BTE-OX biodegradation kinetics with MTBE through bioaugmentation

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Abstract The biodegradation kinetics of BTE-oX and MTBE, mixed all together, in the presence of bioaugmented bacterial populations as high as 880 mg/L VSS was evaluated. The effect of soil in aqueous samples and the effect of Tergitol NP-10 on substrate biodegradation rates were also evaluated. Biodegradation kinetics was evaluated for 36 hours, every 6 hours. Benzene and o-xylene biodegradation followed a first-order one-phase kinetic model, whereas toluene and ethylbenzene biodegradation was well described by a first-order two-phase kinetic model in all samples. MTBE followed a zero-order removal kinetic model in all samples. The presence of soil in aqueous samples retarded BTE-oX removal rates, with the highest negative effect on o-xylene. The presence of soil enhanced MTBE removal rate. The addition of Tergitol NP-10 to aqueous samples containing soil had a positive effect on substrate removal rate in all samples. Substrate percent removals ranged from 95.4–99.7% for benzene, toluene and ethylbenzene. O-xylene and MTBE percent removals ranged from 55.9–90.1% and 15.6–30.1%, respectively.
Keywords Bioaugmentation; biodegradation; bioremediation; BTEX; MTBE; Tergitol NP-10

Introduction

Benzene, toluene, ethylbenzene and mixed xylenes (BTEX) along with methyl tertiary-butyl ether (MTBE) are volatile organic compounds (VOCs) commonly found in petroleum-contaminated sites. Underground storage tanks (USTs), production sites, transfer facilities and accidental spills are often reported as an important source of soil and eventually groundwater contamination by BTEX and MTBE (USEPA, 2000). It is also known that a prevalent cause of MTBE groundwater contamination occurs through MTBE concentrations in storm water runoff due to atmospheric emission fallout (Squillace *et al*., 1996). BTEX are included in the current United States Environmental Protection Agency (USEPA) drinking water standards list under the National Primary Drinking Water Regulations (NPDWRs). The maximum drinking water levels for BTEX are 0.005, 1.0, 0.7, and 10 mg/L, respectively (USEPA, 2001). Additionally, the North Carolina Department of Environment and Natural Resources (NCDENR) has set the risk based maximum soil contaminant concentrations (MSCC) for a number of hydrocarbons including BTEX (NCDENR, 2002). The maximum contaminant levels (MCLs) for BTEX in drinking water in Mexico are 0.01, 0.3, 0.7 and 0.5 mg/L, respectively (DOF, 2000). Also, in Mexico, emerging environmental regulations for BTX-contaminated soil have set maximum contaminant levels (MCLs) (DOF, 2002). In the United States, the MTBE drinking water health advisory level for taste and odor has been set at 20–40 µg/L by the EPA

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(USEPA, 1997). Some studies have shown that among the mixed xylenes (o-, m- and p-xylenes), o-xylene appears to be most recalcitrant (Stewart and Kamarthi, 1997). In addition, it has been reported that revertant strains grown on o-xylene are able to metabolize *meta* and *para* isomers (Di Lecce *et al*., 1997) and that the use of nonionic surfactants offer a potential alternative to enhance substrate apparent solubility (Volkering *et al*., 1995) and dissolution rate (Grimberg *et al*., 1996). New developments in environmental regulations and site cleanup demand the formulation of new and more evolved remediation technologies to treat contaminated sites, including groundwater bodies.

This study was aimed to evaluate the biodegradation kinetics of BTE-oX, all together, in the presence of MTBE by the addition of bioaugmented bacterial populations previously acclimated to unleaded gasoline. The effects of soil and the addition of nonionic surfactant Tergitol NP-10 on BTE-oX and MTBE biodegradation kinetics were also evaluated.

Materials and method

Chemicals and culture conditions

Chemicals, including BTE-oX, MTBE and Tergitol NP-10 (TNP-10, a nonionic surfactant) were purchased from Sigma-Aldrich (Mexico) and were above 98% purity. Unleaded gasoline (UG) Premium was purchased from a local gas station. Mineral medium I (MMI) was prepared in deionized water and maintained in the seed biomass acclimation bioreactor according to the following concentration (in mg/L) (Acuna-Askar *et al*., 2003): KH_2PO_4, 17; K_2HPO_4, 44; $Na_2HPO_4 \cdot 2H_2O$, 67; $MgSO_4 \cdot 7H_2O$, 23; NH_4Cl, 3.4; $(NH_4)_2SO_4$, 40; $FeCl_3 \cdot 6H_2O$, 1. Mineral medium II (MMII) was prepared to resuspend the bacterial cells after centrifugation and had the following composition (in g/L): Na_2HPO_4, 6; KH_2PO_4, 3; NaCl, 1; NH_4Cl 1, $MgSO_4 \cdot 7 H_2O$ 0.5; $CaCl_2$, 0.011; $FeCl_3 \cdot 6H_2O$, 0.001. Substrate mineral medium (SMM) was prepared for the experimental bioassays to evaluate biodegradation kinetics and consisted of MMII, 50 mg/L of each BTE-oX component and 50 mg/L MTBE. The pH of MMII and SMM was 7.0–7.5.

