Biodegradation of MTBE and BTEX in an aerobic fluidized bed reactor

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Abstract An aerobic fluidized bed reactor (FBR) was operated for the removal of methyl *tert*-butyl ether (MTBE) and benzene, toluene, ethylbenzene, and p-xylene (BTEX) from water. The reactor was seeded with a mixed culture adapted to MTBE. Granular activated carbon (GAC) was used as the biological attachment medium. Influent MTBE to the reactor was 7.8 mg/L MTBE, with a flow rate of 22.7 L/day, and an empty bed contact time of 1 hour. The acclimation period required was relatively short, about 30 days before reaching an average stable effluent concentration of 18.5 ± 10 μg/L. BTEX was introduced to the feed at an equivalent chemical oxygen demand (COD) as the MTBE at day 225 and was biodegraded spontaneously with no apparent acclimation period required. The average influent of each of the four BTEX compounds was about 2 mg/L, and the range of the average effluent concentrations was 1.4–2.2 μg/L. After achieving 180 days of stable performance with BTEX addition, the total flow rate to the reactor was gradually increased by 20% increments to 160% of the original flow (36.4 L/day). Increases by 20% and 40% had no apparent effect on reactor performance, but increase by 60% required 30 days before effluent quality returned to previous values. Composition of the culture was monitored throughout operation of the reactor using denaturing gradient gel electrophoresis (DGGE). The culture consisted of *Flavobacteria-Cytophaga* and organisms with high similarity to the known MTBE degrader PM1.

Keywords Biodegradation; BTEX; DGGE; fluidized bed reactor; MTBE; PM1

Introduction

Methyl *tert*-butyl ether (MTBE) is a popular gasoline additive used for boosting octane ratings and for promoting cleaner automobile emissions. MTBE is problematic as a groundwater contaminant primarily because of its high water solubility which causes it to mobilize rapidly when a spill occurs. While the health effects of MTBE are still under scrutiny, there is a need to develop and optimize technologies for removing MTBE from groundwater, especially when downstream drinking water resources are a concern. Near the source of the plume, special consideration must also be taken for the removal of benzene, toluene, ethylbenzene and xylenes (BTEX), which are the most water soluble components of bulk gasoline, and which have known adverse health effects (benzene is a known human carcinogen). Preferential degradation of MTBE or BTEX will lead to depletion of electron acceptors and nutrients, and thus adversely affect the degradation of the less preferred compounds. In addition some studies have shown that BTEX metabolically interferes with MTBE degradation (Deeb *et al*., 2001).

Several studies have demonstrated the importance of biomass retention for effective MTBE removal (Salanitro *et al*., 1994; Wilson *et al*., 2000; Pitre and Steffan, 1999; Morrison *et al*., 2001). Fluidized bed reactors (FBRs) which utilize granular activated carbon (GAC) as a biological attachment medium have excellent biomass retention owing to the extensive surface area and sheltering capabilities of GAC. GAC has the additional advantage of the capability to absorb shock loads of contaminants to the reactor.

The objective of this study was to evaluate the application of an aerobic FBR for the

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removal of MTBE from water. The effect of addition of BTEX to the system as well as increased total flow rate (decreased empty bed contact time (EBCT)) was studied. The composition of the culture attached to the GAC was monitored through the course of operation using denaturing gradient gel electrophoresis (DGGE) in order to observe an effect of the changes in reactor operating conditions on microbial populations.

Material and methods

Operation of fluidized bed reactor (FBR)

The FBR consisted of 10.2 cm diameter acetate with a volume of 7.88 L. The reactor was loaded with 0.5 kg of 16 × 20 US mesh washed virgin GAC. Ten milliliters of a mixed culture enriched in a porous pot reactor with MTBE as the sole carbon substrate (see Pruden *et al.*, 2001a for description) was used to seed the reactor. MTBE or MTBE and BTEX mixture was provided to the reactor in neat form using a Gastight syringe (Hamilton Reno, NV) and a programmable syringe pump (Harvard Apparatus South Natick, MA). All feed lines to the reactor consisted of stainless steel tubing in order to minimize abiotic loss of substrate. Flow to the reactor consisted of bulk flow (deionized water), buffer flow (sodium carbonate/sodium hydroxide solution), and nutrient flow, with final nutrient concentrations as described in Pruden *et al.* (2001a). From days 136 to 275, the nutrient concentration to the reactor was doubled in order to determine if there was an effect. All flows to the reactor were regulated using programmable on/off timers. Total flow to the reactor was initially 22.7 L/d and on day 403 was increased by 20% increments to 36.4 L/day by day 419. Influent MTBE concentration prior to BTEX addition was an average of 7.8 mg/L, and increased slightly to 8.8 mg/L after BTEX addition. BTEX was introduced to the feed on day 225, with the total COD of the four compounds being equivalent to that of the MTBE. The average influent concentrations for each of the four BTEX compounds was 2.0 mg/L. A coolant mixture was thermostatically regulated and continuously recirculated through the jacket of the FBR in order to maintain the reactor temperature at 20°C throughout the study. The pH of the reactor was maintained between 7.4–7.9 through regular monitoring and buffer adjustment. Dissolved oxygen (DO) concentrations were maintained above 2 mg/L using air diffusers placed at the level of the fluidization recycle line. Fluidization was maintained at 150% of the GAC bed volume.

