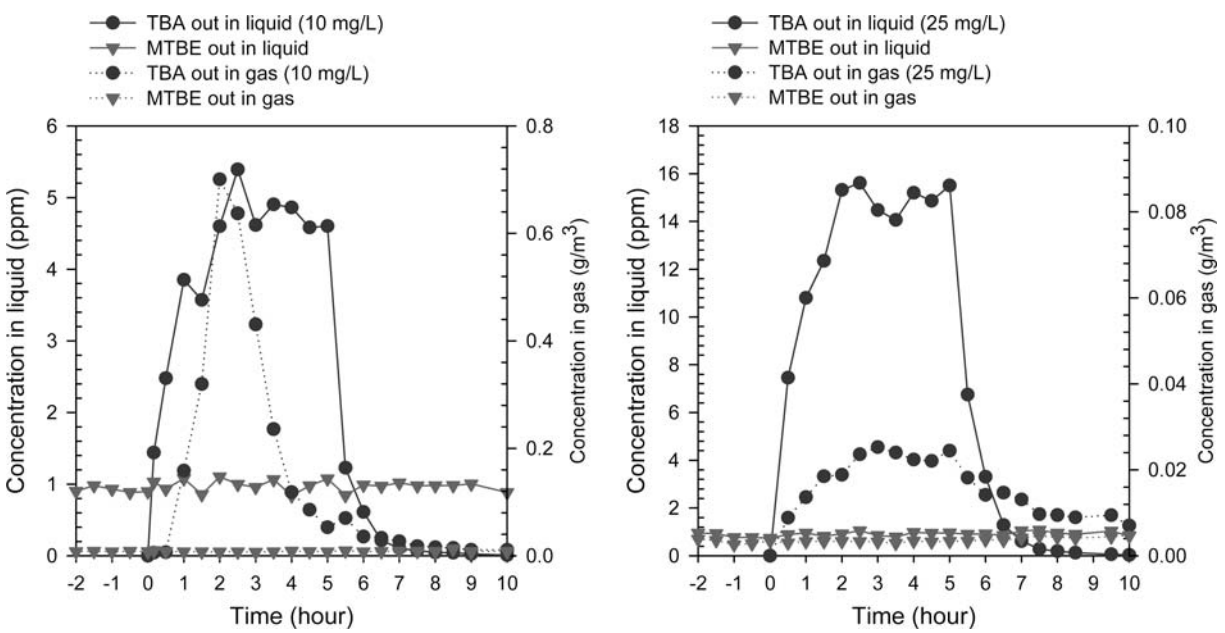


Figure 6. Response of the biotrickling filter degrading MTBE to a 5-h exposure to TBA. Experimental condition: MTBE fed at 10 mg L^{-1}; TBA feeding (0–5 h): 10 mg L^{-1} (left) and 25 mg L^{-1} (right).

Table 1. Summary of MTBE biodegradation in the presence of co-contaminants

Compound	Cumulative removal (%)	Maximum MTBE inhibition[a] (%)
MTBE alone	95	0
MTBE + benzene		
MTBE (w/10 mg L^{-1} benzene)	76.9	19.0
Benzene 10 mg L^{-1}	62.1	
MTBE (w/25 mg L^{-1} benzene)	76.8	19.1
Benzene 25 mg L^{-1}	48.6	
MTBE + toluene		
MTBE (w/10 mg L^{-1} toluene)	60.1	36.7
Toluene 10 mg L^{-1}	93.8	
MTBE (w/25 mg L^{-1} toluene)	29.5	69.0
Toluene 25 mg L^{-1}	61.6	
MTBE + ethyl benzene		
MTBE (w/10 mg L^{-1} ethylbenzene)	92.8	2.3
Ethylbenzene 10 mg L^{-1}	98.6	
MTBE (w/25 mg L^{-1} ethylbenzene)	58.7	38.2
Ethylbenzene 25 mg L^{-1}	94.3	
MTBE + xylenes		
MTBE (w/15 mg L^{-1} xylenes)	51.6	45.7
m- and *p*-xylene 10 mg L^{-1} (total) and	89.0	
o-xylene 5 mg L^{-1}	66.5	
MTBE (w/30 mg L^{-1} xylenes)	31.4	67.0
m- and *p*-xylene 20 mg L^{-1} (total)[b] and	83.0	
o-xylene 10 mg L^{-1}	62.6	
MTBE + TBA		
MTBE (w/10 mg L^{-1} TBA)	94.2	0.8
TBA 10 mg L^{-1}	45.1	
MTBE (w/25 mg L^{-1} TBA)	93.4	1.7
TBA 25 mg L^{-1}	42.5	
MTBE (w/10 mg L^{-1} BTEX mixture)[c]	71.6	24.6
MTBE (w/25 mg L^{-1} BTEX mixture)[c]	42.8	54.9