Critical micelle concentration

The critical micelle concentration (CMC) was chosen as the concentration range of TNP-10 where a sudden variation in the relation between both culture medium density and culture surface tension occurred. The amount of TNP-10 added to experimental bioassays was slightly below the CMC based on prior studies (Acuna-Askar *et al*., 2003).

Biomass acclimation batch reactor

The biomass was grown using a 20 L glass bottle, with 8 L as the working volume, aerated at an inlet flowrate of 50 mL/s and keeping dissolved oxygen at 8.2–8.7 mg/L. Single daily manual additions of 200 mg/L UG as the only source of carbon were made to the bioreactor for 6 months. Culture medium (MMI) was reconstituted once a week throughout the feeding time. Acclimation conditions also included room temperature (17–23°C in Winter and 24 to 32°C in Spring) and pH 7.0–7.5. Enough 1 N NaOH was added daily to keep the pH within range. The conditions described here allowed microbial growth to reach 800–900 mg/L volatile suspended solids (VSS). VSS determination followed Standard Method 2540 E (*Standard Methods*, 1998).

Bioaugmentation and experimental bioassays

A total volume of 560 mL of the mixed liquor was taken from the 20-L biomass acclimation batch reactor using 14 Falcon® tubes (BD No. 352098) filled up to 40 mL each. The acclimated biomass was centrifuged in a Beckman centrifuge (Beckman Instruments, Inc., Palo Alto, CA), model J2MI at 6,000 rpm at 25°C for 5 minutes. The biomass was concentrated

and resuspended in two 50-mL Falcon® tubes with 35 mL of MMII each. An inoculum of 2 mL of concentrated biomass was added to experimental bioassays to reach 880 mg/L VSS, which was a concentration similar to that grown in the 20-L biomass acclimation batch reactor. This procedure was made for each of the three replicates. Bioassays were performed using 50 mg/L as the initial MTBE concentration and 50 mg/L as the initial concentration of each BTE-oX component to evaluate substrate removal capabilities of UG-acclimated biomass. Controls and three sets of samples were evaluated. Controls had only SMM. Set 1 contained SMM and 880 mg/L VSS of microbial inoculum. Set 2 contained SMM, 18.5% sterilized soil (SS) and 880 mg/L VSS of microbial inoculum. Set 3 contained SMM, 18.5% SS, 880 mg/L VSS of microbial inoculum and 25 mg/L TNP-10. MTBE and BTE-oX were monitored for 36 hours every 6 hours. Substrate biodegradation kinetics were conducted using 40 mL Wheaton borosilicate glass EPA vials with Teflon™ fluorocarbon resin-lined top screw caps of GPI thread finish (Wheaton Science Products, Millville, NJ), with a maximum working volume of 22 mL, leaving a headspace available for respiration. Three replicates were run to evaluate substrate biodegradation kinetics.

Sterilization of samples and isolation of acclimated bacteria
5-g soil samples wrapped in aluminum foil were autoclaved in a 21 L Presto autoclave (Industrias Steele, Mexico) following three sterilization cycles. Soil samples were considered sterile at a maximum of 5 CFU/mL in nondiluted samples. Other samples and controls were autoclaved following one sterilization cycle. Standard Methods 9215 A and 9215 B (*Standard Methods,* 1998) were followed for sample preparation and for estimating the number of heterotrophic bacteria. UG-acclimated bacteria were grown in UG agar plates and incubated at 28–30°C for 72 hours.