Analysis

The reactor was monitored daily for pH (Model 720A pH meter, Orion Research Co., Boston, MA) and flow rates. DO was measured using a Checkmate II DO probe (Corning, NY). Effluent concentrations of BTEX, MTBE, and its intermediates: *tert*-butyl alcohol (TBA) and *tert*-butyl formate (TBF) were measured bi-weekly using a heated purge and trap (series 3100, Tekmar, Cincinnati, OH) coupled with an Agilent (Palo Alto, CO) 6890 series plus gas chromatograph (GC) with a flame ionization detector (FID). The trap used was Supelco (Bellefonte, PA) type "K", and the column used was a DB-1 (J&W Scientific, Folsom, CA). Effluent total organic carbon (TOC) concentrations were measured using a Shimadzu TOC 5050 in non-purgeable organic carbon (NPOC) mode, in order to determine if significant concentrations of degradative intermediates remained which were not detected by gas chromatography. COD of the effluent was also monitored using ultra low range Hach vials (Loveland, CO), and a Hach direct read spectrophotometer.

Chemicals Used

The following chemicals were used as supplied: methyl tert-butyl ether (MTBE, M_w = 88.15, 99%, Aldrich, Milwaukee, WI), tert-butyl alcohol (TBA, M_w = 74.12, 99%, Aldrich, Milwaukee, WI), benzene (M_w = 78.11, 99%, Fisher Scientific, Pittsburgh, PA), toluene

(M_w = 92.14, 99%, Fisher Scientific, Pittsburgh, PA), ethyl-benzene (M_w = 106.17, 99%, Fisher Scientific, Pittsburgh, PA), and p-xylene (M_w = 106.17, 99%, Fisher Scientific, Pittsburgh, PA).

Denaturing gradient gel electrophoresis

DGGE was done before introduction of BTEX (day 225), one month after introduction of BTEX, 2 months after introduction of BTEX, 5 months after introduction of BTEX (before increasing flow rate, day 390) and one month after increasing flow rate (day 435). DGGE was carried out as described in Pruden *et al*. (2001a) with the exception that the DNA was initially extracted from the GAC using a FastDNA Spin Kit for Soil following the manufacturer's protocol (Bio101, Hercules, CA). The GAC samples were sonicated for 5 minutes prior to extraction in order to facilitate detachment of biomass from the attachment medium. PCR Primers used were as described in Muyzer *et al*. (1993).

Results and discussion

Initial operation of the FBR

Table 1 reports the averages and standard deviations of MTBE, TBA, and BTEX effluent concentrations before and after BTEX addition. Figure 1 plots the overall performance of the FBR throughout the various operating periods.

The FBR adapted very quickly to the initial conditions, requiring only 30 days before a stable effluent concentration was achieved. The average MTBE effluent concentration from day 30 to day 218 was 18.5 ± 10 µg/L. Although the seed culture was enriched under suspended growth conditions, it was able to adapt to attached growth conditions without any notable delay. Fortin and Deshusses (1999) observed a significantly longer start-up period for another attached-growth system.

The only MTBE degradation intermediate detected in the effluent was TBA, which was consistently measured to be below 1 µg/L. This is a promising finding as TBA regulations are now developing. The MTBE effluent concentration levels, however, fall right on the lower end of the current U.S. EPA advisory of 20–40 µg/L, and well above the California advisory of 5 µg/L. Other biomass retaining reactors have been observed to achieve lower effluent concentrations, such as a membrane bioreactor (MBR) which achieved an average effluent MTBE concentration of less than 0.5 µg/L MTBE (Morrison *et al*., 2001). An MBR, which is capable of retaining even virus particles, is much more effective than the FBR at biomass retention. This suggests that the biomass retaining properties of a GAC-FBR may not be sufficient for meeting strict effluent MTBE standards. The TBA concentrations, however, were significantly lower in the FBR than what is reported for the MBR, which may prove important if the health effects of TBA become a greater concern than the health effects of MTBE. Monitoring of the effluent FBR DOC (Figure 2) confirmed that only a very low concentration of DOC is leaving the reactor. Monitoring of effluent COD gave similar results (data not shown). This suggests that the MTBE fed to the reactor is mineralizing, and not simply being converted to other intermediates. Doubling nutrient concentration to the reactor had no apparent effect, and did not aid in reducing effluent MTBE, TBA, or BTEX concentrations (Figure 1).