[a]Maximum inhibition = (MTBE removal alone − Minimum MTBE removal in the presence of co-contaminants)/MTBE removal alone×100%
[b]*m*- and *p*-xylene combined.
[c]Degradation of individual BTEX compounds in the mixture (values not listed) was generally comparable to removal observed when spiked alone with MTBE.

rate of MTBE clearly decreased during the addition of benzene, but the total CO_2 production increased. Shortly after benzene feed was initiated, there was a sharp rise in CO_2 effluent concentration. This is consistent with the benzene concentration pattern (see Figure 2) and indicates that benzene mineralization readily occurred, because the enzymes necessary for benzene degradation were either available, or readily synthesized. Integration of the mass of benzene leaving the system via the liquid and gas revealed that approximately 60% of total incoming benzene was biodegraded during the experiment. A detailed carbon balance is not possible as benzene had a marked effect on MTBE biodegradation.

In a similar manner, simultaneous feeding of 10 mg L^{-1} of toluene and MTBE led to a clear decrease in elimination rate of MTBE degradation (Figure 8). CO_2 production did not increase until one hour after introducing toluene in the feed, but CO_2 produced reach a much higher level than during the experiment with benzene. The same response pattern was found for the addition of 25 mg L^{-1} of toluene (not shown here). The short lag phase for the increase of CO_2 production might be due to the time required for induction of enzymes for toluene degradation. Cumulative mass flow data reveal that 94% of the input toluene was degraded during the experiment, which is consistent with the high of CO_2 production rates.

Figures 9 and 10 report the dynamic change of CO_2 production and elimination rate of MTBE for the experiment with 25 mg L^{-1} ethylbenzene and 10 mg L^{-1} of each xylene isomer, respectively. In the case of co-feeding of 25 mg L^{-1} of ethylbenzene, CO_2 increase was observed without delay, which contrasts with the 30-min lag observed for the experiment with 10 mg L^{-1} ethylbenzene performed a few days earlier (results not shown). This is probably due to the fact that some induction was involved the first time the microorganisms were exposed to ethylbenzene. The subsequent exposure to ethylbenzene resulted in biodegradation and production of CO_2 without delay. For xylene, no significant delay was observed in CO_2 production at either low or high xylenes concentration, which might indicate that some microorganisms in the reactor could already degrade xylenes. However, the degree of CO_2 increase was lower than that seen for the other tested aromatics.