Sample shaking, sonication and gas chromatography
Samples and controls used for biotransformation studies were shaken using a Lab-line oscillating incubator shaker (Barnstead International, Dubuque, IA) model Orbit. Uniform shaking was maintained at 200 rpm at 30°C. Samples were tested for sonication following the USEPA method 3550, with some modifications, to release potential BTE-oX and MTBE trapped in cell membrane. MTBE and BTE-oX were analyzed by a Varian 3400 GC/FID chromatograph. GC/FID determinations followed standard procedures (USEPA, 1995) with some modifications. A Petrochol™ (Supelco, Bellefonte, PA) 100 m × 0.25 mm ID × 0.5 µm film DH fused silica GC capillary column was used. The initial oven temperature was set up at 60°C and held for 30 minutes, after which the first temperature rate varied 10°C/min from 60°C up to 90°C, at which point the temperature was held for 20 minutes. A second temperature rate followed and varied 30°C/min from 90°C up to 150°C, at which point the temperature was held for 2 minutes. The injector was set up on a split/splitless mode (1:20) and its temperature was set at 250°C. The detector temperature was set at 300°C. 5-mL samples were purged with nitrogen at 25°C for 10 minutes and concentrated prior to injection.

Kinetic models evaluation
For the three sample sets, the overall benzene and o-xylene removal rate constants K were obtained by the first-order one-phase model (Acuna-Askar *et al.*, 2000):

Model I $S_t = S_0 \exp(-Kt)$ (1)

where: S_t = Substrate concentration at time t, (mg/L)
           S_0 = Substrate concentration at time zero, (mg/L)

K = overall first order constant, $K = k X_V$
X_V = VSS, (mg/L)
k = specific rate constant h^{-1}(mg/L)$^{-1}$VSS
t = time (h^{-1})

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The overall removal rate constants K were obtained from the slope by plotting ln S_t versus t.

For the three sample sets, the overall toluene and ethylbenzene removal rate constants K were obtained by the first-order two-phase model (Hu *et al.*, 2004):

$$\text{Model II} \quad S_t = S_1 \exp(-K_1 t) + S_2 \exp(-K_2 t) \tag{2}$$

where: S_t = Substrate concentration at time t, (mg/L)
S_1 = First phase substrate concentration at time zero, (mg/L)
S_2 = Second phase substrate concentration at time zero, (mg/L)
K_1 = First phase kinetic rate constant, (h^{-1})
K_2 = Second phase kinetic rate constant, (h^{-1})

For the three sample sets, the overall MTBE removal rate constants K were obtained by the zero-order model:

$$\text{Model III} \quad S_t = -Kt + S_0 \tag{3}$$

Terms are defined as for model I. The overall removal rate constants K were obtained from the slope by plotting S_t versus t.

Results and discussion
Effect of bioaugmentation, sterile soil (SS) and surfactant on BTE-oX and MTBE
MTBE showed a zero-order removal rate during the time frame evaluation of 36 hours. The presence of soil on MTBE biodegradation had a slight increase on the slope of the curve and the addition of surfactant did not have a significant effect on MTBE biodegradation (Figure 1). All BTE-oX chemicals biodegraded in the presence of bioaugmented bacteria at 880 mg/L VSS. As shown in Figure 2, benzene was removed faster than o-xylene, and these two substrates were removed slower than toluene and ethylbenzene. No significant difference was seen between removal rates of toluene and ethylbenzene.

As indicated in Figure 3, soil had a negative impact on the biodegradation rates of all BTE-oX chemicals, primarily on benzene and o-xylene removal rates. The significant reduction of BTE-oX biodegradation rates by soil can be explained by a decrement of sub-
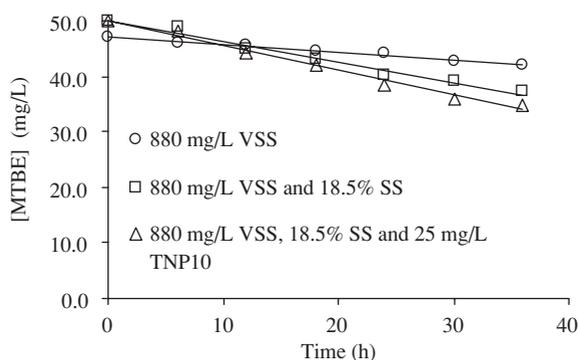
Figure 1 MTBE biodegradation kinetics with 200 mg/L total BTEoX in the presence of 880 mg/L VSS
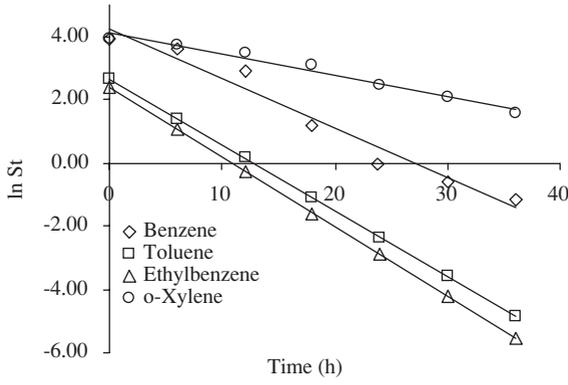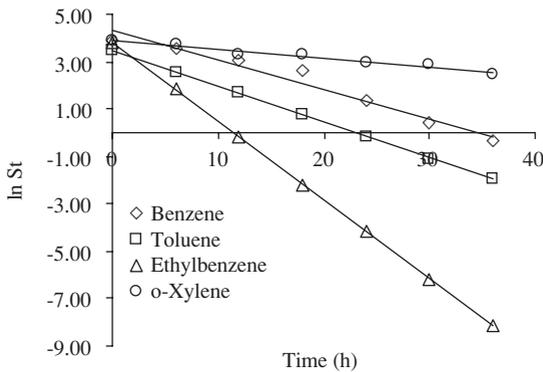
Figure 2 BTEoX biodegradation kinetics with 50 mg/L MTBE in the presence of 880 mg/L VSS
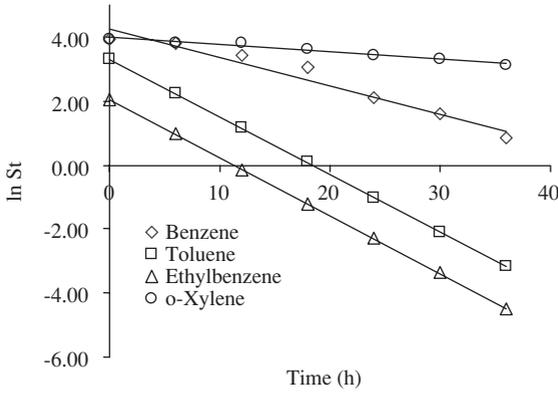
Figure 3 BTEoX biodegradation kinetics with 50 mg/L MTBE, 880 mg/L VSS and 18.5% SS