Table 1 Average and standard deviation of fluidized bed reactor effluent compounds before and after BTEX addition. Values are reported in µg/L

	MTBE	TBA	Benzene	Toluene	Ethylbenzene	p-Xylene
Before BTEX	18.5 ± 10	1.0 ± 1.6				
After BTEX	19.7 ± 8	0.36 ± 0.32	1.37 ± 1.22	1.17 ± 1.28	2.24 ± 1.28	1.90 ± 2.08

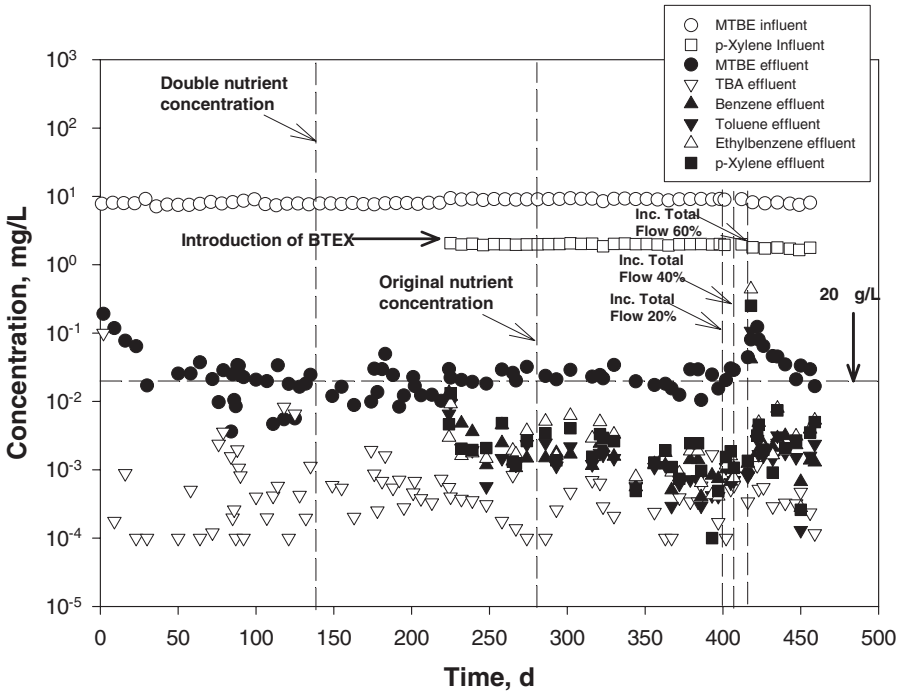

Figure 1 Performance of fluidized bed reactor with time: influent and effluent concentrations are plotted through various stages of operation. For brevity, only p-xylene is plotted of the four influent BTEX compounds. The dashed horizontal line indicates the 20 μg/L effluent concentration level

Cotreatment of MTBE with BTEX

Figure 1 shows that addition of BTEX to the feed did not affect MTBE degradation. Table 1 also reveals that the average effluent MTBE concentration before and after BTEX addition fell within one standard deviation of each other. BTEX achieved stable effluent concentrations immediately after initial addition, with the range of the average concentration of the four compounds being 1.37 to 2.24 μg/L. This is an interesting observation considering that the seed culture had no history of prior exposure to BTEX. This demonstrates that versatile cultures exist capable of both MTBE and BTEX degradation.

Effect of increased flow rate

Increasing the flow rate by 20% and 40% of the original rate had no apparent effect on FBR performance. Increasing flow by 60% of the original flow, however, resulted in immediate disruption of reactor performance (Figure 1). After four weeks, the FBR adapted to these conditions, and effluent quality returned to previous levels. This demonstrated that the FBR can tolerate higher flow rates when given time to recover. Further studies will indicate if there is a limit to the total flow to the reactor.

FBR community analysis

DGGE community fingerprints done with each stage of the study are shown in Figure 3. The identity of the bands marked in Figure 3 are shown in Table 2. Bands 1–4 were stable and present throughout the entire study. Bands 2 and 3 showed very high similarity to the known MTBE degrader, PM1 (Hanson *et al*., 1999; Deeb *et al*., 2001). Bands 1 and 4 belong to the *Flavobacteria-Cytophaga*, which were observed in several other MTBE degrading reactors (Pruden *et al*., 2001a). Bands 5–8 appeared 5 months after the addition of BTEX. It is difficult to speculate the role of the organisms represented by bands 5–8,