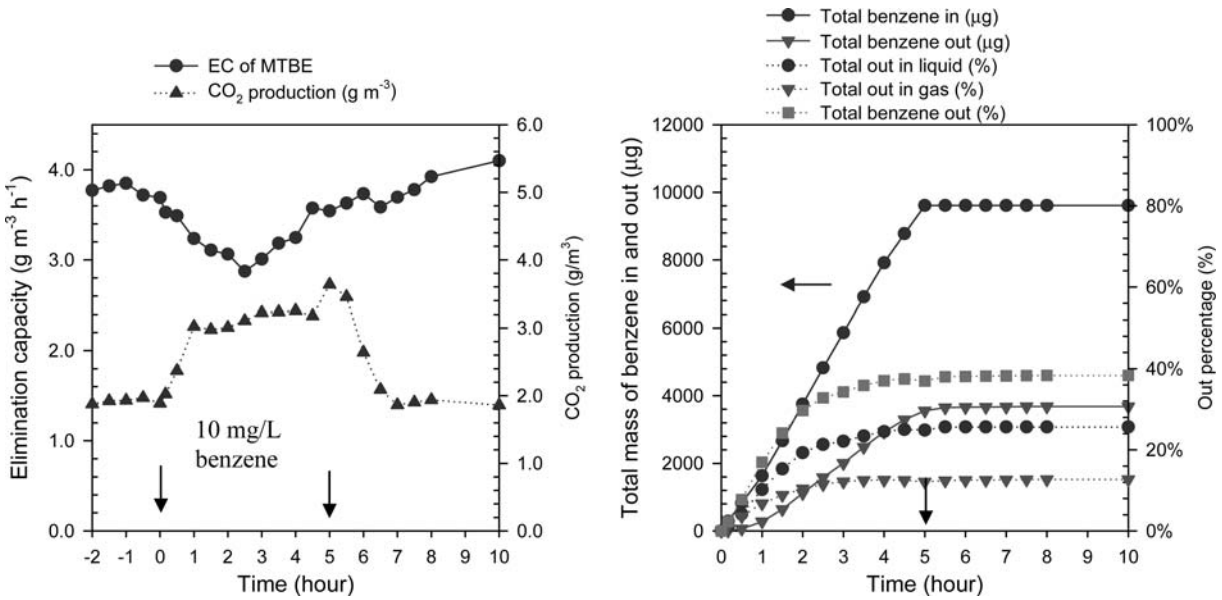


Figure 7. Left: Dynamic change of CO_2 production and elimination capacity of MTBE during the 5-h experiment with 10 mg L^{-1} benzene. Right: cumulative mass flows of benzene in and out of the biotrickling filter (arrows show limits of the 5-h feeding period).

It implies either xylene degrading species were not abundant or that degradation of xylenes was ineffective or incomplete.

Cumulative flows in Figure 8 reveal that only approximately 5% of incoming ethylbenzene exited the biotrickling filter within 5 h of simultaneous loading with MTBE; about another 1% escaped the system in the next 5 h. This latter value is very low and indicates effective biodegradation of ethylbenzene by the enrichment culture.