strate solubility in water, possibly due to the hydrophobic attraction between soil and substrates. As can be seen from comparing Figures 1 and 3, the negative effect of soil on BTE-oX removal rates was higher than the effect of soil on MTBE removal rate, which can be explained by the higher octanol-water partition coeffcients of BTE-oX (Sangster, 1989). As can be seen from comparing Figures 3 and 4, the addition of TNP-10 clearly showed a trend to restore BTE-oX availability in water. Benzene, ethylbenzene and o-xylene removal rates were restored around 50% by the addition of TNP-10 to the slurry samples. Toluene removal rate, however, had a significant negative impact by the addition of TNP-10.

Figure 4 BTEoX biodegradation kinetics with 50 mg/L MTBE, 880 mg/L VSS, 18.5% SS and 25 mg/L of TNP10

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Benzene and o-xylene followed a first-order one-phase removal rate model, whereas toluene and ethylbenzene followed a first-order two-phase removal rate kinetics under the same experimental conditions in the three sample sets evaluated (Table 1). Kinetic models for mixed BTEX and MTBE, all together, are limited in the literature. Reliable fit of data consistently showed that toluene and ethylbenzene had a biphasic removal rate with a strong slope change at 12 hours. First phase kinetic rate constants were significantly higher than the corresponding second phase kinetic rate constants, suggesting that toluene and ethylbenzene removal rates may have been influenced by some type of substrate interaction (Chang *et al.*, 2001). Benzene removal rate constants in all experimental bioassays were consistently higher than o-xylene removal rate constants.

MTBE followed a zero-order removal rate model in the three samples evaluated (Table 2). The presence of other easily assimilated carbon sources such as BTE-oX may have limited MTBE biodegradation. The presence of soil, however, had a positive effect on MTBE removal rate of three-fold. TNP-10 showed a slight increase on MTBE removal rate.

As indicated in Table 3, MTBE biodegradation was 15.6% and increased to 25.1% with the addition of soil and had a slight further increase to 30.1% when surfactant was added to