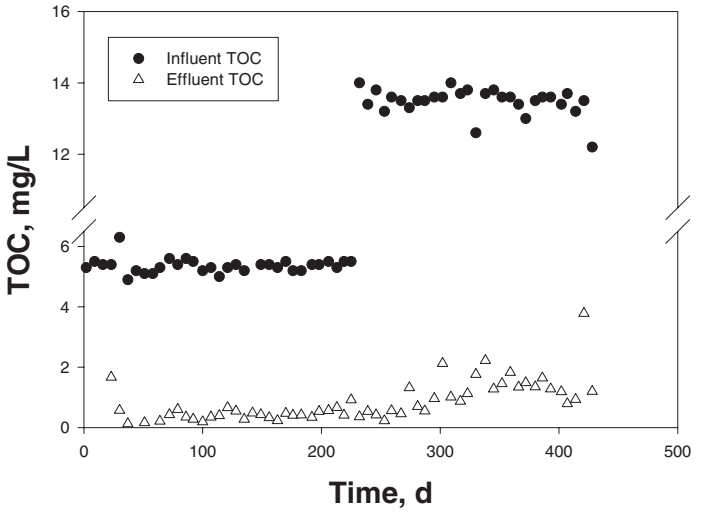

Figure 2 Plot of influent and effluent TOC to the reactor

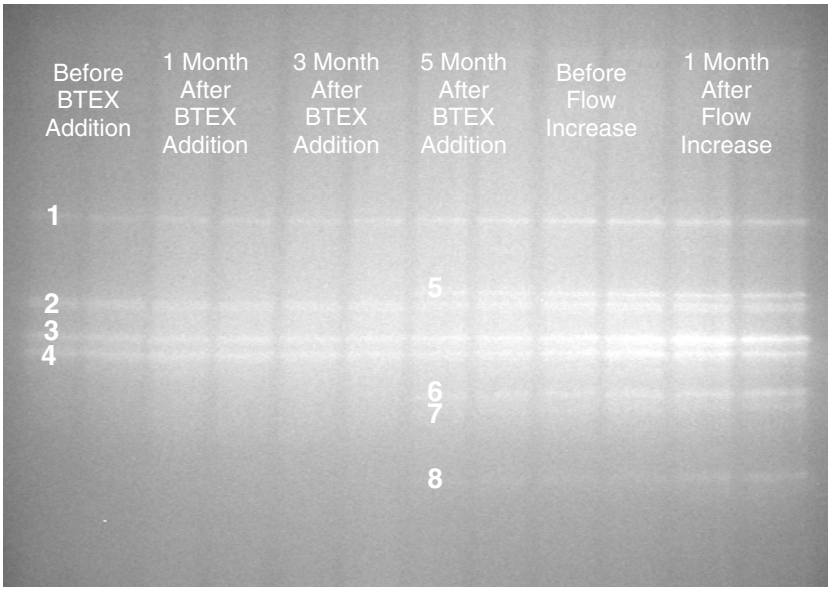

Figure 3 DGGE fingerprinting of the microbial community inhabiting the FBR GAC. DGGE was done in duplicate, individual bands are labeled by number

other than to note that their dominance in the community was not necessary for the stable reactor performance that was observed prior to BTEX addition. The most likely role would be linked with BTEX degradation, with a significant amount of time required before these organisms became visibly enriched in the community. DGGE analysis, however, did confirm that the general structure of the community before BTEX addition and immediately after BTEX addition were identical. PM1-like organisms were also present throughout the study. This demonstrates that in under mixed culture reactor conditions, PM-1 like microorganisms are not inhibited by the presence of BTEX, as was observed in pure culture studies (Deeb *et al*., 2001). This supports the results of other studies that have shown that MTBE and BTEX compounds can be degraded simultaneously in mixed-culture reactors (Pruden *et al*., 2001b, Schroeder *et al*., 2000).

Table 2 Identification of DGGE bands

	Identity
Band 1	Flavobacterium-Cytophaga
Band 2	Beta Proteobacterium – PM1
Band 3	Beta Proteobacterium – PM1
Band 4	*Flavobacterium-Cytophaga*
Band 5	*Flavobacterium-Cytophaga*
Band 6	*Flavobacterium-Cytophaga*
Band 7	*Flavobacterium-Cytophaga*
Band 8	unknown

Conclusions

This study demonstrates that MTBE contaminated water can be biologically treated using a fluidized bed reactor with and without BTEX addition. Effluent concentration of MTBE before BTEX addition and after BTEX addition were comparable. BTEX therefore did not inhibit MTBE degradation. DGGE community fingerprinting revealed that organisms most closely related to the known MTBE degrader PM1, were present in the reactor throughout its operation, as were members of the *Flavobacteria-Cytophaga*. Presence of PM1-like organisms before and after BTEX addition, along with stable removal of MTBE and BTEX suggest that these organisms were not inhibited by BTEX. Gradual increase in total flow to the FBR has demonstrated the feasibility of stable reactor performance at higher flows.

Acknowledgements

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