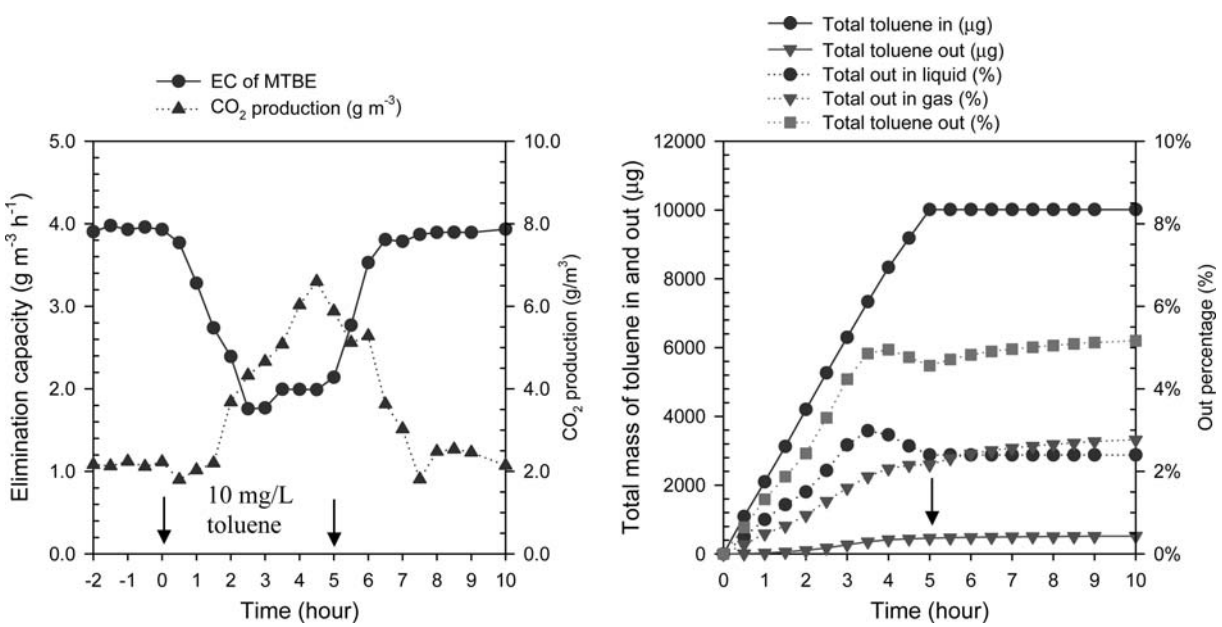


Figure 8. Left: Dynamic change of CO_2 production and elimination capacity of MTBE during the 5-h experiment with 10 mg L^{-1} toluene. Right: cumulative mass flows of toluene in and out of the biotrickling filter (arrows show limits of the 5-h period).

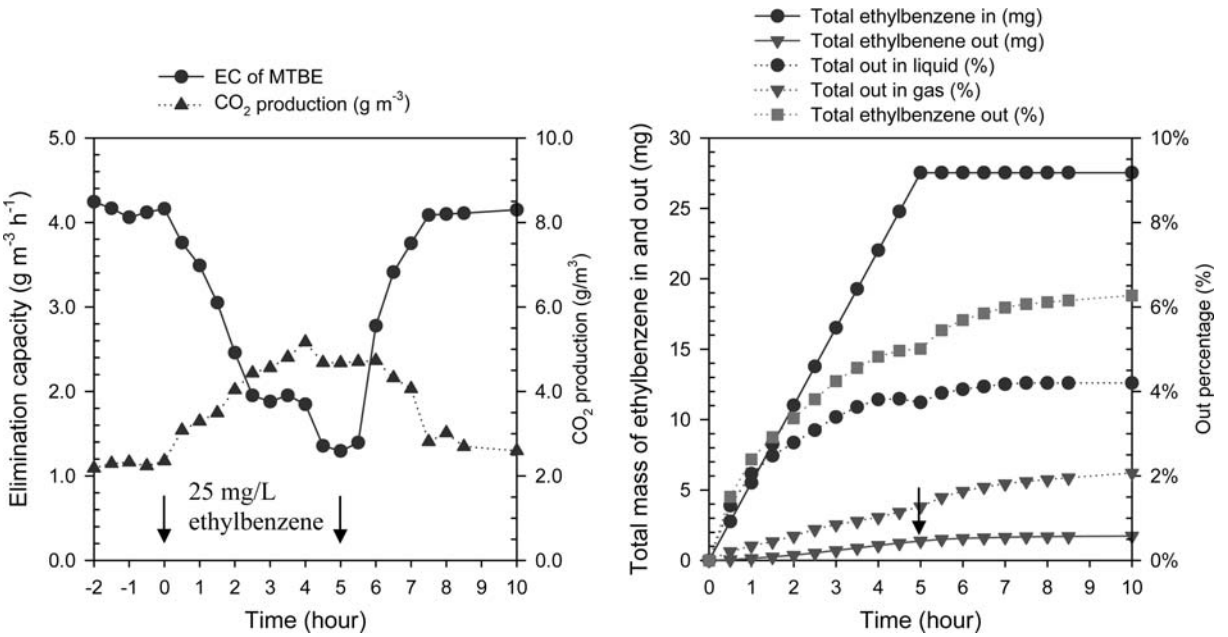


Figure 9. Left: Dynamic change of CO_2 production and elimination capacity of MTBE during the 5-h experiment with 25 mg L^{-1} ethylbenzene. Right: cumulative mass flows of ethylbenzene in and out of the biotrickling filter (arrows show limits of the 5-h period).

Figure 10 shows that MTBE degradation was strongly inhibited by xylenes. The degradation rate of MTBE decreased to about one sixth of the normal level. As a result, nearly 70% of the MTBE fed left the reactor without being degraded, instead of the normal value of less than 10%.