Table 1 Kinetic model reaction rate constants vs. experimental bioassay samples

	Benzene	Toluene		Ethylbenzene		o-Xylene
Set 1 Samples*						
Overall K rate	K	K_1	K_2	K_1	K_2	K
[h^{-1}]	0.1568	0.2088	0.0808	0.2217	0.0926	0.0673
(r)	(0.985)	(0.999)		(0.999)		(0.986)
Specific k rate	k	k_1	k_2	k_1	k_2	k
[h^{-1} (mg/L)$^{-1}$]	1.78×10^{-4}	2.37×10^{-4}	0.918×10^{-4}	2.52×10^{-4}	1.05×10^{-4}	0.764×10^{-4}
(r)	(0.985)	(0.999)		(0.999)		(0.986)
Set 2 Samples**						
Overall K rate	K	K_1	K_2	K_1	K_2	K
[h^{-1}]	0.0889	0.1807	0.013	0.1808	0.1043	0.0228
(r)	(0.978)	(0.999)		(0.999)		(0.985)
Specific k rate	k	k_1	k_2	k_1	k_2	k
[h^{-1} (mg/L)$^{-1}$]	1.01×10^{-4}	2.05×10^{-4}	0.147×10^{-4}	2.05×10^{-4}	1.19×10^{-4}	0.259×10^{-4}
(r)	(0.978	(0.999)		(0.999)		(0.985)
Set 3 Samples***						
Overall K rate	K	K_1	K_2	K_1	K_2	K
[h^{-1}]	0.1239	0.1519	0.0397	0.3333	0.0652	0.0386
(r)	(0.983)	(0.999)		(0.999)		(0.986)
Specific k rate	k	k_1	k_2	k_1	k_2	k
[h^{-1} (mg/L)$^{-1}$]	1.40×10^{-4}	1.7×10^{-4}	0.451×10^{-4}	3.79×10^{-4}	0.74×10^{-4}	0.438×10^{-4}
(r)	(0.983)	(0.999)		(0.999)		(0.986)

r = correlation coefficient ** SMM + 18.5% SS + 880 mg/L VSS
* SMM + 880 mg/L VSS*** SMM + 18.5% SS + 880 mg/L VSS + 25 mg/L TNP-10

Table 2 MTBE kinetic model reaction rate constants vs. experimental bioassay samples

	Set 1 Samples*	Set 2 Samples**	Set 3 Samples***
Overall K rate [mgL^{-1} h^{-1}]	K	K	K
	0.1362	0.3684	0.4501
(r)	(0.994)	(0.989)	(0.991)
Specific k rate [mgL^{-1} h^{-1} (mg/L)$^{-1}$ $_{VSS}$]	k	k	k
	1.55×10^{-4}	4.18×10^{-4}	5.11×10^{-4}
(r)	(0.994)	(0.989)	(0.991)

r = correlation coefficient   ** SMM + 18.5% SS + 880 mg/L VSS
* SMM + 880 mg/L VSS   *** SMM + 18.5% SS + 880 mg/L VSS + 25 mg/L TNP-10

Table 3 Biodegradation percentage vs. experimental bioassay samples

	Benzene (%)	Toluene (%)	Ethylbenzene (%)	o-Xylene (%)	MTBE (%)
SMM + 880 mg/L VSS	99.3	99.5	99.7	90.1	15.6
SMM + 880 mg/L VSS + 18.5% SS	95.4	97.6	99.7	55.9	25.1
SMM + 880 mg/L VSS + 18.5% SS + 25 mg/L TNP-10	98.6	99.4	99.7	75.9	30.1

the mixture. The low biodegradation of MTBE was not unexpected because previous work (Acuna-Askar *et al.*, 2000; Stringfellow and Oh, 2002; Pruden *et al.*, 2003; Hu *et al.*, 2004) has shown that different conditions are required to achieve MTBE biodegradation. Among the BTE-oX, o-xylene biodegradation was significantly affected by the addition of soil with a 50% reduction in removal performance. The addition of TNP-10, however, helped increase o-xylene percentage removal by 35%, suggesting that the addition of nonionic surfactant at a concentration lower than the CMC was able to enhance the interaction of substrate with the microbial population. This is interesting because previous research had indicated that micellization would restrain hydrocarbon availability (Grimberg *et al.*, 1996).

Conclusion

Benzene and o-xylene biodegradation was well described by a first-order one-phase kinetic model, whereas toluene and ethylbenzene biodegradation followed a first-order two-phase kinetic model in all samples. MTBE followed a zero-order removal kinetic model in all samples. Soil significantly slowed down the biodegradation rate of all BTE-oX compounds, having the highest negative effect on o-xylene biodegradation. The presence of soil enhanced MTBE removal rate. The addition of TNP-10 to aqueous samples containing soil showed an increase in removal rates in all samples evaluated. Benzene biodegradation rates were higher than o-xylene biodegradation rates in all samples. Toluene and ethylbenzene removal rates were higher than benzene removal rates in all samples. No significant differences were found between toluene and ethylbenzene biodegradation rates, except when Tergitol NP-10 was added and, therefore, enhancing the ethylbenzene biodegradation rate. MTBE showed the lowest biodegradation rate among the substrates evaluated. Substrate percent removals ranged from 95.4–99.7% for benzene, toluene and ethylbenzene. O-xylene and MTBE percent removals ranged from 55.9–90.1% and 15.6–30.1%, respectively.

Acknowledgements

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