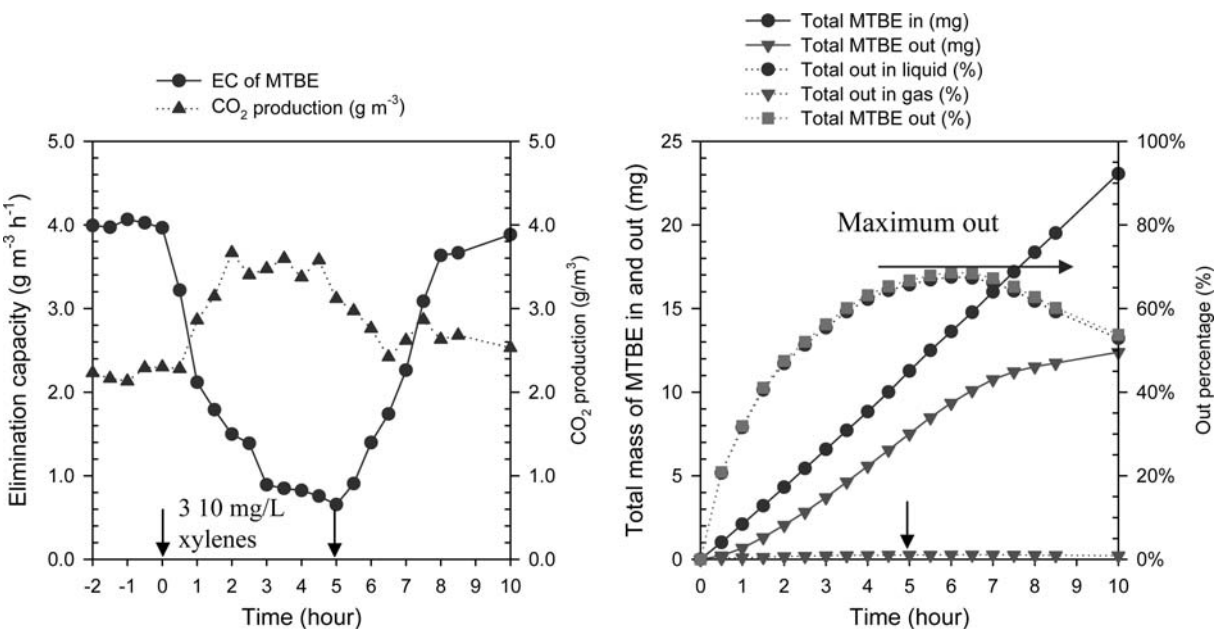


Figure 10. Left: Dynamic change of CO_2 production and elimination capacity of MTBE during the 5-h experiment with 10 mg L^{-1} of each xylene isomers. Right: cumulative mass flows of MTBE in and out of the biotrickling filter (arrows show limits of the 5-h period).

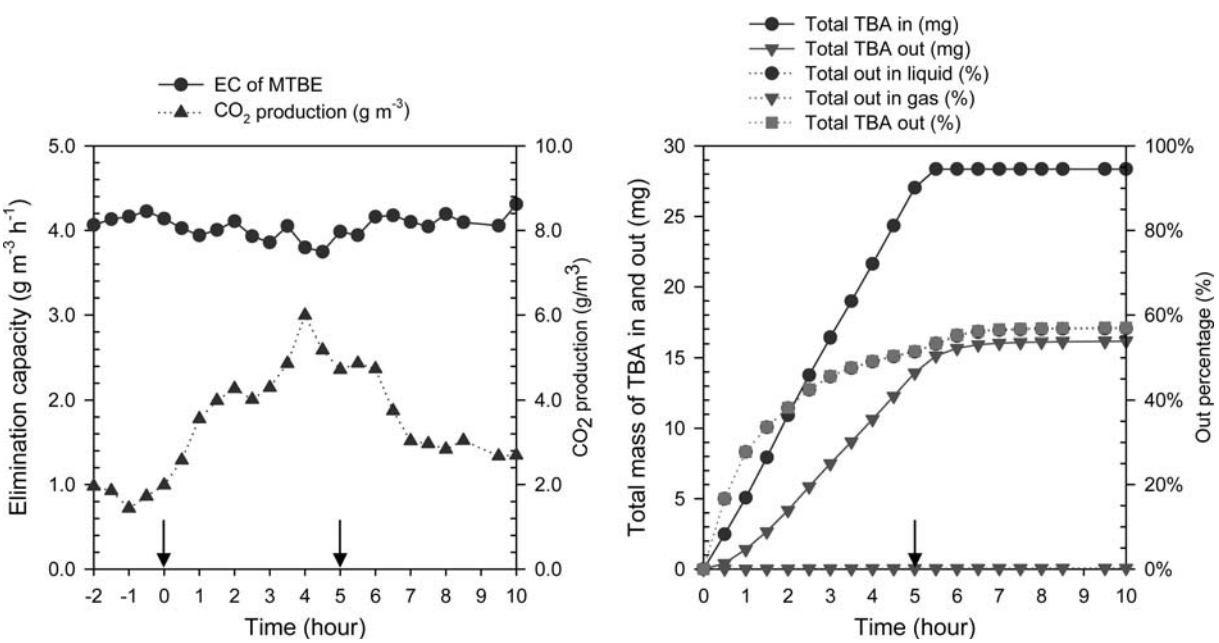
The CO_2 production pattern during exposure to TBA is shown in Figure 11. As mentioned earlier, the effluent TBA concentration was about 10 mg L^{-1} of TBA when 25 mg L^{-1} were fed (see Figure 6). The increase in CO_2 production confirmed that biodegradation of TBA was indeed occurring. Integration of the concentration signal indicated that 42% of total TBA injected was degraded.

General discussion

The results of the impact of BTEX and TBA on MTBE biodegradation are summarized in Table 1 and graphed on Figure 12. Examination of Figures 2–6 reveals significant differences in the breakthrough patterns of the co-contaminants. These are related to the varying degrees of co-contaminant biodegradation which resulted in different levels of MTBE biodegradation inhibition. At the concentrations tested, TBA and ethylbenzene were the least inhibitory compounds, whereas toluene and xylenes were the most inhibitory.

Deeb et al. (2001) had found that the presence of 20 mg L^{-1} of ethylbenzene or xylenes in mixtures with MTBE completely inhibited MTBE degradation by a pure culture of *Rubrivivax* sp. PM1. In our case, examination of the consortium in the biotrickling filter using 16S and 23S rDNA denaturing gradient gel electrophoresis (DGGE) analyses and subsequent sequencing of selected bands revealed that PM1-like organisms (>99% DNA sequence identity) were present in the biotrickling filter (Wang 2003). Thus the difference of Deeb et al. results with those presented inhere suggests that either MTBE degrading species other than PM1 were also in the reactor, and/or that some bacterial members of the consortium degraded benzene to a level that it could not fully inhibit MTBE degradation. As discussed above, CO_2 production increased without a lag (Figure 6) after initiating benzene feed, which would indicate the existence of benzene degrading species in the biotrickling filter. The presence of MTBE degraders other than PM1 could not be determined.

Unlike benzene, significantly distinct responses were achieved with feeding different concentrations of toluene. The degradation rates of both MTBE and toluene were much lower at 25 than at 10 mg L^{-1}. However, this difference may not solely be the result of the difference concentration. As mentioned earlier, the 25 mg L^{-1} toluene experiment was conducted prior to the test with



Figuer 11. Left: Dynamic change of CO_2 production and elimination capacity of MTBE during the 5-h experiment with 25 mg L^{-1} of TBA. Right: cumulative mass flows of TBA in and out of the biotrickling filter (arrows show limits of the 5-h period).

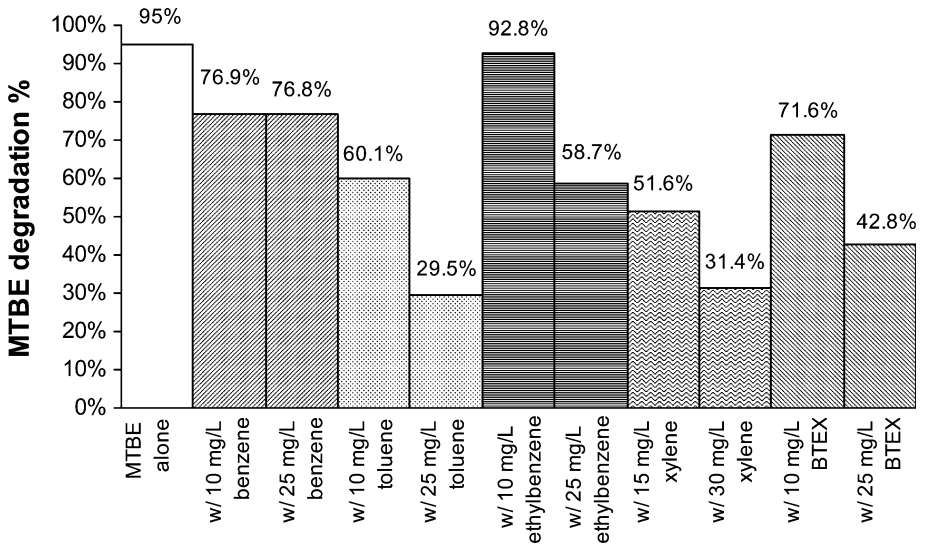


Figure 12. Cumulative percentage of MTBE removal in the biotrickling filter in the presence of BTEX or TBA at different concentrations.

10 mg L^{-1}. This may have had an impact on the results, as toluene degradation required induction of enzymes (see discussion with Figures 3 and 7). Very possibly, the exposure to 25 mg L^{-1} toluene caused some degree of acclimation and therefore, the subsequent experiment with 10 mg L^{-1} toluene resulted in greater degradation of toluene, and possibly a lower degree of interaction with the biodegradation of MTBE.

Among BTEX compounds, ethylbenzene at 10 mg L^{-1} had the mildest effect on MTBE degradation and eventually most of incoming ethylbenzene at either 10 or 25 mg L^{-1} was removed in the reactor. The highest concentration of ethylbenzene caused greater decrease in MTBE degradation probably because some MTBE-degrading bacteria shifted to breaking down ethylbenzene, which is a more favorable substrate.

Mixtures of xylenes and BTEX strongly inhibited MTBE degradation, with increasing effect at increasing concentrations. Interestingly, the inhibitory effect of BTEX mixture appeared to be additive. In other words, the effect of the mixture was very close to the sum of each single compound effect. The causes for such a peculiar observation remain to be elucidated.

Compared to BTEX, TBA at 10 or 25 mg L^{-1} did not result in a noticeable effect on MTBE degradation. This is consistent with previous microcosm studies that indicated that TBA at concentrations below 120 mg L^{-1} had no inhibitory effect on MTBE biodegradation (Fortin et al. 1997). On the other hand, the same studies showed that MTBE at concentrations up to 600 mg L^{-1} had no effect on TBA biodegradation, hence one would have expected that the removal of TBA in the biotrickling filter would have been greater than 50%, the value observed. Especially since the consortium exhibited a faster degradation rate of TBA than MTBE (Wang 2003). The reasons for the lower than expected removal of TBA are still unclear. It is intriguing to think that one possible reason could be that MTBE biodegradation may have taken a pathway that does not produce TBA as metabolite. Earlier, two studies had independently proposed that MTBE and TBA are very likely degraded by two separate, independent metabolic systems (Fortin et al. 1997; Hatzinger et al. 2001), which could be an explanation for the observed phenomena, though it remains to be verified.

The question of the possible relationship between biodegradation of co-contaminant and inhibition of MTBE biodegradation is further explored in Figure 13, where inhibition of MTBE biodegradation is reported vs. the removal of co-contaminant. Ethylbenzene was an obvious outlier, being well degraded without much inhibition of MTBE biodegradation. This is supposedly because ethylbenzene was degraded by organisms

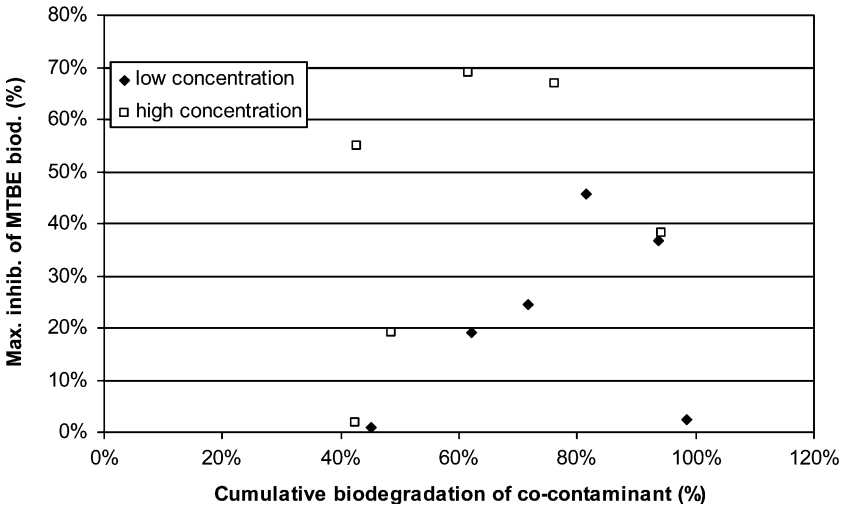


Figure 13. Inhibition of MTBE biodegradation vs. biodegradation of co-contaminant.

that did not degrade MTBE. For all other compounds, there appears to be a clear positive correlation between inhibition and biodegradation at low concentration of co-contaminant, and some trend for the higher concentration cases. Interestingly, there seemed to be a threshold at about 40% biodegradation of the co-contaminant, below which little inhibition occurred. The inhibition was much more severe at the higher concentration. Further detailed metabolic studies, possibly coupled with reactor modeling, would be needed to fully understand the reasons for such behaviors.

Conclusion

The present study demonstrates that biotrickling filtration technology can efficiently remove MTBE from contaminated water. No metabolic intermediates were found in the outgoing liquid and gas. CO_2 emission experiment showed a clear relationship between the MTBE consumption and CO_2 production, and hence confirmed that mineralization of the treated contaminants occurred in the bioreactor.

Co-feeding of single BTEX compounds or BTEX mixtures inhibited MTBE degradation to different degrees, but none of them completely repressed MTBE biodegradation. Compared to the study with the pure culture *Rubrivivax* sp. PM1 (Deeb et al. 2001), the present results suggest that relying on mixed cultures immobilized in biofilms results in a more robust process, less sensitive to the presence of co-contaminants.

The consortium in the reactor was capable of removing over 90% of ethylbenzene and toluene at 10 mg L^{-1}, or partially degrading benzene, xylenes and toluene (at 25 mg L^{-1}) and TBA (at 10 and 25 mg L^{-1}). The increase of CO_2 production during co-feeding of contaminant clearly showed that the BTEX compounds and TBA were mineralized. Prior exposure to a given compound usually enhanced the degradation during subsequent feeding of the compound.

The precise mechanisms of how BTEX and TBA impact MTBE degradation are still not known. One can safely assume that the different microbial members in the consortium serve different roles in MTBE, BTEX and TBA biodegradation, and that the biodegradation of BTEX and MTBE are using different enzymes, either within same bacterium or distributed amongst the different species. Metabolically, MTBE is not a very favorable substrate; therefore, the presence of more easily degradable compounds such as toluene and ethylbenzene would result in preferential use of the more favorable substrates. TBA is a widely acknowledged daughter intermediate of MTBE degradation. However it had no negative effect on MTBE biodegradation, while it was itself well degraded (~50%), though not to the extent one might have expected from TBA alone biotrickling filtration experiments (Wang 2003). The result

challenges whether TBA is an obligatory intermediate of MTBE mineralization, since it is hard to explain why TBA degradation was lower than that of MTBE degradation.

Overall, this study shows that it is feasible to use a mixed bacterial consortium to efficiently biodegrade MTBE, BTEX and TBA at the same time. From an applied perspective, it is probably an advantage to use a consortium rather than a pure culture for the bioremediation of MTBE and other gasoline compounds, as it provides the diversity and robustness needed for field applications.

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